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Preface

This year´s 21st International PhD Students Conference for undergraduate and postgraduate students is hosted by **the Faculty of Agronomy**, Mendel University in Brno, the Czech Republic, in November 19th–20th, 2014. The conference has provided a platform to discuss new trends in plant and animal production, plant and animal biology, agroecology, rural development, food technology, and techniques and technology etc. with participants from European educational and research institutions.
Their success is reflected in the papers received, with participants coming from diverse backgrounds, allowing a real multinational and multicultural exchange of experiences and ideas.
The accepted papers of this conference are published in this full text that will be sent to international indexes.
Conferences such these can only succeed as a team effort, so the Editors want to thank the Committees and the Reviewers for their excellent work in reviewing the papers as well as their invaluable input and advice.

The Editors

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Section – Plant Production

Effect of tillage and crop rotation on weed infestation in maize

SVETLANA CHOVANCOVA[1], JAROSLAV NOVAK[2], JAN WINKLER[1]
[1]Department of Plant Biology
[2]Department of Agrosystems and Bioclimatology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

svetlana.chovancova@mendelu.cz

Abstract: The aim of this study was to determine the effect of different tillage and crop rotation on weed infestation in maize. The observation was made at the experimental site in Žabčice, which belongs to corn production areas, in 2013 and 2014. The soil was treated in two variants. The first option was the traditional method of tillage (plowing). The second variant was shallow tillage to a depth of 0.05 m (minimum tillage). Crop rotation was another factor influencing weed infestation of maize (maize monoculture, Norfolk crop rotation). The obtained data were evaluated by multivariate analysis of ecological data. Different variants of tillage and crop rotation affect the occurrence and number of certain species, it follows from this analysis. The experimental results show, that the lowest weed infestation was on the variant of maize monoculture. Lower infestation was on the variant with traditional tillage.

Key-Words: weeds, maize, soil tillage, crop rotation

Introduction

Maize is one of the most grown crops on the planet today. The reason for this trend is a many-sided utilization of maize (e.g. human nutrition, animal feed, industrial and energy usage), [1].

While the average yield of maize is more than 8 $t.ha^{-1}$ in developed countries, it is only 3 $t.ha^{-1}$ in the developing countries [2]. Cultivation of maize faces a number of problems especially on soils threatened by erosion. A reduction of soil erosion is reliant on the structure of soil surface, which in principle are better at reduced tillage [3, 4]. Soil protective technologies of cultivation create a new framework for maize planting as well as for growth of weeds [5]. Soil cultivation approaches can affect hydraulic soil features. The differences in the porosity between the soil tillage practices are strongest in the depth of 0.08 m from the soil surface [6].

Miscellaneous ways of soil cultivation have influence on the bulk density. No-tillage increases bulk density by 48%, manual hoeing by 61%, disc cultivation by 55% and stubble breaking by 57% [7]. The stability of soil aggregates is then higher when no-tillage is used, which has direct impact on the soil resistance towards the water erosion [8]. Millions of growers of any size use no-tillage to their profit. This technology improves soil productivity by rising biological activity, reducing the use of fertilizers and decreasing manual and financial costs on farming [9]. The use of no-tillage is accompanied with higher content of soil water during dry months and with periods of unevenly distributed precipitation [10]. Interlinear content of soil water is also higher than in the row, due to the significantly lower soil density in the soil row [11].

Based on the experiments from the Illinois state, where adoption of no-tillage was not easily accepted and despite the environmental benefits, maize yields did not achieve satisfactory results. Lower soil temperatures during no-tillage suspended the development of planting and early growth compared to the conventional tillage at the beginning of the growing season [12]. Effect of tillage on weeds has been studied by number of authors [13, 14, 15]. However, most authors focus their attention on the growth of narrow-row cereals (wheat, barley). This is probably associated with the use of herbicides in stands of maize, which greatly limits the occurrence and harmfulness of weeds. Nevertheless, the presence and the spectrum of weed species in maize change depending on tillage.

Maize is crop in which the weed species spectrum is relatively narrow [16]. *Chenopodium*, *Amaranthus*, *Persicaria*, *Echinochloa crus-galli* are typical weeds of Czech Republic. *Setaria pumila* and other late spring weeds such as *Datura stramonium* may also occur in some locations.

Fallopia convolvulus belongs to typical weeds occurring in maize, particularly due to its high resistance against a number of soil and foliar herbicides. Perennial species such as *Elytrigia repens*, *Cirsium arvense* and *Artemisia vulgaris* are relatively easy to enforce in maize. Above-mentioned weed spectrum is typical for maize areas in the Czech Republic [17, 18, 19]. *Tripleurospermum inodorum, Capsella bursa-pastoris* and *Thlaspi arvense* are cosmopolitan species mainly from the group of overwintering weeds. Their incidence is mostly influenced by weather conditions during that year [20, 21, 22], [23]. The results of these studies show that the different intensity of weed infestation by various species may reduce the yield by 12 - 37%.

Material and Methods

Characteristic of Experimental Location

The experimental plots are located in the cadastral of municipality Žabčice (16° 37´ E, 49° 01´ N). Žabčice is located in the corn production area with a flat terrain and an altitude of 184 meters. The village Žabčice is approximately 25 km south from Brno. The geographical surrounding of Žabčice can be considered as very warm and dry. According to weather station, the annual rainfall in the last 30 years was around 483.3 mm with the average annual temperature of 9.2°C. The long-term data of an average rainfall as well as temperatures can be found in Table 1. The measured data were used from an experimental Meteorological station of Mendel University, in Žabčice.

The territory of Žabčice is located in the vicinity of Dyjsko-Svratka valley, which consists mainly of Neogene sediments. Farmland is partially represented by alluvial plain and quaternary gravel. The habitat is located in young flood plain of the gley-soil and is situated in the alluvial area of the river Svratka. These soils have been created in the holocene, calcareous alluvial sediments. Gluing process is significantly growing to a depth of the soil, which is cause due to the permanent groundwater. The color of the gley horizon is grayish brown and at a depth of up to 90 cm has a clayey structure. The soil reaction is in the arable horizon of the soil neutral, pH is 6.9 and the content of humus is average (2.28%).

Description of Field Trial

The first field trial is monoculture of maize, which has been consecutively grown since 2002. The size of one parcel is 5.3 m x 7.0 m. The crop rotation in the second field attempt is designed according to Norfolk crop rotation system and was established in 1970 and partially changed in 2002. The size of one parcel is 5.3 m x 7.0 m. Crop rotation is as follows: alexandria clover, winter wheat, maize (for grain) and spring barley. NK Silitop was used hybrid of maize in 2013 and SY Beautiful in 2014, with sowing rate 90 000 plant / ha.

Evaluation of Weed Infestation

The weed infestation was evaluated by using a numerical method. Weeds were counted in the area of 1 m^2 in 24 repetitions in each variant of tillage and crop rotation. The evaluation of maize growth was held every July two years in row (2013 and 2014). Names of found species were used according to Kubát [24]. A multivariate analysis of ecological data was used to determine the effect of tillage and crop rotation on the weed species in maize. Selection of the optimal analysis followed the length of the gradient (*Lengths of Gradient*), which was detected by segment analysis DCA (*Detrended Correspondence Analysis*). Furthermore, redundancy analysis RDA was used.

A total number of 499 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program called Canoco 4.0 [25].

Table 1 Long-term precipitation totals and temperature averages for each month (1961 to 1990)

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.
Average temperatures (°C)	-2.0	0.2	4.3	9.6	14.6	17.7	19.3	18.6	14.7	9.5	4.1	0.0
Precipitation (mm)	25	25	24	33	63	69	57	54	36	32	37	26

Table 2 The average number of weed individuals found in maize

	Soil tillage		Crop rotation		Year	
	CT	MT	Mono	Norf	2013	2014
Amaranthus sp.	0.17	0.46	0.49	0.14	0.60	0.02
Anagallis arvensis	0.11	0.36	0.18	0.30	0.43	0.05
Cirsium arvense	0.25	0.25		0.50	0.48	0.02
Convolvulus arvensis	0.35	5.09	1.72	3.73	1.48	3.97
Datura stramonium	0.05	0.21	0.26	0.00	0.19	0.07
Echinochloa crus-galli	3.84	5.63	2.55	6.92	4.81	4.66
Euphorbia helioscopia	0.01			0.01		0.01
Fallopia convolvulus	0.82	0.22	0.32	0.72	0.54	0.50
Galium aparine	0.29	0.50	0.04	0.75	0.43	0.36
Chenopodium album	1.56	0.58	0.61	1.53	1.29	0.85
Chenopodium hybridum	0.16	0.00	0.16	0.00	0.14	0.02
Lamium amplexicaule	0.24	0.08	0.00	0.32	0.24	0.08
Malva neglecta	0.18	0.53	0.07	0.64	0.31	0.40
Microrrhinum minus	0.01		0.01			0.01
Persicaria lapathifolia	0.26	0.11	0.02	0.35	0.32	0.05
Polygonum aviculare	0.03	0.26	0.26	0.03	0.22	0.07
Stellaria media	0.11	0.16	0.05	0.22	0.26	0.01
Thlaspi arvense	0.01	0.19		0.20	0.16	0.04
Trifolium incarnatum	0.30	0.25		0.55	0.34	0.21
Triticum aestivum	0.00	0.18		0.18	0.09	0.08
Veronica persica	0.02	0.10	0.03	0.09	0.10	0.02
Veronica polita	0.00	0.04	0.04	0.00	0.03	0.01
Viola arvensis	0.11	0.00	0.02	0.09	0.09	0.02
Number of individuals	8.91	15.21	6.84	17.27	12.56	11.55

Results and Discussion

Twenty-three (23) various species of weeds were found within the monitoring period. The average numbers of weeds are shown in Table 2.

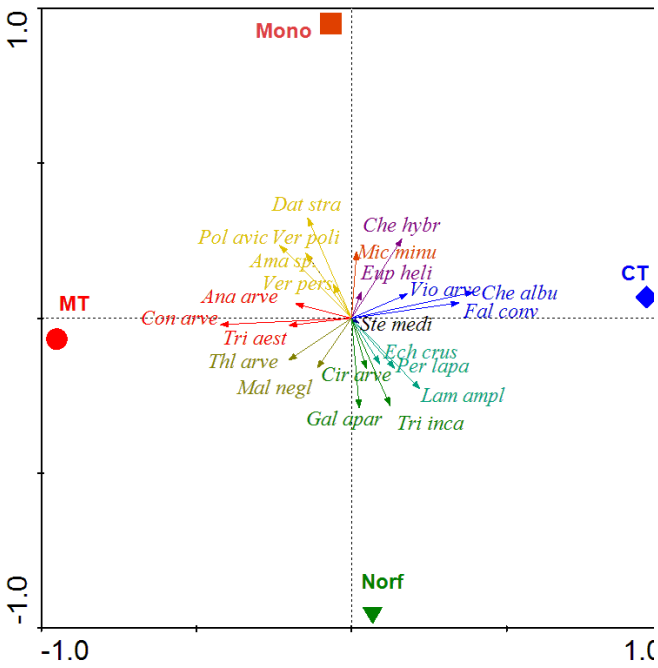
DCA analysis was used as first for statistical evaluation of the results. This analysis calculated the length of gradient (*Lengths of Gradient*), as 3.238. Based on this calculation and for further processing was selected redundancy analysis (RDA) . The RDA analysis defines the spatial arrangement of particular weed species, tillage variants and the crop rotation, on the basis of obtained data, which represent frequency of weed occurrence. This is subsequently graphically displayed in the ordination diagram. Weed species are represented by vectors (arrows), tillage options and crop rotation are displayed as the points of various form and color. In case that the vector of relevant weed species tends to the concrete point of tillage variant or crop rotation means that its occurrence is more bounded to this type of treatment.

Results of redundancy analysis, which evaluated the influence of tillage and crop rotation on weed occurrence are significant at the significant level $\alpha = 0.002$ for all canonical axes. Based on the RDA analysis (Fig. 1) it is possible to divide found weed species into 8 groups.

First group of weed occurred mainly when the minimum tillage (MT) was used and is represented by these species: *Anagallis arvensis, Convolvulus arvensis, Triticum aestivum.* Second group of weed species occurred in connection of minimum tillage together with growing in monoculture: *Amaranthus* sp., *Datura stramonium, Polygonum aviculare, Veronica persica, Veronica polita*. Third group of weeds occurred especially in monoculture: *Microrrhinum minus*. Fourth weed group was formed based on the combination of monoculture and conventional tillage: *Euphorbia helioscopia, Chenopodium hybridum*. Fifth group of weed occurred mainly in connection with conventional tillage: *Fallopia convolvulus, Chenopodium album, Viola arvensis*. Sixth group of weed is represented by the combination of Norfolk crop rotation and conventional tillage: *Echinochloa crus-galli, Lamium amplexicaule, Persicaria lapathifolia*. Seventh group of weeds occurred especially on the variant of Norfolk crop rotation and is represented by these species: *Cirsium arvense, Galium aparine, Trifolium alexandrium*. The last eighth group of

weeds occurred mainly in combination of Norfolk crop rotation system and minimum tillage: *Malva neglecta, Thlaspi arvense*.

Fig. 1 Ordination diagram expressing relations between tillage variants, crop rotation and weed species in growths of maize



Legend: CT – conventional tillage, MT – minimum tillage, Mono – monoculture of maize, Norf – Norfolk crop rotation system.
Ama sp. – Amaranthus sp., *Ana arve – Anagallis arvensis, Cir arve – Cirsium arvense, Con arve – Convolvulus arvensis, Dat stra – Datura stramonium, Ech crus – Echinochloa crus-galli, Eup heli – Euphorbia helioscopia, Fal conv – Fallopia convolvulus, Gal apar – Galium aparine, Che albu – Chenopodium album, Che hybr – Chenopodium hybridum, Lam ampl – Lamium amplexicaule, Mal negl – Malva neglecta, Mic minu – Microrrhinum minus, Per lapa – Persicaria lapathifolia, Pol avic – Polygonum aviculare, Ste medi – Stellaria media, Thl arve – Thlaspi arvense, Tri inca – Trifolium alexandrium, Tri aest – Triticum aestivum, Ver pers – Veronica persica, Ver poli – Veronica polita, Vio arve – Viola arvensis.*

Echinochloa crus-galli was the most frequently delegated species. Its presence was very strong especially in the combination of Norfolk crop rotation and minimum tillage. According to earlier studies, the annual grasses are more often represented on the soils with reduced soil tillage [26]. There have been problems with species *Setaria viridis* in North America, where this species was more frequently observed in the areas with minimum tillage [27].

Weed species *Chenopodium album* occurred the most in the variation of conventional tillage. According to the results of other experiments was its density around 500 plant.m^{-2}, while in the other variations was always lower [28]. Based on the experiment evaluation of four different systems of maize cultivation in the time period of five years monoculture was found, that the cultivation systems established on reduced tillage contained more weed especially by species *Chenopodium album* [29]. The findings of both authors are in disagreement with the results of our experiment. The reason will be most probably in high stock of *Chenopodium album* seeds in soil, which is caused by conventional tillage.

Conclusion

Soil tillage and crop rotation significantly affect both the intensity of weed infestation as well as the spectrum of the weed species. The experimental results show, that the lowest weed infestation was in the variation with maize monoculture. Fewer weed infestation was on the variant of conventional tillage. *Echinochloa crus-galli, Convolvulus arvensis, Chenopodium album* and *Fallopia convolvulus* were the most often occurring weed species.

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Weed species spectrum of chosen field crops

VLADIMIR DANHEL, SVETLANA CHOVANCOVA, JAN WINKLER
Department of Plant Biology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xdanhel@node.mendelu.cz

Abstract: The aim of this thesis was to evaluate weed infestation of chosen crops, to compare weed infestation among each crop and evaluate used regulation interventions, eventually to suggest another solution. Evaluation was made in winter oilseed rape, winter wheat and spring barley. *Viola arvensis, Veronica hederifolia, Veronica persica, Achillea millefolium, Stellaria media, Cirsium arvense, Tripleurospermum inodorum, Thlaspi arvense, Rumex obtusifolius, Triticum aestivum, Geranium pusillum, Lamium purpureum, Euphorbia cyparissias, Agropyron repens* and *Capsella bursa-pastoris* were found in winter oilseed rape. *Viola arvensis, Veronica hederifolia, Galium aparine, Veronica persica, Stellaria media, Matricaria recutita, Polygonum aviculare,Geranium pusillum, Lamium purpurem* and *Apera spica-venti* were in winter wheat. *Viola arvensis, Brassica napus ssp. napus, Rumex obtusifolius, Lamium purpureum, Fallopia convolvulus, Chenopodium album, Stellaria media, Galinsoga parviflora, Thlaspi arvense* and *Galeopsis tetrahit* occurred in spring barley. As conclusion was suggested using different methods of weed regulation.

Key-Words: spring barley, weed, winter oilseed rape, winter wheat

Introduction

In the current agriculture it may seem, that weeds are eliminated by wide range of herbicides offered by many companies and weed control is therefore not a serious problem. But the opposite is true, pay special attention to weed control is important even today [1].

We currently have several methods for determining the actual weed infestation, which are used depending on the objectives of monitoring [2].

Numerical method – individual weed species are counted per unit area (1 m^2) and are not considered their developmental stage. Evaluation may be very imprecise, even when it is repeatedly performed in several places. A relatively small part of the total area is mapped. Monitored area is generally defined by a square frame with a length of 0.25 m or 1 m [3]. The method is accurate enough in suggesting control treatments with knowledge of the damage thresholds of individual weeds [4].

Estimation method – percentages of individual weed species cover were estimated. Evaluation is performed within the plots repeatedly with all disadvantages of previous method [5]. It is fast but subjective method. Estimation method is more efficient than the numerical method, because it is an expression of number of weeds and takes into account their developmental stage at the same time [6].

Weighting method – detects weight of above-ground biomass of weed species per unit area. All above-ground parts of plants are collected close above the ground on the monitored area and dried plant material is weighed. Arrangement and samplings are similar to numerical method [7].

Combined method – is a combination of numerical and weighting method. Weeds are classified according to weed species on the monitored area, quantity is determined and weighed after drying [6].

Bonitation scale EWRC – this scale allows the estimation of the direct herbicide effects on monitored weeds. This method belongs to estimated method and it is burdened with subjective error of evaluator [5].

Phytocenological scanning – it is analysis and description of weedy communities in nature, its resulting report is called phytocenological relevé. Size of the study area is recommended from 25–100 m^2 [7].

These methods are very laborious and for targeted applications are less suitable. Therefore more accurate methods without possible influence of human factor are searched. There is a solution in use of computer technology nowadays [2].

State Plant Protection Administrative carries out an annual survey of the weed occurrence and its distribution in the Czech Republic by using general

phytocenological methodologies. Data are evaluated using computer technology since 1968. Weed monitoring was focused on one or a maximum of two basic crops in previous years. For data processing are used programs as JUICE, Turboveg for Windows, Canoco for Windows a CanoDraw for Windows since 2007 [8].

Phytocenological relevé are entered in web applications called Monitoring – weed infestation. All acquired relevé from that year are transferred from a web application to the program JUICE, where is adjusted the nomenclature according to the publication Key to the flora of the Czech Republic. Using JUICE is calculated the mean vegetation cover and frequency and characteristic species for the production area, previous crops, crops species and areas are determined Ordination methods are used to detect the main environmental gradients influencing species composition of weed communities. Specifically the method of canonical correspondence analysis (CCA). The influence of monitored environment variables on species composition is tested by Monte Carlo permutation test using CANOCO for Windows. By using LPIS applications are compiled maps for selected weed species. Its incidence and vegetation cover is shown in the maps, localization is only approximate [9].

Material and Methods

Characteristic of Experimental Location

Agricultural enterprise Pluhuv Zdar (GPS 49.226480, 14.893355) is located in southern Bohemia in Jindrichuv Hradec district, between cities Kardasova Recice and Destna, approximately 16 km away from Jindrichuv Hradec city. Production area is situated in the transition between potato and cereal production area. It lies at an altitude of 470 to 550 m a.s.l. Long-term precipitation of experimental area were used from www.chmi.cz. Data for 2012 were applied from teranos.ala1.com of Pluhuv Zdar station.

Enterprise focuses on crop and livestock production and operates on the total agricultural area of 2 140 ha till January 1, 2012. 1 750 ha are arable lands and the rest is permanent grassland. Enterprise manages farm lands with different soil surface type (0.15, 0.30 m) and varying granularity. Conventional tillage dominates in this enterprise, minimum tillage is used only for clover growths to save soil moisture establishing in August.

Meteorological values for the year 2012 are shown in Table 1.

Determination and evaluation of weed infestation

Current status of weed infestation of selected lands was conducted in winter wheat, oilseed rape and spring barley by numerical method. All weed species individuals, which were found at a given point, were summed during each observing. Measurements were carried out on randomly selected plots on area of $1m^2$. And each measurements represented 1 ha of selected land area. Overview of evaluated lands is given in Table 2.

A multivariate analysis of ecological data was used to determine the effect of environment factors to weed species occurred on monitored lands. Selection of the optimal analysis followed the length of the gradient (*Lengths of Gradient*), which was detected by segment analysis DCA (*Detrended Correspondence Analysis*). Furthermore, canonical correspondence analysis CCA was used. A total number of 499 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program Canoco 4.0 [10]. Czech and Latin terms of found species are listed according to Kubat [11].

Table 1 Meteorological figures measured in meteorological station in Pluhuv Zdar in 2012

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	average
Average temperature (°C)	-0.4	-5.8	5.3	8.5	14.3	17.2	18.2	18.4	13.5	7.4	4.6	-1.2	8.3
Precipitation total (mm)	91	41	22	59	62	129	166	145	64	59	45	67	950

Table 2 Characteristic of observed fields of monitored agricultural enterprise in 2012

Plot	Acreage	Crop
Stražky	19.94 ha	Oilseed rape
U Remizu	10.08 ha	Oilseed rape
Za Zastavkou	12.33 ha	Oilseed rape
Zahumenky - I	3.14 ha	Oilseed rape
Zahumenky - II	1.12 ha	Oilseed rape
Pod Stražky	25.26 ha	Winter wheat
Padělka	25.26 ha	Winter wheat
Travniky	50.76 ha	Spring barley

Table 3 Average number of found weeds in selected crops

Species of weeds (pcs.m^{-2})	Crop		
	Oilseed rape	Winter wheat	Spring barley
Agropyron repens	0.2		
Achillea millefolium	0.1		
Apera spica-venti		0.3	
Brassica napus ssp. Napus			9.5
Capsella bursa-pastoris	0.4		
Cirsium arvense	0.2		
Euphorbia cyparissias	0.6		
Fallopia convolvulus			0.2
Galeopsis tetrahit			0.5
Galinsoga parviflora			0.5
Galium aparine		0.7	0.3
Geranium pusillum	0.3	0.4	
Chenopodium album			3.1
Lamium purpureum	0.3	1.0	1.6
Matricaria recutita		0.2	
Polygonum aviculare		0.1	
Rumex obtusifolius	0.1		
Secale cereale	0.2		
Stellaria media	0.3	2.6	01
Thlaspi arvense	0.4		1.7
Tripleurospermum inodorum	0.1		
Triticum aestivum	0.3		
Veronica hederifolia	0.4	3.3	
Veronica persica	1.2	0.8	0.2
Viola arvensis	5.3	18.0	11.8

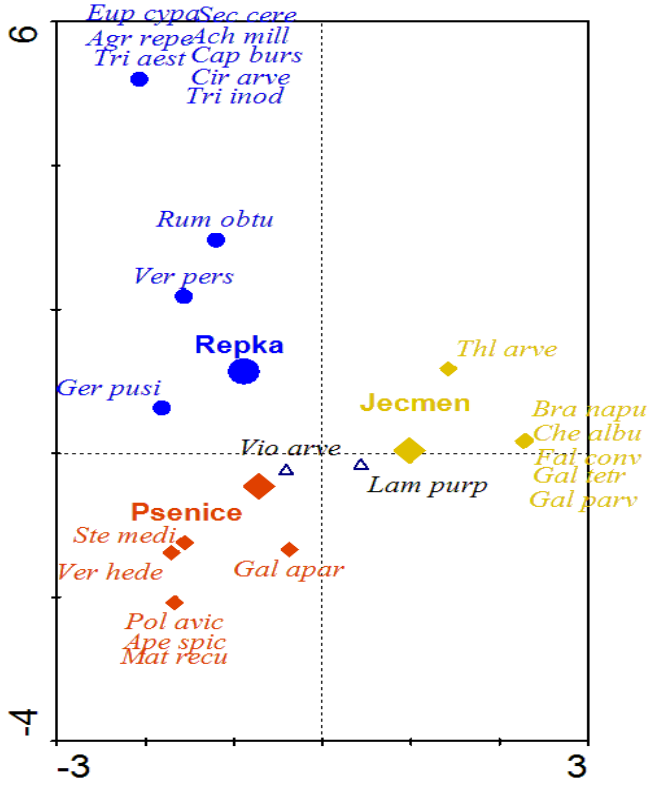
Results and Discussion

25 weed species were found within the monitoring period. Average numbers of weeds in selected crops were shown in Table 3.

Results of evaluation of weed infestation were initially processed by DCA analysis, which figured out the length of gradient (*Lengths of Gradient*). The length of gradient is 4.646. Based on this calculation was to further processing selected Canonical Correspondence Analysis CCA. Analysis CCA defines the spatial arrangement of particular weed species and variants of factors on the basis of the data that was on the frequency of occurrence of detected weed species. This is subsequently graphically displayed in the ordination diagram (Fig.1). Weed species and monitored crops are represented by points of different shapes and colors. Results of analysis CCA, which evaluated the influence of crop on weed occurrence are significant at the significant level $\alpha = 0.002$ for all canonical axes. Based on the analysis CCA (Fig. 1) is possible the found weed species divided into 4 groups. First group of weeds occurred mainly in stands of oilseed rape, it were species: *Agropyron repens*, *Euphorbia cyparissias*, *Secale cereale*, *Achillea millefolium*, *Capsella bursa-pastoris*, *Triticum aestivum*, *Cirsium arvense*, *Tripleurospermum inodorum*, *Rumex obtusifolius*, *Veronica persica* and *Geranium pusillum*. Second weed group was tied to the growth of winter wheat and these species occurred: *Stellaria media*, *Veronica hederifolia*, *Galium aparine*, *Polygonum aviculare*, *Apera spica-venti* and *Matricaria recutita*. Third group of weeds occurred mainly in spring barley: *Thlaspi arvense*, *Brassica napus ssp. napus*, *Chenopodium album*, *Fallopia convolvulus*, *Galeopsis tetrahit* and *Galinsoga parviflora*. And fourth weed group was more affected by different factors and is represented by these species: *Viola arvensis* and *Lamium purpureum*.

Low to medium weed infestation by species *Viola arvensis* may not cause yield losses, but there is a large seed production, which significantly enriches the soil seed stock and in subsequent years the weed infestation may be mass, which is confirmed by Jursik et al. [13]. Kazda et al. [14] state, that *Viola arvenis* is quickly spreading in cereals and oilseed rape in recent years and occurs in all crops. This statement can be confirmed in the given conditions, because *Viola arvensis* was present in all monitored crops and plots.

Fig. 1 Ordination diagram expressing relations between weed occurrence and monitored crops



Legend: Explanatory notes of used abbrevations in ordination diagram:
Crops: Repka – oilseed rape, Psenice – winter wheat, Jecmen – spring barley
Weeds: *Cap burs – Capsella bursa-pastoris, Cir arve – Cirsium arvense, Tri inod - Tripleurospermum inodorum, Tri aest - Triticum aestivum, Agr repe - Agropyron repens, Ach mill - Achillea millefolium, Eup cypa - Euphorbia cyparissias, Sec cere - Secale cereale, Rum obtu – Rumex obtusifolius, Ver pers – Veronica persica, Ger pusi - Geranium pusillum, Thl arve – Thlaspi arvense, Bra napu - Brassica napus* ssp.*napus,Che albu - Chenopodium album, Fal conv - Fallopia convolvulus, Gal tetr – Galeopsis tetrahit, Gal parv – Galinsoga parviflora, Ste medi - Stellaria media, Verhede - Veronica hederifolia, Gal apar - Galium aparine, Pol avic - Polygonum aviculare, Ape spic – Apera spica-venti, Mat recu – Matricaria recutita,Lam purp - Lamium purpureum, Vio arve – Viola arvensis*

Species *Stellaria media* is often weed in winter cereals. *Stellaria media* begins growth in early spring, is rapidly expanding and with species *Lamium* sp., *Capsella bursa-pastoris* and *Veronica* sp. generates characteristic community at this time. It can significantly compete just in early spring or fall, because later on is very fast overgrowth of cereals. Despite its negative character is considered as less important weeds according to Mikulka, Kneifelova et al. [15].

Brassica napus ssp. napus is crop with high risk of second growth, because seeds fall freely on the ground. Seeds can withstand alive in soil for long period. Dvorak, Smutny [16] reported that under field conditions weed infestation by oilseed rape after 24 years of its seeding was observed. It follows that the weed infestation caused by oilseed rape can be expected in a series of subsequent crops. However, it is well herbicide controllable in most of the crops except of oilseed rape growths.

Conclusion

Viola arvensis, *Veronica hederifolia*, winter barley, *Veronica persica*, *Achillea millefolium*, *Stellaria media*, *Cirsium arvense*, *Thlaspi arvense*, *Tripleurospermum inodorum*, *Rumex obtusifolius*, *Triticum aestivum*, *Geranium pusillum*, *Lamium purpureum*, *Euphorbia cyparissias*, *Agropyron repens* and *Capsella bursa-pastoris* were found in growths of oilseed rape. *Viola arvensis* was the most numerously species here, 5.34 pcs.m^{-2} in average.

Weed species as *Viola arvensis*, *Veronica hederifolia*, *Galium aparine*, *Veronica persica*, *Stellaria media*, *Matricaria recutita*, *Polygonum aviculare*, *Rumex obtusifolius*, *Brassica napus* ssp.*napus*, *Geranium pusillum*, *Lamium purpureum* and *Apera spica-venti* were identified in growths of winter wheat. *Viola arvensis* was the most occurred weed species, found in 18.04 pcs.m^{-2} in average.

Viola arvensis, *Brassica napus* ssp.*napus*, *Rumex obtusifolius*, *Lamium purpureum*, *Fallopia convolvulus*, *Chenopodium album*, *Stellaria media*, *Galium aparine*, *Veronica persica*, pet'our maloúborný, *Thlaspi arvense* and *Galeopsis tetrahit* were determined in spring barley growths. *Viola arvensis* was again the most occurred weed species in average of incidence 11.8 pcs.m^{-2}.

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Evaluation of the effect of synthetic brassinolide on the seedlings of lettuce in different moisture conditions

JITKA DOLEZALOVA, MARTIN KOUDELA, JOSEF SUS
Department of Horticulture
Czech University of Life Sciences in Prague
Kamycka 129, 165 21 Prague 6
CZECH REPUBLIC

dolezalovajitka@af.czu.cz

Abstract: The aim of this work was evaluation of synthetic brassinolide influence on growth of the lettuce seedlings (cultivar ´Maršálus´) in optimal and reduced moisture conditions. The experiments were established in the growth chamber. Irrigation was based on EWC; 75 % for optimally irrigated trays (OPT), and 60 % for reduced irrigation (STR). The four variants of treatment were in both conditions: c0 – no application of synthetic brassinolide (SB), c11 - application of SB concentration 1.10^{-11} M, c9 - concentration 1.10^{-9} M, c7 - concentration 1.10^{-7} M. The plants were treated with SB 15th day after the sowing. The growth was assessed by harvesting 20 individuals per treatment (upper-part; root fresh weight; root length, root neck diameter; greater leaf length and dry matter content were measured. On 21st day the highest value of average leaf length (50.1 mm) was observed in STR, c9. The highest value of average length of roots (52.6 mm) was measured in OPT, c11. The parameters were not statistically significantly different from those measured in the control variants. On 28th day, the highest average value of length of upper-parts (65.5 mm) was found out for variant STR, c9. This value was statistically significantly different from the control value (c0), variants STR and OPT. The highest average value of length of roots (81.5 mm) was measured at OPT, c11 (same as 21st day). The maximum value of dry matter content (4.95 %) was found in variant STR, c9 treatment - statistically significantly different from other treatments in STR variant. The laboratory study indicates a tendency of treatment SB 1.10^{-9} M (c9) to support the aboveground plant size and the dry matter content in condition with reduced irrigation. But we cannot submit that the effect would be also valid for root growth of plants or root neck diameter.

Key-Words: lettuce, draught, brassinosteroids, vegetable, juvenile plant

Introduction

The insufficient water supply may cause wilting plants. The absence of accessible water in the soil profile may lead to irreversible wilting [1]. The most of vegetable species have a high transpiration rate (from 280 to 830 ml of water per 1 g dry matter). As limit value of efficient water capacity is reported at 50 %. If there is a drop below this level, vegetables suffer from lack of water [2]. Sensitivity of plants to drought differs among species, populations and varieties and depends upon physiological stage of the plant and duration of stress impact [3]. Water shortage considerably lowers plant dry matter production and thus final yield [4]. Drought stress as well as UV irradiation is the most adverse factors for plant growth and productivity [5]. In this context it is necessary to look for means to reduce the negative impact of water deficit for grower praxis.

The application of brassinosteroids could be one way to reduce the negative impact of water deficit in vegetable production. These hormones help by reducing of environmental stress impact of plant physiology, e.g. in connection to temperature [6] and to water deficiency [7, 8, 9]. This growth regulators on the basis of sterols not only promotes growth [10], but they also have the potential to increase yield and economic parameters in horticultural crops [11, 12, 13].

The hypothesis of this work was: application of synthetic brassinolide can minimize negative influence of water deficit in cultivation of head lettuce seedlings.

Material and Methods

The trials were carried out in the laboratory belonging to Department of Horticulture, CULS in Prague in 2012 and 2013. The experiments were established in the growth chamber BINDER KBW 400 with illumination, that allows to create constant lighting and temperature conditions .The synthetic analogue of the natural brassinosteroids - substance 2 α, 3 α, 17 β – trihydroxy – 5 α – androstan – 6 – one (SB) was used for testing (patent pending No. 252605 Industrial Property Office). The seeds of cultivar ´Maršálus´ (seed category S; producer Semo a.s.) were sown (5 mm depth) in plastic seedling trays TEKU JP3050 160 (1 seed per cell; 20 cell per replication; four replications per treatment; 16 ml cell capacity) in ready-mixed seed-sowing compost based on peat (Agro CS a.s.).

Irrigation was based on current values of efficient water capacity (EWC); the critical value of the EWC was 75 % for optimally irrigated trays (irrigation OPT), and 60 % for variants with reduced levels of irrigation (irrigation STR). The total quantities of irrigation water during test were as follows: the variant with optimal levels of irrigation (OPT) had 39 mm; the variant with reduced levels of irrigation (STR) had 28 mm. The irrigation was done by hand sprayer. Together with the first irrigation fungicide Previcur (concentration 0.25 %) was applied. The seedlings were cultivated in accordance with techniques recommended by Petříková et al. [14]: 20 °C /full illumination 13000 lx - 12 hours / day; 15 °C / 12 hours night.

The experiment was carried out in randomized design. Four variants were in both (OPT and STR) conditions: c0 – no application of SB, c11 - application of SB concentration 1.10^{-11} M, c9 - application of SB concentration 1.10^{-9} M, c7 - application SB concentration 1.10^{-7} M. The plants were treated with SB 15th day after sowing. Just before the using of SB, the controls out measurements of plants were carried. On 21st and 28th day (DC 19 – 29, according to Vogel et al. [15]) the seedling growth was assessed by harvesting 20 individuals per treatment (five plants per each replication). The juvenile plants were counted, cleaned, and upper-part and root fresh weight (g / plant) as well as the root length (mm / plant), root neck diameter (mm / plant) and the greater leaf length (mm / plant) were measured. For upper-part of plant dry matter content was determined [16].

The entire experiment was repeated again. The measured values were statistically analyzed using the STATISTICA CZ, version 12.0 software system for data analysis.

Results and Discussion

The first evaluation of plants was done 15th day. The differences between the lengths of upper-parts of plants (leaf length) were not significant. The average length of the plants in OPT was 33.1 mm and 31.4 mm in STR variant of irrigation.

The average values of the measured lettuce seedlings parameters are given in Table 1.

On 21st day (sixth day after the treatment) juvenile plants were in stage of two true leaves (DC 11 - 19). The highest value of average leaf length (50.1 mm) was observed in variant with reduced irrigation, treatment c9. The highest value of average length of roots (52.6 mm) was measured at optimally irrigated variant, treatment c11. The average root neck diameter (0.746 mm) has a maximum in the control (c0) variant STR. The maximum measured average parameters were not statistically significantly different from those measured in the control variants. The detailed statistical survey results are given in Table 1. The average values of the dry matter content of the upper parts of the plant are shown in Fig. 1. The highest average dry matter content (4.92 %) was found in variant STR, c9 treatment. Conversely, the lowest average dry matter contained samples of both non-treated (c0) variants (STR 3.78 %, OPT 3.85 %). There were not statistically significant differences between treatments. In case of the average fresh weight of upper-parts (Fig. 2), the highest average weight was observed at treatment c11 (0.145 g), irrigation OPT. The lowest average values were obtained for variant STR - treatment c7 (0.103 g).

On 28th day (13th day after the treatment) seedlings were in stage DC 21 – 29. When the measurement was carried out 28th day (Table 1), the highest average value of length of upper-parts (65.5 mm) was found out for variant STR, treatment c9. This value was statistically significantly different from the control value (c0), variants STR and OPT. The highest average value of length of roots (81.5 mm) was measured at optimally irrigated variant (OPT), treatment c11 – as in the case of measurements made 21st day. The average value of root neck diameter (0.970 mm) has a maximum in the variant OPT, treatment c9. The average values of the dry matter content in the upper-parts of the plant are shown in Fig.3. The highest average value of dry matter content (4.95 %) was found in variant STR, c9 treatment - as in the case of measurements made 21st day. This value is statistically significantly different from other treatments in condition with reduced irrigation. In case of average weight of fresh matter (Fig. 4) the highest

value (0.358 g) was found in variant OPT, c9 treatment. However, this value did not differ statistically significantly from the value for the treatment c9 (in STR) and all other treatments in conditions with optimum irrigation (OPT). In case of measurements carried out 21st day values of treated variants do not differ from the control (c0), but during measurement carried out 28th day there are some differences already evident. It is mainly the average length of upper-parts (leaf length) with treatment c9, where the variants with reduced and optimum irrigation statistically significantly differed from the control variants.

Table 1 Average values of measured parameters (21st and 28th day)

		21st day			28th day		
		Leaf length (mm)	Root length (mm)	Root neck diameter (mm)	Leaf length (mm)	Root length (mm)	Root neck diameter (mm)
c0	OPT	48.6^{a}	47.9^{ab}	0.644^{ab}	56.8^{a}	74.6^{ac}	0.875^{ab}
	STR	48.3^{a}	44.1^{abc}	0.746^{b}	51.6^{b}	68.8^{a}	0.854^{ab}
c11	OPT	49.4^{a}	52.6^{b}	0.705^{ab}	57.9^{a}	81.5^{c}	0.945^{b}
	STR	47.0^{ab}	41.3^{ac}	0.686^{ab}	51.6^{b}	67.0^{ab}	0.814^{a}
c9	OPT	49.4^{a}	42.7^{abc}	0.629^{ab}	58.8^{ac}	74.4^{ac}	0.970^{b}
	STR	50.1^{a}	45.6^{abc}	0.581^{a}	65.5^{c}	67.0^{ab}	0.848^{ab}
c7	OPT	47.4^{ab}	48.9^{ab}	0.690^{ab}	59.4^{ac}	67.2^{ab}	0.878^{ab}
	STR	45.5^{b}	37.6^{c}	0.662^{ab}	51.9^{b}	60.7^{b}	0.782^{a}

Legend: In each column; values followed by the same letter did not differ significantly (P < 0.05) according to Fisher´s LSD test.

Fig. 1 Dry matter 21st day, upper-parts (in %)

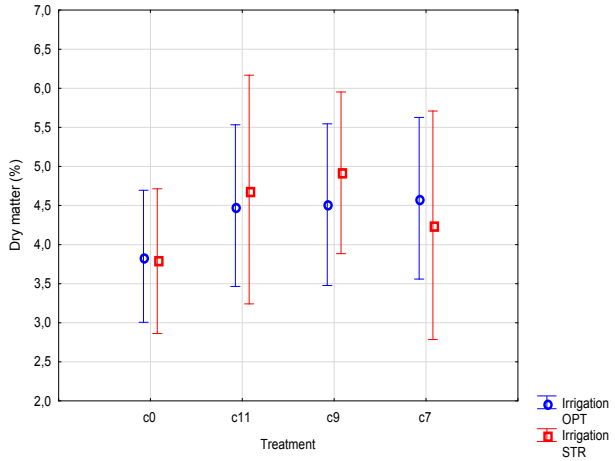


Fig. 2 Fresh weight 21st day, upper-parts (in g)

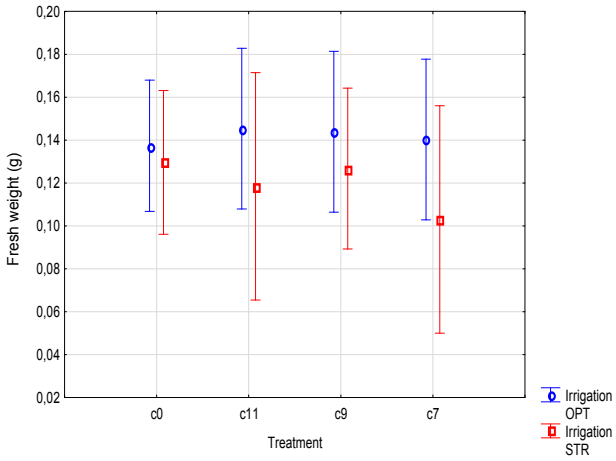


Fig. 3 Dry matter 28th day, upper-parts (in %)

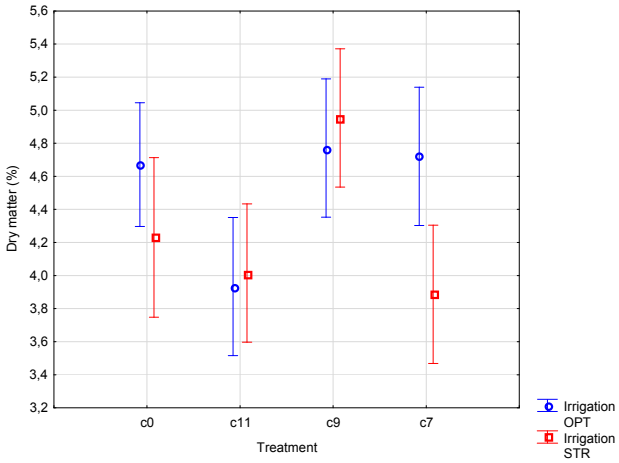
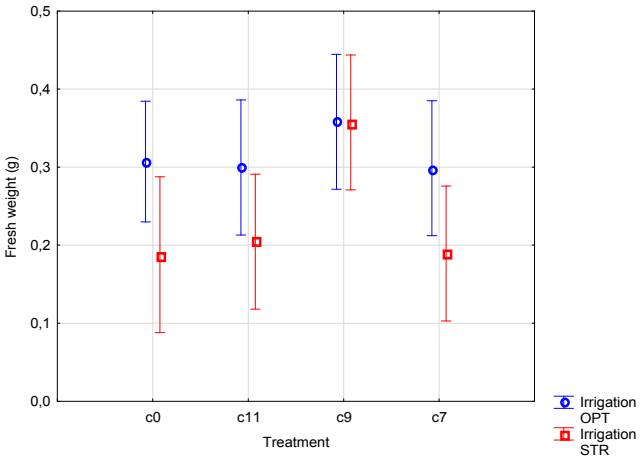


Fig. 4 Fresh weight 28th day, upper-parts (in g)



Also in this case, the treatment c9 variant STR is showed promising. However, the value of dry matter - treatment c9 in variant STR is not statistically significantly different from the values of c0, c7 and c9 treatments in optimum irrigation conditions (OPT).

As indicated by numerous studies, application of brassinosteroids can reduce negative environmental impacts, e.g. negative influence of pesticides [17] or biotic stressors [18]. The positive effect of synthetic brassinolide is reflected by increase of value of leaf length and together with increase of dry matter content. The obtained data show a positive effect of treatment c9 in both - reduced and optimal irrigation conditions.

Although there are not always statistically significant differences, the tendency is evident. This finding is consistent with data of Procházka et al. [19], who reported effective concentration from 10^{-8} to 10^{-11}M. We can also agree with the conclusions of Mussig [10] and Pavlová et Fischer [20], who talks about supporting effect of brassinosteroids on growth and the size of plant, respectively.

Conclusion

The study indicates a tendency of treatment with synthetic brassinolide concentration 1.10^{-9} M (c9) to support the aboveground plant size and thus the growth of the plant. We cannot, however, clearly state that the effect would be valid completely for root growth of plants or root neck diameter. The positive effect of treatment c9 was observed in both - reduced and optimal irrigation conditions. The effect of application of synthetic brassinolide was reflected in measuring carried out 28th day (i.e. 13th day after the treatment). The highest average value of length of upper-parts (65.5 mm) was find out for variant with reduced irrigation, treatment c9. This value was statistically significantly different from the control value (c0), both variant - with optimal and reduced irrigation. It is known that lack of water in the cultivation leads to a decrease of dry matter content [4]. The results (treatment c9, variant STR), however, show that this effect can be limited by application of synthetic brassinolide.

It would be appropriate to verify results, obtained in the laboratory, in cultivation of seedlings in the greenhouse.

Acknowledgement

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Effect of herbicide treatment on milk thistle (*Silybum marianum* (L.) Gaertn) germination

IVA DRAPALOVA, HELENA PLUHACKOVA
Department of Crop Science, Breeding and Plant Medicine
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

helena.pluhackova@mendelu.cz

Abstract: The effect of herbicide treatment on milk thistle has been observed in the years 2010 and 2011 in the experimental field station Žabčice. The milk thistle variety "Silyb" was sown. In the first year the seeds were obtained from the company IREL, Ltd.; in the second year the seed harvested in the year 2010 were used. The phytotoxicity of used herbicides was observed during the vegetation period in both years (Stomp 400 SC, Refine, Butisan Star, Afalon 45 SC, Fusilade Forte, Glean + Starane and untreated control plot. In 2010 the harvest was performed in two terms due to the gradual ripening; in 2011 only once due to excessive rainfall. The achenes harvested in 2010 were evaluated according to following characteristics: yield ($g.m^{-2}$), overflow of the sieve (%) (2.2, 2, and the rest), weight of thousand seeds (g), light and dark achenes germination in the year of harvest and light and dark achenes germination after storage (%). In 2011 following characteristics were evaluated: yield, germination ability and weight of thousand of seeds.

Key-Words: germination, milk thistle, yield, weight of thousand seeds

Introduction

Milk thistle, *Silybum marianum* (L.) Gaertn, belongs to the Asteraceae family; it is an annual herb of a height 150 – 200 cm. There are two varieties protected by the law in the Czech Republic - Silyb and Mirel [1]. Milk thistle is grown mostly for pharmaceutical use, for the flavonolignans found in fruits that protect liver cells, stabilise cell membranes and prevent toxins to enter hepatocytes [2]. The growing area varied in the range 500-5000 ha during last 5 years according to the need of processing companies [3, 4].

Milk thistle is suitable for potato-growing or beet-growing regions. Uneven maturation time of individual anthodia is typical and the achenes tend to fall out from ripe anthodia, so optimal time of harvest must be selected [5, 6].

Although the growing technology is known from 70s and 80s, it was never solved in complex including the plant protection; no plant protection products are registered for milk thistle [7].

Herbicides for milk thistle haven't been investigated yet in the framework on modern technologies. Milk thistle has good weed-removing qualities thanks to quick growth and high leaves cover, but decreased competitive ability during germination to extensive growth. It is an unpleasant weed itself. The seeds maintain their germination ability for a long time. According to Czyž [8] it is possible to sow milk thistle repeatedly in the same plot, but Andrzejewska and Lamparski [9] warn against the expansion of *Cleonis pigra*.

Most common weeds are *Sonchus arvensis*, *Elytrigia repens*, *Cirsium arvense, Chenopodium album*, *Raphanus raphanistrum, Sinapis arvensis, Galium aparine, Mentha crispa, Stachys arvensis, Convolvulus arvensis* and *Atriplex patula*. Milk thistle itself is often an unpleasant weed for next crops. To diminish the weeding, it is good to use a following crop that won't let germinated weed plants to spread seeds and weed the plot again, like grass or clover-grass mixtures where weed moving is used or monocotyledonous crops allowing the use of herbicides against dicotyledonous weeds. Weed removing should be based on mechanical cultivation. Herbicides can be given into the soil before sowing. However, the herbicides against dicotyledonous weeds and annual grasses must be applied not sooner than three days one after the other. There isn't any registered weed-regulation product for milk thistle growth at the moment in the Czech Republic, but this area is widely researched.

Material and methods

Experimental methodology

Milk thistle experiments were established in the field experimental station MENDELU in Žabčice, using the method of split parts. The position of field experimental station Žabčice is north latitude 49°0´ and east longitude 16°16´. The cadastral area is situated in corn-breeding region, subregion K_2, that belongs to the warmest in the Czech Republic. The locality altitude is 179 m above the sea level. This dry south-moravian region has typical inland climate with dryness caused by large evaporation of soil moisture due to stronger winds [10].

According to BPEJ classification it is very warm and dry climatic district. The value of Lang rain factor is ca. 57; that means one of the most dry localities. Average yearly temperature is 9.2°C, July is the warmest month with average daily air temperature 19.3°C, January is the coldest month with average temperature -2.0°C. As for the rainfall, the locality belongs to dry regions, 30-year average total rainfall is 480 mm. The rainfall is distributed very unevenly during the vegetation period. The length of sunlight varies in the range of 1800-2000 hours per year.

Table 1 Temperature and rainfall values in I.-XII. 2010 and 2011 at the experimental plot [10]

Year	Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	$\bar{x}$
2010	Average temper.	-3.9	-0.6	4.8	10.2	14.0	18.7	21.9	19.3	13.7	7.3	6.7	-3.9	**9.02**
	Total rainfall	46.8	22.8	9.8	53.1	102.4	79.8	87.9	75.8	57.8	10.4	32.8	11.1	**49.2**
2011	Average temper.	-0.4	-0.9	5.4	12.4	15.3	19.4	19.2	20.5	17.1	9.3	2.5	2.2	**10.2**
	Total rainfall	21.4	4.6	39.3	33.2	46.2	42.9	79.8	42.4	31.3	22.6	1.6	14.6	**31.6**

Source: The report of the experiment with milk thistle (Silybum marianum (L.) Gaertn.) in 2010 and 2011. The average temperature is given in °C, the total rainfall in mm

As shown in Table 1, average month temperatures and especially total rainfalls were very different in experimental years. In 2011 the average yearly total rainfall was 31.6 mm (per month), which is by 17.6 mm less than in 2010, but also less than the long-term average monthly rainfall (40 mm). As for the temperatures, in the year 2011 were found higher temperatures during the vegetation period compared to 2010, especially in June. These differences could affect both the effectiveness of herbicide treatment and the formation of yield components in milk thistle. The soil type in the locality is gleyic fluvisoil (FLq). Redoximorf characteristics are found from the depth of 70 cm. The level of underground water is in the depth of 100 cm. In terms of particle size distribution it is a heavy soil [10]. The experimental plot was prepared for sowing a common way and wasn't fertilized. The crop before milk thistle was winter wheat in 2010 and milk thistle in 2011.

The sowing was performed on April 1st 2010; treated milk thistle seeds were obtained from the company IREL, Ltd. The seed germination rate was 78.13 % (determined according to Methodologies of Seed Testing of the Ministry of Agriculture No. 34349/04-17220). For the sowing on March 28th 2011 the seeds gained from the cultivation in 2010 were used. The seeds were stored in laboratory conditions and the segmentation by the 2.2 mm sieve was performed. The seeds used for sowing had the germination rate 96.45%.

In both years the sowing was performed by the means of sowing machine KVERNELAND ACCORD PNEUMATIC DA-l into 0.25 m rows; with the seed rate 6 $kg.ha^{-1}$.

In the year 2010 the growth emerged on April 21st 2010. Total area of the experiment was 650 m^2. The area was divided to 18 plots of the size 24 m^2. In 2011 the growth emerged on April 18th 2011. Total area of the experiment was 730 m^2. The area was divided to 21 plots of the size 24 m^2.

Herbicides were applied on individual plots in below-given variants, always repeated triplicate.

1. Control,
2. STOMP 400 SC – 2.5 $l.ha^{-1}$
3. REFINE – 10 $g.ha^{-1}$
4. BUTISAN STAR – 1.5 $l.ha^{-1}$
5. AFALON 45 SC – 1.5 $l.ha^{-1}$
6. FUSILADE FORTE EC – 1.5 $l.ha^{-1}$
7. GLEAN + STARANE – 7 $g.ha^{-1}$ + 3 $l.ha^{-1}$

Evaluation of the experiment was performed in three terms according to the Methodology for the determination of preparation phytotoxicity [EPPO

No. 135/1988]. Part of the area was flooded in the end of May 2010 due to the cloudburst.
Because of consecutive ripening the harvest was performed in two terms in the year 2010: July 16th 2010 and July 23rd 2010. Hand cutted ripe anthodia were harvested (white pappus and brown achenes). In 2011 there was only one harvest term - July 20th 2011. Due to excessive rainfall the harvest was only performed with two repetitions and the rest of the growth was ploughed.

The anthodia with achenes were dried after the harvest at the temperature 20°C and the achenes were removed from anthodia by the trashing machines MOVIS and HALTRUB 20 in 2010 and 2011, resp., and manually cleaned. The weight of thousand kernel (TKW) was determined as well as the germination ratio from individual harvests and plots and for the harvest 2010 also the seed segmentation was performed according to the size by 2200 and 2000 μm sieves on Steinecker sieving machine.

Data were evaluated in the program STATISTICA CZ 10 by the means of one-factor and two-factor variance analysis and consequent testing.

Results and Discussion

Results from the harvest year 2010
Phytotoxicity
Toxicity evaluation, May 27th 2010
1. CONTROL - in the phase BBCH 14-18 the phytotoxicity was zero and a wide range of weeds were present, like emerging barnyardgrass, field penny-cress and shepherd's purse; the average amount of weeds in a control plot was 5 pcs.m^2.
2. STOMP 400 SC - in the phase BBCH 14-17 the phytotoxicity was up to 5%, edges of leaves where the preparation was applied were damaged, but newly grown leaves were unharmed. Necroses were observed in damaged leaves (15 – 30%).
3. REFINE - in the phase BBCH 14 – 18 the phytotoxicity was up to 5%; leaves where the preparation was applied were damaged, but newly grown leaves were unharmed. Necroses (15 – 25%) occurred on whole area where the preparation was applied.
4. BUTISAN STAR - in the phase BBCH 14 – 18 the phytotoxicity was 2 – 3%. Some leaves were harmed, the damage was 10 – 15%. Newly grown leaves were unharmed. Necroses occurred on whole area where the preparation was applied.
5. AFALON 45 SC - in the phase BBCH 14-16 the phytotoxicity was 15 – 20%. The growth delay was up to 40%. Necroses (30 – 40%) occurred on whole area where the preparation was applied. The leaves were drying from the edges in the direction to centre. Newly grown leaves were unharmed.
6. FUSILADE FORTE 150 EC - in the phase BBCH 14 – 16 the phytotoxicity was 4 – 5%. Necroses (30 – 40%) occurred on whole area where the preparation was applied. The leaves were drying from the edges in the direction to centre.

Toxicity evaluation, June 10th 2010
1. CONTROL - in the phase BBCH 18 – 51 the phytotoxicity was zero and a wide range of weeds were present, like emerging barnyardgrass, bedstraw, field penny-cress, shepherd's purse, doorweed, creeping thistle, wild radish, sorrel, earth smoke and common lambsquarters.
2. STOMP 400 SC - in the phase BBCH 18 – 32 the phytotoxicity faded without harm of the growth top, wide range of weeds occurred, the same as those in the control plot.
3. REFINE STAR - in the phase BBCH 18 – 32 the phytotoxicity also faded, part of the plot was flooded.
4. BUTISAN - in the phase BBCH 18 – 32 the phytotoxicity was 0%, without necroses.
5. AFALON 45 SC - in the phase BBCH 18 – 32 the phytotoxicity was 5%, without necroses and damage.
6. FUSILADE FORTE 150 EC - in the phase BBCH 18 – 32 the phytotoxicity was 0%, occurrence of dicotyledonous weeds.

Toxicity evaluation, July 15th 2010
The phase BBCH 63 – 87 is valid for all variants. There aren't visible differences among the beginning of flowering and ripening. The differences among plant height and density are given rather by the soil and moisture characteristics of the stand.

Measurement results

Table 2 Average values of the yield, TKW and size selection (overflow over a sieve) of the milk thistle achenes in two harvest terms and selected herbicide treatments in the harvest year 2010

Date	Variation	Yield ($g.m^{-2}$)	TKW	Grading %			Germination		Germination next year	
				2.2	2	remain	light achenes	dark achenes	light achenes	dark achenes
1. - 16. 7. 2010	1 – control	22.50 ^{abc}	28.70 ^{a}	4.96 ^{a}	80.29 ^{d}	14.75 ^{ab}	88.67 ^{ab}	92.67 ^{ab}	91.33 ^{abc}	92.67 ^{ab}
	2 - STOMP 400 SC	19.23 ^{ab}	28.06 ^{a}	13.63 ^{a}	75.77 ^{d}	10.60 ^{ab}	91.33 ^{b}	100.00 ^{b}	86.00 ^{a}	100.00 ^{b}
	3 – REFINE	20.49 ^{abc}	28.66 ^{a}	69.94 ^{bc}	23.05 ^{ab}	7.01 ^{ab}	94.00 ^{b}	96.67 ^{ab}	89.33 ^{ab}	96.67 ^{ab}
	4 - BUTISAN STAR	22.42 ^{abc}	28.12 ^{a}	50.38 ^{b}	42.11 ^{bc}	7.51 ^{ab}	74.67 ^{a}	96.00 ^{ab}	85.33 ^{a}	96.00 ^{ab}
	5 - AFALON 45 SC	7.43 ^{a}	28.88 ^{a}	19.63 ^{a}	67.13 ^{cd}	13.24 ^{ab}	93.33 ^{b}	90.67 ^{a}	90.00 ^{ab}	90.67 ^{a}
	6 - FUSILADE FORTE	13.89 ^{a}	28.79 ^{a}	12.55 ^{a}	73.48 ^{d}	13.97 ^{ab}	97.33 ^{b}	92.67 ^{ab}	94.67 ^{bcd}	92.67 ^{ab}
	Mean	**17.66**	**28.54**	**28.52**	**60.31**	**11.18**	**89.88**	**94.78**	**89.44**	**94.78**
2. - 23. 7. 2010	1 – control	39.43 ^{bcd}	26.56 ^{a}	63.27 ^{bc}	19.92 ^{ab}	16.80 ^{b}	85.33 ^{ab}	98.67 ^{b}	98.00 ^{cd}	98.67 ^{b}
	2 - STOMP 400 SC	43.40 ^{bcd}	26.63 ^{a}	91.33 ^{c}	4.25 ^{a}	4.42 ^{a}	92.67 ^{b}	100.00 ^{b}	97.33 ^{cd}	100.00 ^{b}
	3 – REFINE	44.86 ^{cd}	27.02 ^{a}	84.45 ^{c}	8.93 ^{a}	6.62 ^{ab}	85.33 ^{ab}	98.67 ^{b}	98.67 ^{d}	98.67 ^{b}
	4 - BUTISAN STAR	41.60 ^{bcd}	27.03 ^{a}	83.14 ^{c}	4.28 ^{a}	12.58 ^{ab}	87.33 ^{ab}	98.00 ^{ab}	98.00 ^{cd}	98.00 ^{ab}
	5 - AFALON 45 SC	31.04 ^{abc}	28.60 ^{a}	71.71 ^{bc}	16.04 ^{ab}	12.25 ^{ab}	86.00 ^{ab}	100.00 ^{b}	94.67 ^{bcd}	100.00 ^{b}
	6 - FUSILADE FORTE	52.27 ^{d}	28.38 ^{a}	75.79 ^{bc}	15.36 ^{ab}	8.85 ^{ab}	97.33 ^{b}	98.00 ^{ab}	98.67 ^{d}	98.00 ^{ab}
	Mean	**42.1**	**27.37**	**78.28**	**11.46**	**10.25**	**88.99**	**98.89**	**97.55**	**98.89**

Note: Average values in the same columns marked with different numbers varies statistically at p=0.05; germination ability is given in [%]

In the first term of harvest (July 16th 2010) the total average values were lower in comparison with the second term (July 23rd 2010, see Table 2). The highest yield was in the second term of harvest in the plot treated by Fusilade forte, but it didn't vary significantly from all other preparations in the second term of harvest. The grading made by the means of Steinecker sieving machine showed in the 2.2 mm sieve overflow highest values for the second harvest term and treatment with STOMP 400 SC (91.33%), but it didn't vary significantly from all other preparations in the second term of harvest.

The germination ability of milk thistle was evaluated for selected achenes according to sensory selection to light and dark colour of achenes. The colour of achenes was supposed to be the characteristics of their ripeness and thus could affect the germination ability.

The germination ability of light achenes was lower than the one of dark achenes both in the first harvest and second term comparison. The conclusion is that the germination ability of dark achenes is higher in general.

Germination ability of achenes was evaluated also after a year of storage. The lowest germination ability was found after the treatment by herbicide preparations Butisan Star (85.33%) and Stomp 400 SC in the first harvest term (July 16th 2010). In the second harvest term (July 23rd 2010) the lowest germination ability was found after the treatment by herbicide preparation Afalon 45 SC (94.67%), but it didn't vary significantly from all other values. The lowest germination ability of dark achenes was found in the first harvest term (July 16th 2010) after the treatment by herbicide preparation Afalon 45 SC (90.67%). As shown in the table, the germination ability was generally higher for both light and dark achenes compared to the first harvest term; it is also apparent that the germination ability of dark achenes is higher. The comparison of germination ability after the harvest and after one year storage showed that the germination ability didn't change much, the trend stayed the same even after a year of storage (see Table 3). However, it was found out that after one year of storage the average germination ability of light achenes was increased in the second harvest term by almost 8%.

Results from the harvest year 2011

Phytotoxicity

Toxicity evaluation, May 9th 2011

1. CONTROL - in the phase BBCH 14 – 16 the phytotoxicity was zero and a wide range of weeds were present, like field mustard, wild radish, barnyardgrass, bedstraw, common lambsquarters, field penny-cress, pigweed and creeping thistle.

2. STOMP 400 SC - in the phase BBCH 14 – 16 the phytotoxicity was 5 %, slightly delayed growth of leaves, older leaves damaged, necrosis from the edges of leaves.

3. REFINE - in the phase BBCH 14 – 16 the phytotoxicity was up to 5%, leaves without necrosis.

4. BUTISAN STAR - in the phase BBCH 14 – 16 the phytotoxicity was 0%, resistant weed - wild radish.
5. AFALON 45 SC - in the phase BBCH 14 – 16 the phytotoxicity was 85-100%, spots on plants after the application.
6. FUSILADE FORTE 150 EC - in the phase BBCH 14 – 16 the phytotoxicity was 5-7%, the same damage like in the case of STOMP 400 SC, including wild radish.
7. GLEAN + STARANE - in the phase BBCH 14 – 16 the phytotoxicity was 10-15%, chlorotic plants in comparison with the control, slower growth, plants without necrotic spots.

Toxicity evaluation, May 27th 2011
1. CONTROL - in the phase BBCH 29 – 32 the phytotoxicity was 0% and following weeds occurred: field mustard, wild radish, barnyardgrass, bedstraw, common lambsquarters, field penny-cress, pigweed, creeping thistle.
2. STOMP 400 SC - in the phase BBCH 29 – 32 the phytotoxicity was 0%, the plot was clean, total herbicide efficiency was 85 – 90%, with rare occurrence of wild radish, barnyardgrass and creeping thistle.
3. REFINE - in the phase BBCH 29 – 32 the phytotoxicity was 0%, total herbicide efficiency was 95-97%, just barnyardgrass and creeping thistle.
4. BUTISAN STAR - in the phase BBCH 29 – 32 the phytotoxicity was 0%, total herbicide efficiency was 90%, the plot looks weedy, contains grown cruciferous weeds.
5. AFALON 45 SC - in the phase BBCH 29 – 32 the phytotoxicity was 90 – 95%, total herbicide efficiency was up to 95%.
6. FUSILADE FORTE 150 EC - in the phase BBCH 29-32 the phytotoxicity was 0%, plant damage including wild radish.
7. GLEAN + STARANE - in the phase BBCH 29 – 32 the phytotoxicity was 0%, total herbicide efficiency was 95%, barnyardgrass and damaged creeping thistle.

Toxicity evaluation, June 6th 2011
The phytotoxicity faded for all treated plants except herbicide Afalon, where the phytotoxicity varied in the range 90 – 95%.

Measurement results
The analysis of variance for the selected qualitative characters of milk thistle achenes grown in 2011 shows a highly significant impact of varieties on germination and a highly significant effect on the yield.

Table 3 Average values of the yield, TKW and germination ability of milk thistle achenes treated by selected herbicides in the harvest year 2011

Variety	Yield ($g.m^{-2}$)	TKW (g)	Germination (%)
1-Control	50.71 ^{a}	28.55 ^{a}	97.0 ^{d}
2-STOMP 400 SC	166.46 ^{d}	27.15 ^{a}	91.0 ^{b}
3-REFINE	152.17 ^{cd}	26.41 ^{a}	93.0 ^{c}
4-BUTISAN STAR	140.67 ^{cd}	27.41 ^{a}	98.0 ^{d}
5-AFALON 45 SC	78.65 ^{ab}	28.97 ^{a}	92.5 ^{bc}
6-FUSILADE FORTE EC	118.77 ^{bc}	26.98 ^{a}	86.0 ^{a}
7-GLEAN+STARANE	149.40 ^{cd}	26.26 ^{a}	97.0 ^{d}
Average	**122.40**	**27.39**	**93.5**

Note: Average values in the same columns marked with different numbers varies statistically at p=0.05

As can be seen in Table 3, the lowest yield gave the control plot (50.71 g); the value varied significantly from the yields from plots treated with the preparations Stomp 400 SC (166.46 g), Refine (152.17 g), Butisan Star (140.67 g), Glean + Starane (149.40 g) and Fusilade Forte EC (118.77 g). The control didn't vary statistically significantly from the treatment with Afalon 45 SC (78.65 g). There weren't any statistically significant differences among the TKW values. The lowest germination ability was found for the treatment with herbicide preparation Fusilade Forte EC (86.00%) and it varies statistically significantly from the treatments with Butisan Star (98.00%), Glean + Starane (97.00%) and from the control plot (97.00%).

Conclusion

The effect of herbicide treatment to the milk thistle was studied in years 2010 a 2011 in the field experimental station in Žabčice. In both years the milk thistle variety Silyb was sown; the first year the seeds were gained from the company IREL, Ltd., and in the second year the seeds from previous harvest were sown. Phytotoxicity of used herbicides was observed during the vegetation for Stomp 400 SC, Refine, Butisan Star, Afalon 45 SC, Fusilade Forte, Glean + Starane and untreated control plot. In the year 2010 the harvest was performed in two terms due to consecutive ripening. In 2011 the harvest was performed only once due to excessive rainfall during the harvest period.

The achenes harvested in 2010 were evaluated for following characteristics: yield in $g.m^{-2}$, size separation in % (2.2; 2 and the rest), TKW in g, germination ability of light and dark achenes in the year of harvest and after a year of storage in %. In the year 2011 following characteristics were evaluated: yield in $g.m^{-2}$, TKW in g and germination ability in %.

The results were influenced by the weather course; because of that, the effect of herbicides wasn't unambiguous in the observed years. Following conclusions result from the findings:

2010

The yield of achenes in the first harvest was highest from the control plot (22.5 $g.m^{-2}$), in the second harvest the yield was highest from the plot treated with the herbicide Fusilade Forte EC (52.27 $g.m^{-2}$). Total average harvest yield in 2010 was 59.8 $g.m^{-2}$.

TKW was highest in the plot treated with the herbicide Afalon 45 SC in both harvests (28.88 g and 28.6 g, resp.). TKW is the characteristics that showed the lowest variability in all observations (in the range 26.6 g – 28.9 g).

Separation of the achenes according to the size. The highest ratio of achenes gained in the 2.2 mm sieve was in the first harvest from the plot treated with the herbicide Refine (69.94%) and in the second harvest from the plot treated with the herbicide Stomp 400 SC (91.33%).

Germination ability after the harvest. In both cases the dark-coloured achenes had higher germination ability than the lighter ones (94.78% and 98.89%, resp.). The difference between germination abilities of light and dark achenes was 7.4%.

Germination ability after one year of storage was also higher for dark achenes, but the difference compared to light achenes was only 3.3%.

2011

The yield of achenes was highest from the plot treated with the herbicide Stomp 400 SC (166.46 $g.m^{-2}$) and lowest from the control plot (50.71 $g.m^{-2}$). Total average harvest yield in 2011 was 122.4 $g.m^{-2}$.

TKW was highest in the plot treated with the herbicide Afalon 45 SC (28.97 g) and lowest in the plot treated with the herbicides Glean + Starane (26.26 g). The average TKW value is comparable with the results from the year 2010 (27.39 g).

The germination ability was highest from the plot treated with the herbicide Butisan Star (98.00%). The average germination ability in the year 2011 was 93.5%.

From all used herbicide preparation, highest values of observed characteristics were reached repeatedly for the herbicides Stomp 400 SC and Fusilade Forte. These herbicides were evaluated from the viewpoint of phytotoxicity in three terms in various BBCH phases. During the first evaluation in 2010 and 2011 the phytotoxicities of herbicide preparations Stomp 400 SC and Fusilade Forte were found to be 5% and 4 – 5%, resp. In both following controls the phytotoxicity faded. The results indicate that the herbicide preparations Stomp 400 SC and Fusilade Forte can be considered suitable for the milk thistle growing. However, further examination of these herbicides is necessary.

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Studying the Adaptability of *Zea mays* ssp. Peruvian Morado and *Chenopodium quinoa* Willd. to Temperate Conditions for European Agricultural Diversification

LEITER GRANDA[1], AMPARO ROSERO[2], DEISY ROSERO[2], GUILLERMO CORREDOR[3], BLANKA KOCOURKOVA[1], RADIM CERKAL[1]
[1]Department of Crop Science, Breeding and Plant Medicine
Mendel University in Brno, Zemedelska 1, 613 00 Brno,
Czech Republic
[2]Indigenous Organization for Research ORII "Tierra y Vida", Street 16b #28ª -113, Palmira, Colombia
[3]Agriculture University, Street 170 #54ª -10, Bogota, Colombia

Leiter.granda@gmail.com

Abstract: Adaptability is considered as plant ability to respond positively to specific environmental conditions. Sustainable and resilient agriculture systems could mitigate the adverse effects of climate changes to ensure food supplies for a growing population. Thus, alternative crops are potential strategy to ensure the food security and increase the diversity in crop species. Alternative plant species which have been traditionally grown in Peru, Purple corn, *Zea mays* ssp. Peruvian Morado, and quinoa, *Chenopodium quinoa* Willd, were cultivated in Czech Republic. The growth of purple corn and quinoa was monitored in Žabčice experimental station. Temperate conditions affected significantly the plant growth, plant architecture and flowering induction, however, significant differences were observed between early, intermediate and late flowering varieties to long day conditions. Early flowering varieties showed potentiality to be adapted to temperate conditions. Purple corn and quinoa could be cultivated in the Czech Republic and the understanding of the response of tropical plants to temperate conditions is a first step in the plant adaptation.

Key-Words: alternative crops, *Chenopodium quinoa*, *Zea mays* ssp. Peruvian Morado, adaptability, agricultural diversification, temperate conditions, developmental stages.

Introduction

While atmospherical changes were recognized several years ago, the alteration in the earth's climate is clearly perceptible today. The planet is constantly in a state of flux, the climate is warming and weather patterns are shifting. These alterations have severe implications in the agriculture [1, 2]; therefore the actual challenge is to ensure adequate food supplies for a growing population under the changes in climatic conditions, which affect the plants especially through rising temperatures, elevated atmospheric CO_2 concentrations and changes in precipitation patterns [2, 3]. However, the domesticated crops are at a disadvantage due to the selection has been mainly done favoring traits such as high yield rather than adaptability. Moreover, the reduction of used crops increases the risk of the climate change.

The homogeneity of the used crops in the human diet is closer to a global standard composition [4]; this increase in similarity affects the human health by increasing high-energy and low-nutrients diets, [4, 5] and also produces genetic erosion in crops [6]. Micronutrient deficiency is a health problem widespread among 2 billion people in developing and in developed countries [7]. Dietary deficiencies of zinc and iron cause a loss of 63 million life-years annually [8]. The main sources of zinc and iron are C_3 grains and legumes; however, the elevated atmospheric CO_2 produced lower concentrations of protein, zinc and iron in C_3 crops and legumes, whereas C_4 crops seem to be less affected. Breeding for decreased sensitivity to atmospheric CO_2 concentration is a part of new challenges to global health [8].

The diversification of the crop scope as food source is the main approach to guaranty a balanced diet [5] and its potential to produce a sustainable and resilient agricultural system has to be recognized [9]. Decreased diversity of crops should be avoided by growing alternative crops and by greater genetic diversity of cultivated varieties [10]. Alternative crops complements and extends the range of food production. Most of them are characterized by specific qualitative properties (taste, nutrition, health, etc.), they are part of the

rational nutrition, therapeutic diets and are recognized as functional food [11].
Two important and traditional Andean crops, purple corn (*Zea mays* ssp. Peruvian morado) and quinoa (*Chenopodium quinoa* Willd). have been cultivated for centuries in the Andean Region and have shown great potential as food and crop. Purple corn, is a corn landrace frequently used for people, it is an important source of anthocyanins [12]. Anthocyanins in purple corn cobs and seeds include cyaniding-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and their respective malonated counterparts [13]. Purple corn is a crop with great adaptability and can be considered as a potential genetic resource for breeding to increase the spectrum of utilization.

Quinoa has been cultivated by many civilizations for over 7.000 years, it is a strategic crop that contribute to food security due to its highly nutritional composition, with a good protein quality (12.9-21.9%, depending on the variety), balanced amino acid spectrum which include a high lysine and methionine contains, carbohydrates (77.6%), lipids (6.5%), high content of a range of vitamins, antioxidants [14]. Quinoa is rich in dietary fiber and not contains gluten [15]. Its mineral nutrients contents (K, Ca, Mg, P, Zn and Fe) are much higher than those of conventional cereals [16]. This pseudo-cereal is one of the main food crops in the Andean mountains, but recently, the interest for this crop around the world has been increased. Quinoa was selected by FAO as one of the crops destined to offer food security in the next century [17]. This annual, self-pollinating C_4 plant [18], is a crop with great adaptability to the conditions of the Andes region, where the most harmful abiotic adverse such as drought, frost, soil salinity, hail, snow, wind, flooding, and heat are present. Thus, quinoa shows great drought and high salt tolerance, has a high degree of frost resistance and grows in highly acidic, alkaline, heavy metals soils [19].

This study reports the adaptability of two important and traditional Andean crops, purple corn and quinoa under temperate conditions.

Material and Methods

Experimental site and conditions

The experimental plots were carrying out on Žabčice experimental station (GPS Loc. 49°C1´18.656´´N, 16°36´56.150´´E) of Mendel University in Brno. This region is part of the geomorphological area of Dyje-Svratka river valley. The experimental station is located at corn production region, barley subtype, situated at an altitude of 184m above sea level. This area is reckoned as a very hot and dry. The average annual temperature is 9.2°C and average annual rainfall 483.3mm. Purple corn and quinoa were evaluated during 2012 and 2014, respectively.

Plant material

One variety of purple corn from Peru (PMV-581) and 9 diverse set of quinoa varieties from different sources were used in the experiment. The Danish quinoa variety Titicaca (QTC) is a hybrid between southern Chilean and Peruvian lines, bred and selected at the University of Copenhagen. Pasankalla (QP), Rosada de Huancayo (QRH), Blanca de Hualhuas (QBH) are commercial varieties in Peru; Amarilla de Marangani (QAM), Blanca dulce (QBD), Tunkahua (QT) and Blanca Sajama (QBS) are commercial varieties in Colombia and a variety Černá (QČ) commercialized in Europe.

Experimental design

Purple corn was stablished in a row of 5m length with 50 plants separated by 10cm between plants, 70 cm between rows. It was cultivated together with Czech hybrid corns. Urea was applied (100 kg/ha N) previous to the experiment and Laudis herbicide (2.25 L/ha) was applied after 23 days of sowing.
Randomized block with three replications was used in quinoa evaluation. The plots size for each replication was 3m x 1.50m. Each plot had 4 rows spaced 50 cm apart and each row had 20 plants separated at 15cm from each other. No chemical fertilizer was applied. Weeds were removed manually once in 30 days.

Parameters evaluated and data analysis

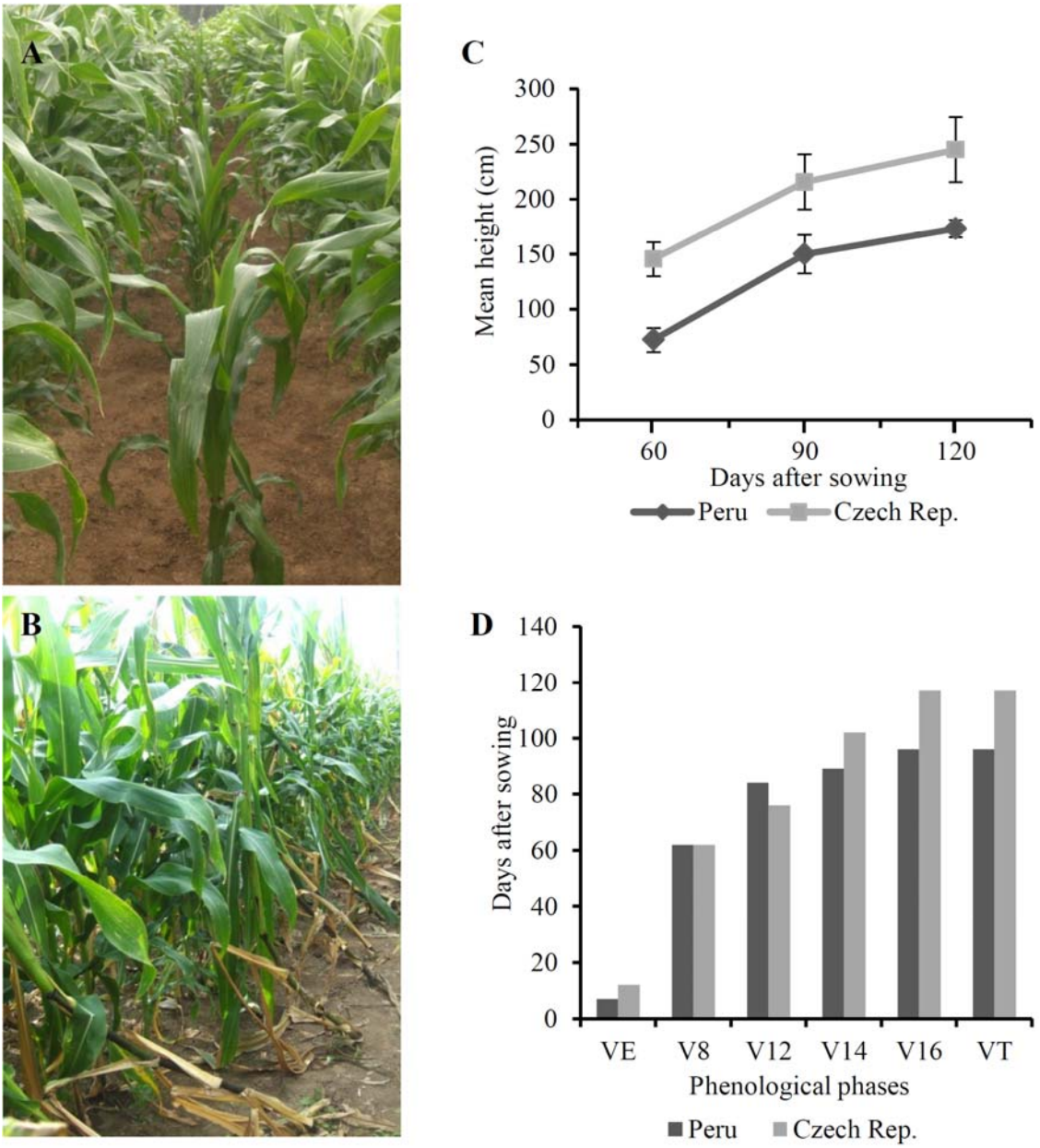
Twenty plants of purple corn were tagged and phenological and growth parameters were measured twice per month until VT phase (beginning of male flowering), according to stages of corn development previously reported [20]. The results were compared with previous studies evaluating PMV-581 variety [21, 22]. In quinoa, 30 plants per replication were randomly tagged and data was recorded every 15 days. Growth parameters were measured after establishment such as plant height, number of leaves and days to flowering. Pest infection was registered using a scale from 0 to 9 according to descriptors for quinoa [23]. Simple statistical parameters were obtained and Tukey test was used to determine significant differences using Minitab software.

Results and Discussion

Purple corn (*Zea mays* ssp. Peruvian Morado) under temperate conditions

At first date of evaluation, purple corn grown under temperate conditions slower than Czech hybrid corns, thus, purple corn plants were smaller (Figure 1A). However, the plants grown until 120 days due to a delay in the time to flowering, therefore the plants exceeded the Czech hybrid corn in plant height and bending was induced due to excessive height (Figure 1B). Similarly, the comparison with previous studies of purple corn phenology carried out in Peru [21, 22], confirmed the excessive growth under temperate conditions (Figure 1C) and delay in phenological phases (Figure 1D). Phenological phases such as emergency (VE), eight true leaves (V8), and twelve true leaves (V12) showed no significant differences, however, after fourteen true leaves (V14), sixteen true leaves (V16) and visible male flowering (VT), a significant delay was observed.

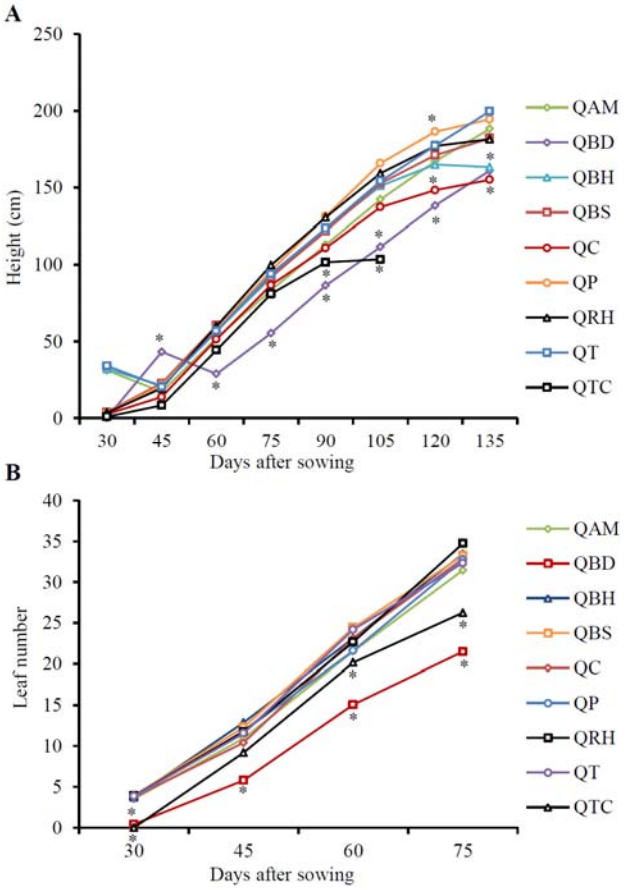
Fig. 1 Purple corn grown under temperate conditions. (A) Plants at V6 stage between Czech hybrid corns. (B) Abnormalities in plant architecture that induce bending. (C) Mean plant height of purple corn cultivated in Peru and Czech Republic. (D) Delay of phenological phases of purple corn cultivated in Czech Republic compared with Peru.



Quinoa (*Chenopodium quinoa* Willd.) under temperate conditions

Most of the quinoa varieties grew up to 150cm, being extreme values compared to these varieties cultivated in South America [24, 25], probably due to a delay in the flowering initiation. The variety Titicaca (QTC) is adapted to temperate conditions, thus, the flowering finished the plant growth at 90 days after sowing (Figure 2A).

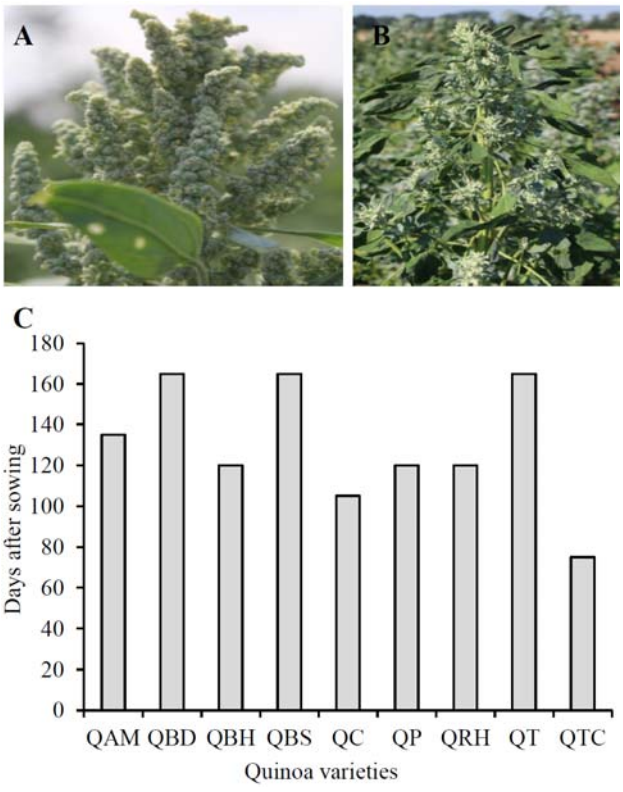
Fig. 2 Growth of quinoa varieties under temperate conditions. (A) Plant height of nine varieties of quinoa. (B) Increase of leaf number. Statistically significant differences are marked *, Tukey test $p< 0.05$.



The varieties QBH, QAM and QBD showed a reduction in the averaged plant height due to beetles infestation. QBH and QAM tolerated the pest attack, while the growth of QBD was significantly affected. The number of leaves was similar among the varieties, QBD and QTC showed the lower values, QBD due to pest infestation previously mentioned and QTC variety showed early induction of reproductive phase. These results are consistent with the differences found among several photoperiod conditions [26]. The increase in the number of leaves in quinoa plants under long day conditions was confirmed in an intermediate flowering cultivar that showed significant differences compared to short day conditions which were visible 40 days after sowing [27].

Quinoa shows three shapes of panicle, glomerulate, intermediate and amarantiform. Varieties with panicle glomerulate shows glomerules inserted in the primary axis showing a globose shape, i.e. QBS, QP, QTC. Varieties with panicle amarantiform have glomerules inserted directly in the secondary axis and have an elongated shape, i.e. QRH, QAM, QBH. Varieties with panicle intermediate show both shapes [23]. However, the inflorescence of some varieties were strongly affected by temperate conditions, producing abnormalities in its architecture (Figure 3A,B).

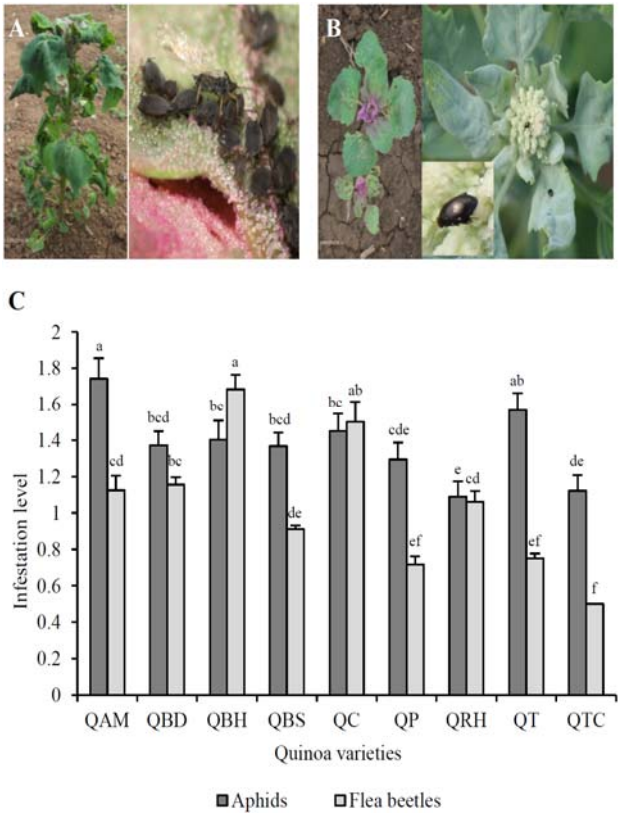
Fig. 3 Quinoa flowering under temperate conditions. (A) Normal amarantiform inflorescence of QBH variety. (B) Abnormal extra-laxed inflorescence of QBH variety. (C) Days to flowering of nine evaluated varieties, date at which 50% in a plot show blooming.



Flowering time was variable (Figure 3C); QTC had the earlier time of flowering as European adapted variety. The varieties QBH, QC, QP and QRH showed less than 120 days to flowering and showed potentiality to be adapted to temperate conditions.QAM, QBD, QBS and QT as late flowering varieties showed more than 140 days to

flowering. The delay in flowering initiation due to long day conditions was previously reported in intermediate flowering cultivar, cv. Blanca de Junin [27].

Fig. 4 Main pests attacking quinoa under temperate conditions. (A) Quinoa plant infested with black bean aphids (*Aphis fabae*) and detailed view. (B) Quinoa plant infested with flea beetles (*Chaetocnema concinna and Ch. tibialis*) and its detailed view. (C) Infestation levels of both aphids and beetles in nine quinoa varieties. Significant differences are marked by different letters, Tukey test $p< 0.05$.



The main pests in the field were black bean aphids (*Aphis fabae*) (Figure 4A), flea beetles (*Chaetocnema concinna* and *Ch. tibialis*) (Figure 4B) and leaf-miner flies (*Agromyzidae* spp.). Beetles affected quinoa plants from emergency until four true leaves, while aphids affected later (from six true leaves until ear formation). QAM and QT were sensitive varieties to aphids attack; however, it did not pass to intermediate level of infection. Aphis are not recognized as quinoa pest in South America, however, it is an important pest in Europe [28]. QRH showed high level of tolerance to aphids. QBH and QC were more affected for beetles, and QP, QT and QTC showed lower level of infestation.

Conclusion

Temperate conditions affected significantly the plant growth, plant architecture and flowering induction. Increase in vegetative phenological phase and significant delay of flowering induction were observed in both plant species. However, significant differences were observed between early, intermediate and late flowering varieties to long day conditions. Early flowering varieties showed potentiality to be adapted to temperate conditions. Purple corn and quinoa could be cultivated in the Czech Republic and the understanding of the response of tropical plants to temperate conditions is first step in the genotype adaptation.

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Evaluation of the occurrence of weeds in orchard

MARKETA JEZOVA, JIRI HANZL, JAN WINKLER
Department of Plant Biology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xjezova@node.mendelu.cz

Abstract: The aim of this study was classification of weed species diversity in a chosen orchard in the company ZEAS Lysice in Blansko region. Exploration took place in an apple orchard by means of phytocoenology relevé in tracks and in strips at ten specific locations. The relevé was carried out during three terms - in spring, summer and autumn. The results were processed with statistical analysis DCA and CCA. The research was done during year 2012. The following plant species had the highest coverage in tracks: *Taraxacum officinale*, *Poa pratensis, Plantago major, Poa trivialis, Lolium perene*. The highest coverage in strips had following plant species: *Taraxacum officinale, Stellaria media, Urtica dioica, Capsela bursa pastoris, Poa annua* and *Triforium hybridum*. 69 plant species were observed and recorded in total.

Key-Words: orchards, weeds, species diversity

Introduction

Orchards have always created the countryside atmosphere and its harmonious character. The villages would not look so picturesquely without these gardens [1].

Currently we realize that the orchard is part of ecosystem. It is therefore necessary to include it as part of environment that can stabilize the landscape. The importance of grassed inter-row was identified in the context of the above-mentioned treatment of inter-row soil cover. Among other things it forms the soil protective functions. However we must realize that weedy species except negative effects have also positive influences. One of them is their importance to increase the species diversity of environments called biodiversity [2].

Mitchem [3] mentioned competition in the struggle for light as another possibility of weed harmfulness. Viny weeds are the biggest threat on orchards, because these species are able to wrap the entire tree. The competitiveness of weeds within the nutrients exploitation brings reduction of crop yield. Intensive growth in early developmental stage, production of large quantities of seeds belong among the other influences of weed harmfulness.

Lipecki [4] argues that machines are still moving in same tracks in modern orchards. Vegetative cover helps to protect soils against the excessive compaction. The ability of weeds to bind toxic compounds and heavy metals is one of the other benefits of weeds for orchards and environment. Weeds by their presence also prevent problems associated with the cultivation of monocultures.

According to Falta and Prazak [5] occur both annual and perennial weeds species in growths of trails. Control of perennial weeds is difficult not only for seeds reproduction (generative) but also for roots, stolons and rhizomes (vegetative reproduction). Perennial weeds create massive root system. If the root system is not removed, usually by chemical means, becomes a source of further weed infestation [6].

Taraxacum officinale, Cirsium arvense, Epilobium montanum, Agropyron repens, Convolvulus arvensis, Urtica dioica, Equisetum arvense and *Rumex* sp. are the most occurred weed species [5].

Material and Methods

Characteristic of Experimental Location

Agricultural enterprise ZEAS Lysice a. s. owns the monitored orchard. Experimental location is situated in cadastre of city Lysice in Blansko district (GPS 49.4515975N, 16.5371575E), Czech Republic.

Blansko district is located in central Moravia in the northern part of South Moravian region. The area belongs to the geomorphological Czech Highlands [7]

Lysice lies in mildly warm climate area, namely MT 7, where is normally long summer and winter. The climate allows successful cultivation of fruit

trees, apricots and grapes in protected locations as well. According to Culka [8] precipitation are generally low because Lysice lies in the rain shadow of the highlands.

Climatic and meteorological data were obtained from website of ČHMÚ, meteorological station Brno – Tuřany (Table 1 – Table 2).

Characterisation of agricultural company

ZEAS Lysice, a.s. was established in Decemeber, 1996. The company employes approximately 100 people and operates on a total area of 2200 ha in 14 village cadastres in Blansko district and partly in Brno-contryside. The lands are situated at an altitude of 350 to 650 m a.s.l.

Fruit cultivation is a special part of crop production, which focuses not only on apple production, but additionally on planting strawberries and currants. Intensive orchards have a total area of 90 ha. Currently 38 ha of apple orchards and 36 ha of red currant are in full fertility. Other areas are planted with new plantings.

Weeds are regulated mechanically and chemically in strips next to roots. Their vegetation cover is maintained below 10 % at the time of sprouting to development of intensive growth.

Methodology of evaluation of the vegetation

Measurement was conducted by phytocenological scanning in selected orchard. Relevé were set in strips next to roots and in inter-rows at ten stands. Scanning was performed in three repetitions. The first term was made in spring, from May 9 to 11, 2012. Second repetition was acquired in summer, from July 9 to 10, 2012. Last term took place in autumn, from September 16 to 17, 2012. Size of scanned area was 15 m^2. After the identification of the species, it was possible to estimate the area, which is covered by particular species. Czech and Latin terms of each weed species were used according to Kubat [9].

Table 1 Data of weather conditions from the meteorological station Brno – Tuřany, in period 1961-1990

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	average
Average temperature (°C)	-2.5	-0.3	3.8	9.0	13.9	17.0	18.5	18.1	14.3	9.1	3.5	-0.6	8.7
Precipitation (mm)	24.6	23.8	24.1	31.5	61.0	72.2	63.7	56.2	37.6	30.7	37.4	27.1	490.1

Table 2 Data of weather conditions from the meteorological station Brno – Tuřany, based on data from 2012

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	average
Average temperature (°C)	0.0	-4.5	5.7	9.2	15.5	18.3	19.7	19.8	14.4	8.4	5.9	-2.5	9.2
Precipitation (mm)	44.2	9.9	2.8	11.7	54.6	74.4	43.2	19.1	55.4	59.4	21.8	16.0	412.5

A multivariate analysis of ecological data was used to determine the effect of environment factors on weed species, which were found in selected lands. Selection of the optimal analysis followed the length of the gradient (*Lengths of Gradient*), which was detected by segment analysis DCA (*Detrended Correspondence Analysis*). Furthermore, Canonical Correspondence Analysis CCA was used. A total number of 499 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program Canoco 4.0 [10].

Table 3 The sum of vegetation cover of found weed species in orchard

Weed species	Stands	
	Inter-row	Strip next
Agropyron repens	64	12
Achillea millefolium	29	3
Ajuga reptans	29	0
Amaranthus retroflexus	0	2
Anagallis arvensis	0	3
Bellis perennis	0	1
Bromus mollis	1	0
Bromus sterilis	0	3
Bromus tectorum	0	1
Calamagrostis epigejos	0	2
Capsella bursa-pastoris	23	44
Carex rostrata	0	1
Carlina acaulis	1	0
Cerastium vulgatum	2	3
Cirsium arvense	2	1
Conyza canadensis	0	1
Crepis biennis	7	0
Dactylis glomerata	4	1
Echinocloa crus-galli	0	12
Fallopia convolvulus	5	5
Festuca pratensis	32	4
Fragaria moschata	0	2
Fragaria vesca	0	1
Fumaria officinalis	0	1
Galeopsis tetrahit	0	2
Galium aparine	3	11
Galium odoratum	0	5
Geranium pratense	1	0
Geranium pusillum	0	1
Geranium robertianum	0	1
Geum urbaneum	4	1
Holcus lanatus	2	9
Holosteum umbellatum	9	30
Chenopodium album	10	22
Chenopodium hybridum	1	3
Lamium album	1	1
Lamium purpureum	1	0
Leontodon autumnalis	2	0
Leontodon hispidus	2	2
Lolium perene	202	0
Malus domestica	1	23
Myosotis arvensis	3	12
Pastinaca sativa	2	4
Picris hieracioides	24	10
Plantago lanceolata	18	0
Plantago major	412	4
Poa annua	92	38
Poa pratensis	443	19.5
Poa trivialis	370	6
Polygonum aviculare	25	12
Ranunculus acris	0	1
Rosa canina	27	26
Sambucus nigra	0	1
Senecio vulgaris	0	7
Silene vulgaris	2	0
Solanum nigrum	0	1
Sonchus arvensis	0	2
Sonchus asper	0	3
Stellaria media	29	307
Taraxacum officinale	540	752
Trifolium dubium	3	2
Trifolium hybridum	46	36
Trifolium medium	57	2
Trifolium pratense	5	0
Trifolium repens	46	5
Urtica dioica	49	68
Urtica urens	13	9
Veronica hederifolia	2	1
Veronica persica	0	2

Results and Discussion

69 of plant species were found within the observation in total. The sums of vegetation cover are listed in Table 3.

Results were evaluated by DCA analysis, which set the length of gradient (*Lengths of Gradient*), 3.594. Based on this calculation was for further processing chosen CCA analysis. Analysis CCA

defines the spatial arrangement of individual weed species, variants of stands and term. This is then graphically expressed using the ordination diagram. Weed species, stands and dates are displayed in a different shapes and colors.

Results of CCA analysis, which evaluated the influence of stands on weed occurrence are significant at the significant level $\alpha = 0.002$ for all canonical axes. Based on the analysis CCA (Fig. 1) is possible found weed species divided into 2 groups.

Ajuga reptans, Poa trivialis, Plantago lanceolata, Poa pratensis, Plantago major, Lolium perene, Achillea millefolium, Trifolium medium, Festuca pratensis, Agropyron repens, *Picris hieracioides*, *Polygonum aviculare*, *Trifolium repens* and *Poa annua* are weeds of first group, which occurred mainly in inter-rows.

Second group of weeds were found mainly in strips next to roots: *Malus domestica, Echinocloa crus-galli, Myosotis arvensis, Galium aparine, Stellaria media, Rosa canina, Urtica dioica, Chenopodium album, Urtica urens, Trifolium hybridum, Fallopia convolvulus, Taraxacum officinale, Capsella bursapastoris, Holcus lanatus* and *Holosteum umbellatum.*

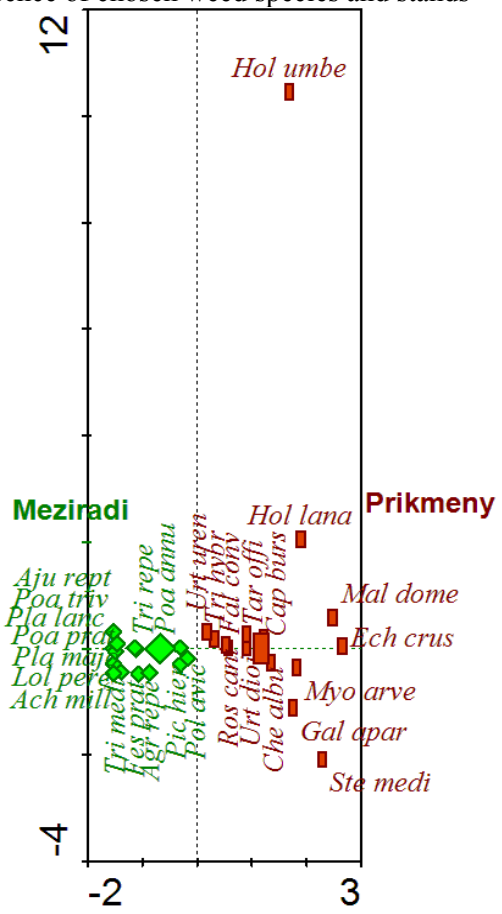
48 plant species occurred in inter-rows, which are grassy. Due to the fact that some species occurred only once within entire observation period, for diagram processing were used 14 species. Most of them were species of *Taraxacum officinale* 20 %, *Poa pratensis* 17 %, *Plantago major* 15 % and *Poa trivialis* 14 %. The largest representation of *Taraxacum officinale* in inter-rows, stated Stratilova [11].

In my opinion the greatest importance for inter-rows have grass. Apart from the above mentioned species of family Poaceae, there were also other monocot species as *Lolium perene*, *Festuca pratensis* and *Dactylis glomerata*. The significance of these species according to Hejduk [2] is especially in their soil protect erosion control function.

58 plant species were observed in strips next to roots within the monitored period. 15 of them with greatest representation we can find in the diagram. *Taraxacum officinale* 49% and *Stellaria media* 20% were recorded in strips next to roots as the most common.

Given to the current development of global climate can be expected from the hottest area of our country, gradual emergence of these species: *Arhenatherum elatius*, *Calamagristis epigejos* and *Robinia pseudacacia* and other species, which states PYŠEK [12]. Together with these species we can assume pest pressure, which are tied to these species (aphids).

Fig. 1 Ordination diagram expressing relations of occurrence of chosen weed species and stands



Legend: Explanatory notes of used abbrevations in ordination: Meziradi – inter-rows, Prikmeny – strips next to roots.

Weeds: *Agr repe - Agropyron repens, Ach mill - Achillea millefolium, Ach mille – Achillea millefolium, Aju rept - Ajuga reptans, Cap burs - Capsella bursa-pastoris, Ech crus - Echinocloa crus-galli, Fal conv - Fallopia convolvulus, Fes prat - Festuca pratensis, Gal apar - Galium aparine, Hol lana - Holcus lanatus, Hol umbe – Holosteum umbellatum, Che albu - Chenopodium album, Lol pere - Lolium perene, Mal dome - Malus domestica, Myo arve - Myosotis arvensis, Pic hier - Picris hieracioides, Pla lanc - Plantago lanceolata, Pla majo - Plantago major, Poa annu - Poa annua, Poa prat - Poa pratensis, Poa triv - Poa trivialis, Pol avic - Polygonum aviculare, Ros cani – Rosa canina, Ste medi - Stellaria media, Tar offo - Taraxacum officinale, Tri hybr – Trifolium hybridum, Tri medi - Trifolium medium, Tri repe - Trifolium repens, Urt dioi – Urtica dioica, Urt uren - Urtica urens.*

Conclusion

A total of 69 plant species was found during the observation. Most plants occurred in strips next to roots, a total of 58 plant species and 48 species in inter-rows. Among the species at all stands was the most represented species *Taraxacum officinale*.

Taraxacum officinale, Poa pratensis, Plantago major, Poa trivialis, Lolium perene, Poa annua, Agropyron repens, Trifolium medium, Urtica dioica and *Trifolium repens* were the most occurred species in inter-rows.

Taraxacum officinale, Stellaria media, Urtica dioica, Capsella bursa-pastoris, Poa annua, Trifolium hybridum, Holosteum umbellatum, Rosa canina and *Chenopodium album* were the most observed species in strips next to roots within the monitored period.

Each plant growing in nature has its unique importance, sometimes even this meaning is not detected and therefore not appreciated. Significance of weeds is both positive and negative. Weeds are artificially created group of plants, which in every way interferes humans. It is important to find limit when weed is harmful and when is not in the agricultural landscape. It certainly will have a different effect on weed infestation of inter-rows in apple orchard, than in the wheat field. Depending on the situation it is necessary to plan their regulation or maintaining them at the stands.

Acknowledgement

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Uptake of Selenium of Red Clover *(Trifolium pratense)*

IVA KLUSONOVA[1], JIRI SKLADANKA[1], VOJTECH ADAM[2], PETR SKARPA[3], PAVEL HORKY[1], LUCIA HODULIKOVA[1], MICHAL KVASNOVSKY[1]

[1]Department of Animal Nutrition and Forage Production
[2]Department of of Chemistry and Biochemistry
[3] Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

iva.klusonova@mendelu.cz

Abstract: The aim of this study was to determine the ability uptake of red clover *(Trifolium pratense)* forage of selenium after foliar application. There was established the pot experiment. Plants were placed in climabox with controlled-environment. In the experiment was selenium used in different forms (selenite and selenate) at a concentration equivalent to 4 $mg.m^{-2}$ Se. Samples of aboveground biomass were taken 14th, 28th and 42nd day after foliar application of selenium. Samples were analyzed by atomic absorption spectrometry with continuous radiation source with high resolution ContrAA 700. The content of selenium was affected ($P < 0.05$) also date of taking green matter and form of the applied selenium.

Key-Words: perennial forage crops, selenite, selenate, pot experiment

Introduction

Selenium is an important factor influencing the health status of animals and humans. Together with zinc and copper are classified as non-enzymatic antioxidants, but the function of enzymes related [1]. The main selenoenzymes is glutathione peroxidase, but the selenium is present in other proteins antioxidant [2].

Adequate intake of selenium in the diet is necessary for the protection of biological membranes from oxidative destruction [1]. As a result of the involvement of selenium compounds in many biological functions, disrupts its deficit overall health status of the animals. Deficiency may manifest as increases the susceptibility of cubs to infectious diseases, reproductive disorders caused or may be directly cause disease (eg. Nutritional myopathy) [3]. Simultaneously, however, be noted that in this micronutrient is the difference between the received and the maximum daily dose of toxicant is very small and its excess can cause serious poisoning which can lead to death [4]. According Panek [5], the content of selenium in plants depends on its content in the soil. In our conditions is an element deficient, and therefore no applied by spraying 25 days after sowing. After application of each group were regular 14 day intoxication or if they are contained in the feed plant species that are capable of selenium in their tissues actively accumulate [4]. The amount of selenium deposited in animal products supply the body depends on this element of the feed, the organic form is better utilized than inorganic [6].

For this reason, Gupta et al. [7] recommended as one of the possible delivery of organic selenium to feed its foliar application.

The aim of the study was to investigate the accumulation of selenium foliar application in forage clover.

Material and Methods

The experiment was included red clover *(Trifolium pratense)*, a variety Amos. For foliar application solution was used at a concentration of selenium which corresponds to the amount of 4 $mg.m^{-2}$ Se in the form of selenate or selenite.

Red clover was sown in pots located in climabox with automatically controlled light, temperature and water regime. Two experimental groups (selenium as selenite, selenium as selenate) and one control group (no treatment) were created.

Selenium in the above concentration and form was intervals (day 14, day 28 and day 42 after application) sampled green mass. The samples were

weighed immediately after collection and subsequently sent out for analysis of organic selenium content present. Selenium was analyzed by atomic absorption spectroscopy with a continuous source of radiation with high resolution ContrAA 700 (ANALYTIK JENA, Germany, 2012). The results were processed using the program STATISTICA 10, multifactor ANOVA and Tukey test.

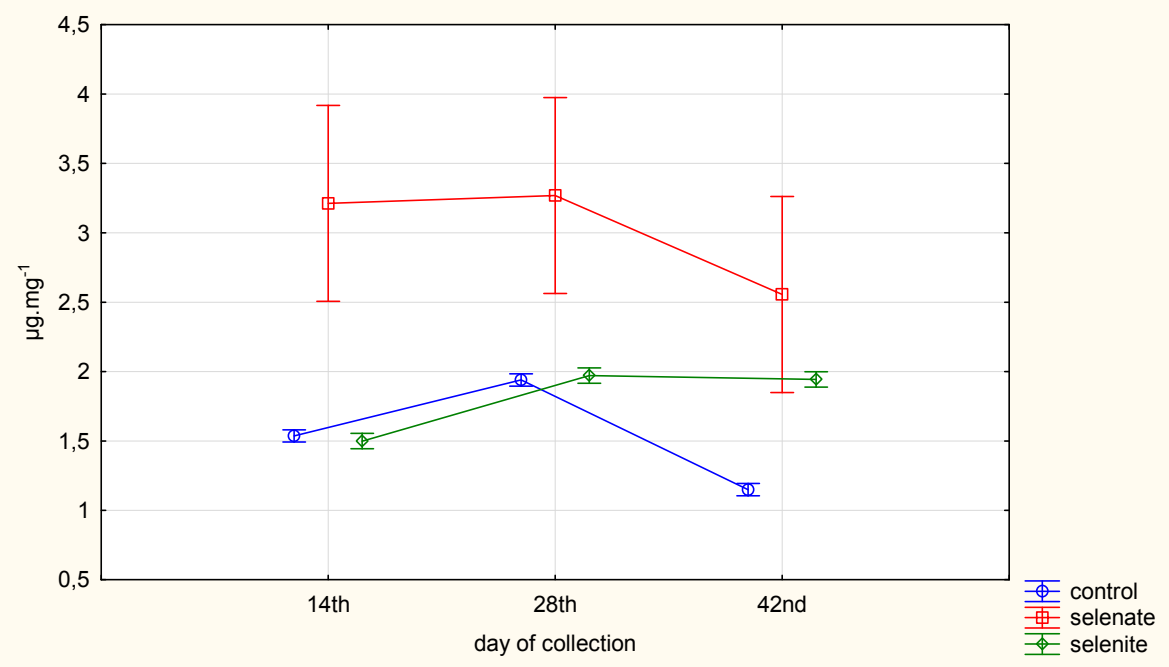
Results and Discussion

The observed selenium content in red clover forage was 1.54 μg.mg^{-1}. The selenium content was affected (P <0.05) date of taking and used form of selenium (Tab. 1). After application of selenite to selenium content in red clover increased (P <0.05) compared with the control group until day 42nd after application. By contrast, selenate led to an increase (P <0.05) the content of selenium in clover already after 14 days compared with control group (Fig. 1). Uptake of selenium in the form of selenate was significantly higher, which corresponds to the findings of the other authors [8]. Experiments that conducted Hu et al. [9] for alfalfa also confirmed that foliar application increases the selenium content in forage plants. Polakova [10] in her work indicates that the chemical similarity of sulfur and selenium. Therefore, they are better able to accumulate selenium plants which have higher demands on sulfur. Red clover is considered a plant which has higher requirements on the sulfur content in the soil [11]. Therefore we can expect greater ability sulfur, respectively selenium receive.

Table 1 Effect of selenium used forms and date of collection aboveground mass detected on the selenium content

	SC	PC	F	p
sampling	1,0747	0,5373	4,753	0,022015
form	8,8058	4,4029	38,943	0,000000
sampling*form	2,1558	0,5390	4,767	0,008452
error	2,0351	0,1131		

Fig. 1 The content of selenium after the foliar application in red clover forage



Conclusion

From the results it was evident that selenium intake influenced the form of selenium used a term sampling aboveground mass after by foliar application. Red clover reacted particular selenate fertilization. The lowest selenium content was observed by the 42nd day after foliar application.

Acknowledgement

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The impact of vermicompost application on the maize (*Zea mays* l.) phytomass creation at the growth stage 16 (BBCH-scale) and on the selected yield parameters of maize

MARIA KMETOVA, PETER KOVACIK
Department of Agrochemistry and Plant Nutrition
Slovak University of Agriculture in Nitra
Trieda Andreja Hlinku 2, 949 76 Nitra
SLOVAK REPUBLIC

kmetova.maria0307@gmail.com

Abstract: The impact of vermicompost application was assessed in one year pot experiment carried out in a vegetation cage located in the area of the SUA in Nitra. The model crop was grain maize. Treatment 1 was a non-fertilized treatment. In treatments 2 and 3, vermicompost was applied in autumn at the same dose of 170 kg ha^{-1} N. In treatments 4 and 5, vermicompost was applied in spring at the same dose of 170 kg ha^{-1} N. In treatments 3 and 5 was added nitrogen in spring to vermicompost as the AND (ammonium nitrate with dolomite) fertilizer at dose of 60 kg ha^{-1} N. The observed parameters (phytomass taken at growth stage 16 (BBCH-scale), yield of phytomass, grain yield, starch content) were most positive influenced in treatment where vermicompost was applied in spring with the addition of the AND. In the treatments, in which mineral nitrogen was added, observed parameters were affected more positively than in the treatments where only vermicompost was applied. Overall, the spring application affected observed parameters more positively than the autumn application.

Key-Words: vermicompost, maize, yield parameters, mineral nitrogen

Introduction

Low livestock numbers are the cause of the lack of cattle manure and slurry, which in many cases causes the reduction of soil fertility [1]. One of the possible alternatives for these fertilizers is vermicompost. Vermicompost is an improved compost (fermented compost mass is biodegraded with help of earthworms, *Eisenia foetida* is the most common genus). Earthworms through their enzymes and hormones transform the nutrients in compost to available forms and they enrich the compost with growth promoters such as auxins, cytokinins and gibberellins, through their secretions (also called "casts").

Vermicompost application positively affects the soil reaction, reduces the hydrolytic acidity [2] and enhances the enzyme activity of the soil [3]. Easily available organic residues and microorganisms coming from vermicompost stimulate in soil degradation of polycyclic aromatic hydrocarbons (PAHs) [4, 5, 6]. Valuable part of vermicompost are humic substances, which make an important contribution to increases yield of crops such as maize, oats, tobacco, soybeans, peanuts, clover and cucumber [7, 8, 9]. Vermicompost application has a positive effect on yield parameters of tomatoes [10, 2], strawberry [11], maize [12], peppers [13] and cucumber [9].

The aim of experiment was to determine the impact of different term (autumn/spring) of vermicompost application and impact of addition of mineral nitrogen to vermicompost on the phytomass taken at growth stage 16 (BBCH-scale) and on selected yield parameters observed after harvest (yield of phytomass, grain yield, starch content).

Material and Methods

The pot experiment was carried out in the vegetation cage at the Slovak University of Agriculture in Nitra (48°18´ S, 18°06´ V). 22 kg of soil were placed into pots of 0.38 m height and of 0.38 m diameter. The soil (Haplic Fluvisol) was taken from the growing areas of Agrokomplex Nitra from the upper 0.25 m of the humus horizon.

The tested vermicompost was produced from cow dung (about 50%), sheep manure (about 10%), green grass (about 10%) and wood chips (about 30%). After 3 - 4 months fermentation, earthworms were introduced into the compost produced from these materials. Earthworms were left in the compost for two months and were fed through an amount of 400 kg per ton of compost fodder per

month. The fodder was mainly fruit and vegetables, and the fodder was mashed before the application. Basic agrochemical parameters of soil and vermicompost are presented in Table 1.

Table 1 Agrochemical characteristics of soil and vermicompost

Material	pH_{KCl}	N_{an}	P	K	Ca	Mg	N_t	C_{ox}	Org. s.
		[mg.kg^{-1}]						%	
Soil (100% drymass)	6.52	17.6	26.2	300.5	4670	1096	3234	2.29	9.59
VC (100% drymass)	7.36	477.1	5642	14285	8535	4893	29400	20.51	48.53

VC – vermicompost, Org. s. – organic substances, P, K, Mg, - available nutrients, C_{ox} - total organic carbon, N_{an} – inorganic nitrogen, N_t – total nitrogen

The experiment consisted of 5 treatments with a threefold repetition. Treatment (tr.) 1 was a non-fertilized control treatment. In treatments 2 and 3 vermicompost was applied in autumn before the sowing of the maize at the same dose of 8.26 t ha^{-1} (170 kg ha^{-1} N). In treatments 3 was added nitrogen to vermicompost as the AND (ammonium nitrate with dolomite) fertilizer at dose of 218.18 kg ha^{-1} (60 kg ha^{-1} N). In treatments 4 and 5, vermicompost was applied in spring, one month before the sowing of the maize at the same dose of 8.26 t ha^{-1} (170 kg ha^{-1} N). In treatments 5 was added mineral nitrogen to vermicompost as the AND fertilizer at dose of 218.18 kg ha^{-1} (60 kg ha^{-1} N). Nitrogen as the fertilizer AND was applied shallowly into the soil one week before the maize sowing. The experiment treatments are shown in Table 2.

Table 2 Treatments of the experiment

Treatments		Dose of N			Dose of VC		Dose of AND		Term of application	
		in VC	in AND	Total						
no.	labelling	kg ha^{-1}			t ha^{-1}	g pot^{-1}	kg ha^{-1}	g pot^{-1}	VC	AND
1	control	0	0	0	0	0	0	0	-	-
2	VC_{aut170}	170	-	170	8.26	202	-	-	autumn	-
3	$VC_{aut170} + N_{spr60}$	170	60	230	8.26	202	218.18	5,33	autumn	spring
4	VC_{spr170}	170	-	170	8.26	202	-	-	spring	-
5	$VC_{spr170} + N_{spr60}$	170	60	230	8.26	202	218.18	5,33	spring	spring

VC – vermicompost, Org. s. – organic substances, P, K, Mg, - available nutrients, C_{ox} - total organic carbon, N_{an} – inorganic nitrogen, N_t – total nitrogen

The doses of vermicompost and nitrogen fertilizer were calculated from per hectare application doses to the application doses for the containers. In accordance with the principles of nutritionist experiments, the doses were increased fivefold [14].

Before the sowing of the seeds (24 April 2013), washed perlite was applied to the soil surface in the container up to 0.05 m, in which the maize seeds were sown. The sowing of the Pionner (PR38V91, FAO 310) type of maize was carried out in the third decade of April. It was seeded in a rate of 10 seeds per pot. The sowing depth was 0.05 m. By the beginning of July, the number of plants kept in the pot was 3 specimens per pot.

During the whole period of vegetation, the experiment was regularly checked and monitored for the overall health of the plants.

During vegetation the phytomass taken at the growth stage 16 (BBCH-scale) was determined by weighing. After harvesting the maize, the yield of phytomass and the yield of grain were determined by weighing. The starch content in maize grain was determined by Ewers polarimetric method [15].

The achieved results were evaluated by analysis of variance (One-Way ANOVA). Averages of treatments were tested by LSD test at significance level 95 % ($p<0.05$) using the computer program Statgraphics Plus, version 4.0. The significance of correlations was assessed using the correlation coefficient.

Results and Discussion

Table 3 shows that the statistically significantly lowest phytomass taken at the phenological growth stage 16 (BBCH-scale) was created in the control, non-fertilized treatment (tr. 1). The highest phytomass was achieved in the treatment 5, in which vermicompost (170 kg ha^{-1} N) was applied in spring along with the addition of AND fertilizer (60

kg ha^{-1} N). The obtained knowledge is in accordance with the knowledge of Tognetti et al. [16], who reports that plants positively respond to rational nitrogen fertilization.

The joint application of vermicompost and AND fertilizer (tr. 3, 5) affected the phytomass significantly more positively than a standalone application of vermicompost (tr. 2, 4). The spring application of vermicompost, either alone or with the addition of mineral nitrogen, influenced the phytomass taken at the phenological growth stage 16 (BBCH-scale) significantly more positively than the autumn application.

The yield of phytomass taken after harvest has developed by similar way as phytomass taken at the phenological growth stage 16 (BBCH-scale). The statistically significantly lowest phytomass yield was created in the control, non-fertilized treatment (tr. 1), i.e. in the treatment, where the lowest phytomass taken at the phenological growth stage 16 (BBCH-scale) was created. The highest yield of phytomass was achieved in the treatment 5, in which vermicompost (170 kg ha^{-1} N) was applied in spring along with the addition of AND fertilizer (60 kg ha^{-1} N). As in the case of phytomass taken at the phenological growth stage 16 (BBCH-scale), the joint application of vermicompost and AND fertilizer (tr. 3, 5) affected the phytomass yield significantly more positively than a standalone application of vermicompost (tr. 2, 4). The spring application of vermicompost, either alone or with the addition of mineral nitrogen, influenced the phytomass yield significantly more positively than the autumn application.

The impact of treatment fertilization on the grain yield was almost completely identical to its impact on the phytomass yield (Table. 3). The statistically significantly lowest grain yield was created in the control, non-fertilized treatment. The highest yield of grain was achieved in the treatment 5, in which vermicompost (170 kg ha^{-1} N) was applied in spring along with the addition of AND fertilizer (60 kg ha^{-1} N). In this treatment was created the highest phytomass taken at the phenological growth stage 16 (BBCH-scale) and also the highest yield of phytomass was achieved.

From the viewpoint of the application term, the autumn application of vermicompost exclusively (tr. 2) influenced the grain yield significantly more positively compared to the spring application (tr. 4). This confirmed the general recommendation of the practice to apply organic fertilizers in autumn, when enough organically fixed nutrients are discharged via mineralization. Even in the treatment in which vermicompost was applied in spring (tr. 4) the grain yield was on the level of the control non-fertilized treatment (tr. 1), which was surprising, because the phytomass taken in the growth stage 16 (BBCH-scale) and also phytomass yield belonged to the significantly highest. This indicates that the application of vermicompost in spring at a dose of 170 kg ha^{-1} N (tr. 4) may have been able to significantly positively affect the phytomass yield, but has also shown an obvious deficiency of nitrogen in the grain yield. This is also reflected in Kováčik's research [17], where it is stated that the application dose of 170 kg ha^{-1} N in compost or cattle manure cannot saturate the nitrogen need for maize.

Table 3 The impact of the treatments of the experiment on the phytomass taken at phenological growth stage 16 (BBCH-scale), yield of phytomass, grain yield and on the starch content

Treatment		Phytomass (BBCH 16)		Yield of phytomass (harvest)		Grain yield		Starch content	
		100 % drymass				86 % drymass		86 % drymass	
no.	labelling	g pot^{-1} (3 pieces)	rel. %	g pot^{-1} (3 pieces)	rel.%	g pot^{-1}	rel. %	[%]	rel. %
1	control	16.76 a	100.0	68.55 a	100.0	33.63 a	100.0	60.77 c	100.0
2	VC_{aut170}	24.14 b	144.1	109.64 b	159.9	50.84 b	151.2	59.59 b	98.1
3	$VC_{aut170} + N_{spr60}$	41.02 d	244.7	134.93 d	196.8	66.69 c	198.3	58.47 a	96.2
4	VC_{spr170}	24.70 c	147.4	113.79 c	166.0	33.82 a	100.6	59.35 ab	97.7
5	$VC_{spr170} + N_{spr60}$	59.42 e	354.5	170.24 e	248.4	71.51 d	212.7	60.88 c	100.2
$HD_{0.05}$		0.4919		2.2796		1.5641		1.0079	

no. - number, VC_{aut} – autumn application of vermicompost, VC_{spr} – spring application of vermicompost, N_{spr} – spring application of AND fertilizer

Different letters (a, b, c, d, and e) between the factors show statistically significant differences (P <0.05) – LSD test

The addition of mineral nitrogen to the vermicompost in treatments 3 and 5 affected the height of grain yield (Table. 3) significantly more positive than the standalone application of vermicompost. The highest grain yield was achieved in the treatment 5, where the vermicompost was applied in spring with the addition of mineral nitrogen. This explains the popularity of the use of mineral fertilizers for farmers as was proposed by Kováčik [18]. The same results was also deducted by the authors Jeyabal and Kuppuswamy [19] where the joint application of vermicompost and nitrogen fertilizers increased rice yield by 15.6% compared to the standalone application of mineral nitrogen fertilizer. The rationality of organo-mineral fertilization was confirmed, when the most significant effect on plant growth parameters was achieved with the combined use of organic and mineral fertilizers [20, 21]. In this case, fertilizers are an instantaneous source of nutrients and can also accelerate mineralization of organic compounds [19], as can also be seen in our results presented in Table 3.

A negative finding is that the application of vermicompost significantly reduced the content of starch in the maize grain (Table. 3), as was confirmed by Marschner [22], where generally with the increase in the application dose of nitrogen, the content of starch in the maize grain and also in the cereal and root crops decreases. Similar results are also mentioned by the authors Sharma and Arora [23], where the application of mineral nitrogen, together with the organic fertilizers reduced starch content. On the other hand Szukaiski and Sikora [24] report an increase in the starch content of potato tubers after the application of fertilizer which was rich in nutrient content. Also Srikumar and Öckerman [25] reported an increase in starch content in potato tubers after the application of fertilizers which were rich in the content of trace elements. The surprising finding is that the highest content of starch in the grain in our experiment has been spotted in the treatment 5 where vermicompost was applied in spring (170 kg ha^{-1} N) with the addition of AND fertilizer (60 kg N ha^{-1}). The second highest starch content in the grain was achieved in the control, non-fertilized treatment (tr. 1). The standalone autumn application of only vermicompost (tr. 2) affected the starch content more positively than the spring application (tr. 4). On the contrary, the addition of nitrogen has affected starch content in an opposite way in comparison with the treatments where only vermicompost was applied. The spring application of vermicompost with the addition of the AND fertilizer affected starch content significantly more positively than the autumn application of vermicompost with the addition of mineral nitrogen and also compared with other treatments.

Table 4 The relationship between dose of nitrogen an and selected yield parameters of maize expressed as a correlation coefficient (r)

Parameter		Correlation coefficient
Dependent	Independent	(r)
Dose of nitrogen	Phytomass (BBCH 16)	0.7479**
	Yield of phytomass (harvest)	0.8466**
	Grain yield	0.7657**
	Starch content	- 0.4172

*$p<0.05$, **$p<0.01$*

A very strong positive correlation was found between the doses of nitrogen, the phytomass yield and the grain yield (Table. 4). With the increasing doses of nitrogen, the phytomass and grain yield were also increased. Similar results were also reported by the authors Koul [26] and Omer [27] where plants positively responded to the addition of nitrogen, which led to the increase of their height and therefore their phytomass.

The negative impact of the nitrogen on starch content was confirmed partially in our results shown in Table 4. Between the starch content in the maize grain and the dose of nitrogen, there was a trend of negative (inconclusive) correlation.

Conclusion

The results show that the application of vermicompost, either alone or together with AND fertilizer, positively influenced observed parameters (the phytomass taken at the phenological growth stage 16 (BBCH-scale), yield of phytomass, yield of maize grain), while the addition of mineral nitrogen influenced observed parameters more positively than standalone application of vermicompost.

The spring application of vermicompost, either alone or together with AND fertilizer affected the phytomass taken at the phenological growth stage 16 (BBCH-scale) and yield of phytomass more positively than the autumn application. From the observed parameters (the phytomass taken at the phenological growth stage 16 (BBCH-scale), yield of phytomass, yield of maize grain and the starch content) the highest valueswere found in the treatment, where vermicompost was applied in spring (170 kg ha^{-1} N) with the addition of AND fertilizer (60 kg ha^{-1} N).

With increasing of nitrogen dose, was increased the yield of phytomass and grain yield. On the contrary, was partially confirmed the depressant effects of the nitrogen on the starch content in the maize grain.

The rationality of organic-mineral fertilization was confirmed.

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Influence of crop management on winter oilseed rape yield formation - evaluation of first year of experiment

VITEZSLAV KRCEK[1], PETR BARANYK[1], JOSEF PULKRABEK[1], JAROSLAV URBAN[1], MICHAELA SKERIKOVA[2], VACLAV BRANT[2], PETR ZABRANSKY[2]
[1]Department of Crop Production
[2]Department of Agroecology and Biometeorology
Czech University of Live Sciences in Prague
Kamýcká 129, 165 21 Praha 6 - Suchdol
CZECH REPUBLIC

krcekv@af.czu.cz

Abstract: This project aims on the assessment of how appropriate are different ways of sowing rapeseed (*Brassica napus* L. var. *napus*) in the conditions of the Czech Republic. The main focus of this work is to quantify the influence, which has the way of sowing into differently broad lines on the final crop output. The basis of this project is a large half-operational experiment, based in two locations within the company AGRA Řisuty s.r.o. In this experiment we are testing sixteen different variants. There are four different rows spacings (12.5, 25, 35, 45 cm), two sowing rate (30, 40 seedes per m^2 on variant 12.5 and 25 cm and 20, 30 seedes per m^2 on variant 35 and 45 cm) and two different hybrid varieties of OSR. The highest yield from both sites was accomplished by DK Exquisite variety in Řisuty locality, 12.5cm row spacing and seeding rate 40 plants per square metre. Contrarily, the lowest yield was recorded by PR44D06 variety in Slabce area, 25 cm row spacing and seeding rate 40 plants per square metre. There was no demonstration of influence yield of rapeseed by changing the row width or changing the seeding rate in the first year of our experiment.

Key-Words: oilseed rape, row spacing, seeding rate, yield, oil content, thousand-seeds-weight

Introduction

Oilseed rape (Rapeseed) is the most grown oilseed crop both in Europe and the Czech Republic. Areas on which oilseed rape is grown are regularly increasing since 80's and currently there are more than 400 thousand hectares grown in the Czech Republic. This marks that we have already reached an upper boarder line of possible spread of this crop. Through maintaining crop rotation rules and proper agricultural practice futher production growth of oilseed rape seeds is possible only by greater yield. There we have still sizable room for improvement measured, for example, by outcomes of our German colleagues. And it is from Germany that the new technology of seeding into wider rows than 12.5 cm standard is being introduced to us.

Although oilseed rape has good compensation capacity, it can be employed only in case of even plant distribution per area [1]. Therefore stand organization and methods of crop establishment may have significant impact on living space of each plant as well as vegetation microclimate thus can greatly influence health, formation of yield components and finally total yield of grown crop. Boelcke et al. [5] even states that seedling development, winter survival and yield of winter oilseed rape is directly depending on seeding rate and date and method of sowing. This is in agreement with the research of Bagheri et al. [4] who observed notable differences in yield between various (15, 30 and 45 cm) row spacings. On the contrary, in his work, yield was not affected by the distance of plants within the row. Older research of Morrison et al. [3] notes that number of pods per plant is the most affected component forming the yield. Number of pods is decreasing quadratically (squared) with increasing seeding rate. The largest plant height, the greatest dry matter, LAI, seed and straw yield was observed experimentally with lowest harvest index at 30 cm row spacing [6].

More and more of our agricultural businesses establishes their oilseed rape crops by seed drills primarily designed for sugar beets sowing, or by specialized drills of Horsch Focus type. This agronomical decision can seem as a step circa 40 years back when oilseed rape was grown as a root crop. Nevertheless new vigorous hybrid varieties require enough space for their development, which narrow rows cannot provide. In our conditions optimal number of individuals after winter should

be 40 - 60 plants per square metre, lower quantity is recommended for intense technology, circa 30 – 50 plants per square metre [1]. Alpmann [2] however states that lower seeding rate is suitable also in relation with plant habitus. Hybrids have good ability of branching which is positive for yield formation. On the other hand, higher competition among plants plays negative role in development of lateral branches and therefore in number of pods per plant. Too dense stands are more prone to lodging.

Material and Methods

Given that in the agricultural practices of the Czech Republic cases of sowing in rows wider than the traditional 12.5 cm are more common, we decided to check the merits of the agronomic decision in a half-operational experiment. Total number of 16 variants, four different row widths, two hybrid varieties of oilseed rape, each in two diverse seeding rates, were included in this experiment.

Seeding drills Vaderstadt Rapid were used for 12.5 and 25 cm row spacings, Horsch Focus TD for 35 cm row width and for 45 cm Monosem Meka seed drill for oilseed rape with specialized disc was employed. Seeding rate varied from 20 to 40 plants per square metre. Experiment was established on 25th August 2012 on two localities within the company AGRA Řisuty s.r.o. in Slánsko and Slabecko area. This locations are situated in a grain production area with medium soils and the climate in this region is classificated as slightly warm dry. Locations were prepared with minimal tillage of two different stubble depths. Vast drought during last August, made conditions on plots even more difficult because of large quantities of not decomposed crop residues, even though the gap between harvest and liquid nitrogen fertilizer application was more than 14 days.

As it was stated above, two different hybrid varieties of oilseed rape were selected as test variants, vigour hybrid DK Exquisite by Dekalb and semi-dwarf hybrid PR44D06 by Pioneer.

The stands were subsequently treated with identical methods including fertilization and pesticide application according to indication and need of crop, perpendicularly to seeding direction. During the year, vegetation was monitored and samples were collected. Unfortunately Ledce site was hit by hail and we have recorded 15% loss, however the field was affected evenly therefore we have decided to evaluate experiment in spite of the loss. Plots were harvested on 8thand 12th August, circa 14 days after desiccant and sealant application, using modern technique with active dividers on full lenght of cutter bar. Harvested material of each variant was weighted separately, moisture level was measured and samples for evaluation of thousand-seed weight and oil content were collected. Harvested area was calculated by multiplying frame of the combine and plot length, subsequently yield of each variant was gained.

Diagram 1 Indicative layout of experiment organization

Results and Discussion

During the first year of experiment, over 18 tons of oilseed rape seeds were harvested on more than 6 hectares. Average yield of all variants was 2.99 tons per hectare, namely 3.10 tons per hectare in Řisuty locality and 2.88 tons per hectare in Slabce area. The highest yield from both sites was accomplished by DK Exquisite variety in Řisuty locality, 12.5cm row spacing and seeding rate 40 plants per square metre. Contrarily, the lowest yield was recorded by PR44D06 variety in Slabce area, 25 cm row spacing and seeding rate 40 plants per square metre.

Tables 1 and 2 represent yield outcome of each variant. Last part of tables as well as the following Figure 1 display percentage growth, decrease respectively compared with control, which is always

the corresponding variety on each locality sown traditionally in 12.5 row spacing and seeding rate of 40 plants per square metre.

As it is evident from the results, the first year of experiments proved no significant effect on yield by varying row spacing or seeding rate. Variants with wider rows showed rather yield decrease, usually up to 10 %. Vigorous variety DK Exquisite was less influenced, in Slabce area we have recorded increase of yield by circa 10%, however this was not confirmed on second locality. Half-dwarf variety PR44D06 shown substantial decrease of yield, up to 20 % in some cases, but it displayed certain growth in yield in the widest row spacing, therefore we cannot speak about general decrease.

We haven't recorded any significant effect of row spacing on thousand-seed weight, however it clearly decreased with lower seeding rate in DK Exquisite variety on both localities. In PR44D06 variety thousand-seed weight was stable across all variants (see Figure 2). Oil content in seeds varied among pots only in units of percent, and it can be saidt hat it was more affected by variety and especially locality than the row spacing or seeding rate variability (see Figure 3).

Conclusion

First year results haven't shown any effect on yield by increasing row width or seedling rate yet, whereas results from each experimental locality are considerably variable. Therefore it is not possible to issue clear recommendation for agricultural practice, which would suggest certain row spacing for the best oilseed rape growth. In general we can say, that the outcome will depend on the chosen variety and specific locality. Thereafter for wider rows can be recommended sowing rather hybrid varieties with vigour growth type, for example DK Exquisite. Currently second year of this trial is established.

Fig. 1 Effect of row spacing and seeding rate on yield of oilseed rape in proportion to control (%)

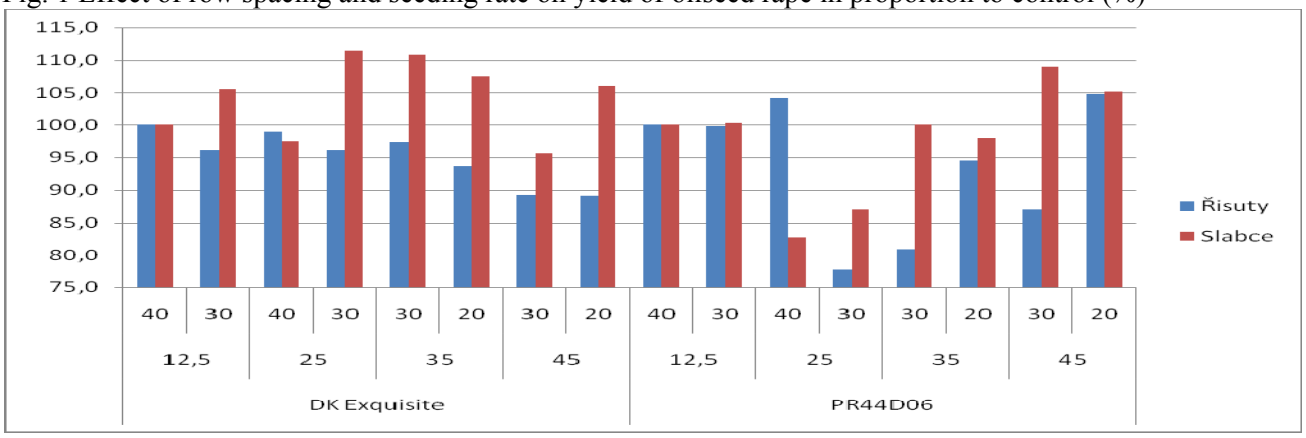

Fig. 2 Effect of row spacing and seeding rate on thousand-seed-weight of harvested oilseed rape (g)

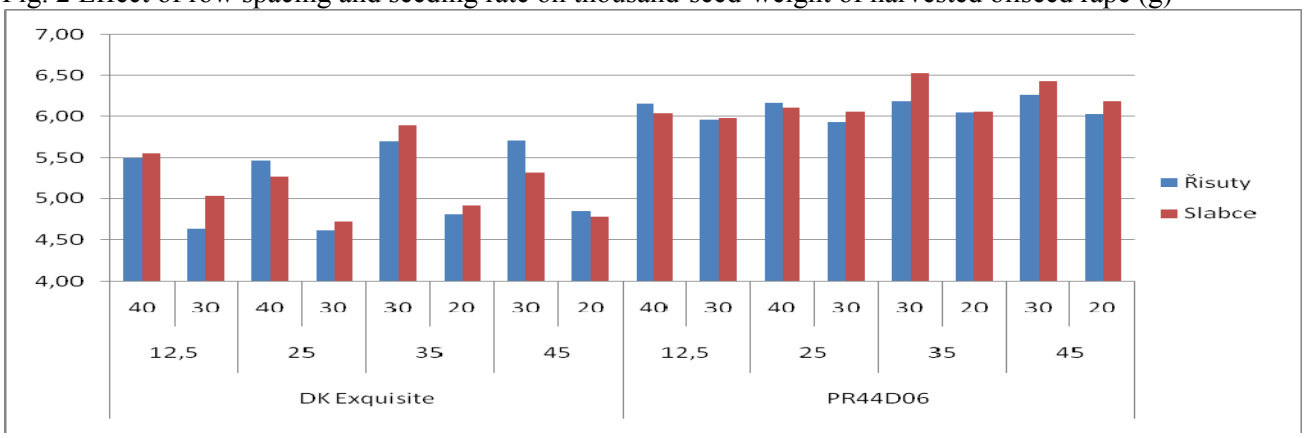

Fig. 3 Effect of row spacing and seeding rate on oil content of harvested oilseed rape (%)

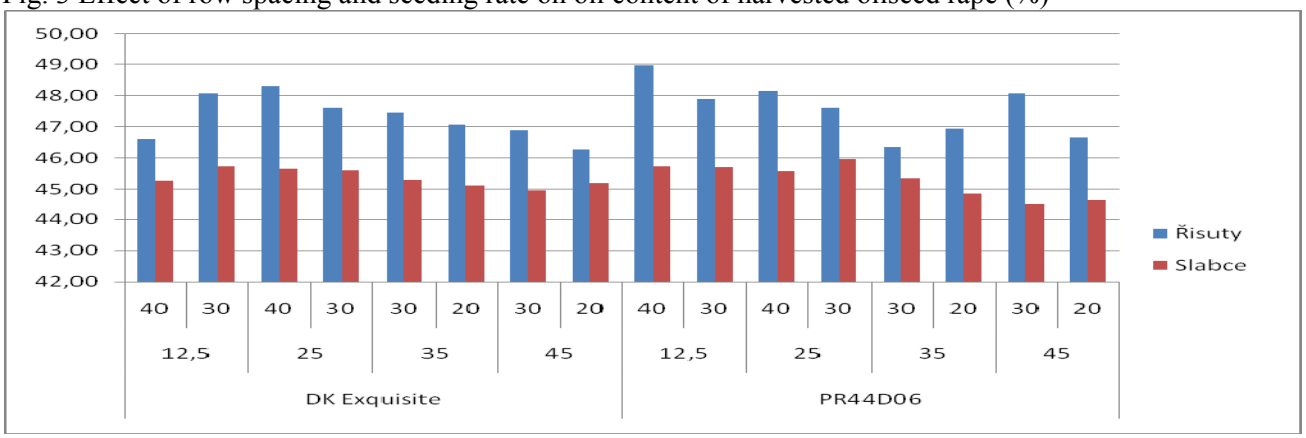

Table 1 Effect of row spacing and seeding rate on yield of oilseed rape, DK Exquisite variety

Variant	*Row spacing (cm)*	*Seeding rate (plants/m²)*	*Yield (t/ha moisture 8%)*	*Yield (%)*
DK Exquisite Řisuty	12.5	40	3.37	100.00
DK Exquisite Řisuty	12.5	30	3.24	96.08
DK Exquisite Řisuty	25	40	3.33	98.98
DK Exquisite Řisuty	25	30	3.24	96.13
DK Exquisite Řisuty	35	30	3.28	97.40
DK Exquisite Řisuty	35	20	3.16	93.65
DK Exquisite Řisuty	45	30	3.01	89.22
DK Exquisite Řisuty	45	20	3.00	89.14
DK Exquisite Slabce	12.5	40	2.79	100.00
DK Exquisite Slabce	12.5	30	2.95	105.55
DK Exquisite Slabce	25	40	2.72	97.45
DK Exquisite Slabce	25	30	3.11	111.45
DK Exquisite Slabce	35	30	3.10	110.87
DK Exquisite Slabce	35	20	3.01	107.57
DK Exquisite Slabce	45	30	2.67	95.62
DK Exquisite Slabce	45	20	2.97	106.12

Table 2 Effect of row spacing and seeding rate on yield of oilseed rape, PR44D06 variety

Variant	*Row spacing (cm)*	*Seeding rate (plants/m²)*	*Yield (t/ha moisture 8%)*	*Yield (%)*
PR44D06 Řisuty	12.5	40	3.21	100.00
PR44D06 Řisuty	12.5	30	3.20	99.78
PR44D06 Řisuty	25	40	3.34	104.18
PR44D06 Řisuty	25	30	2.49	77.71
PR44D06 Řisuty	35	30	2.60	80.87
PR44D06 Řisuty	35	20	3.03	94.57
PR44D06 Řisuty	45	30	2.79	87.01
PR44D06 Řisuty	45	20	3.36	104.83
PR44D06 Slabce	12.5	40	2.91	100.00
PR44D06 Slabce	12.5	30	2.92	100.26
PR44D06 Slabce	25	40	2.41	82.75
PR44D06 Slabce	25	30	2.53	87.01
PR44D06 Slabce	35	30	2.91	100.09
PR44D06 Slabce	35	20	2.85	97.96
PR44D06 Slabce	45	30	3.17	109.06
PR44D06 Slabce	45	20	3.06	105.21

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The course of soil temperature under oilseed rape canopy

JANA KRCMAROVA[1], RADOVAN POKORNY[1], TOMAS STREDA[1], JAN BROTAN[2]
[1]Department of Crop Science, Breeding and Plant Medicine
[2]Department of Agrosystems and Bioclimatology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jana.krcmarova@mendelu.cz

Abstract: The course of soil temperatures was determined under winter oilseed rape canopy of two varieties during the spring growth season in 2014. The first variety, Sherpa, was so called classic variety with typical height and canopy architecture for rape. The second variety was hybrid PX 104 with semi-dwarf height. Automatic sensors were positioned at two levels (50 and 100 mm) under the soil surface. The course of soil temperature varied under two tested varieties and their vegetation period. The regression between soil temperature in both depth and ground air temperature in the rape stand was established. The same relationships were determined between soil temperature under rape canopy and grass cover. These findings can be used in making more accurate prediction models of pathogens and pest occurrence on winter rape.

Key-Words: microclimate, soil temperature, air temperature, rape

Introduction

Specific microclimate develops in different plant species stands. Vertical distribution of air temperature and humidity are fluctuating and there are differences in these data recorded on the climatological station and in the different heights of canopy [1]. The soil temperature under crop canopy can differ from ones recorded on standard meteorological station, also.

The knowledge of temperature course in different soil depth under crop canopy is important from the root growth point of view. The appropriate temperature and humidity are inevitable for development of several pathogens stages, too. E.g., important pathogen, *Sclerotinia sclerotiorum,* which causes Sclerotinia stem rot of rape, develops sexual ascospores in fruiting bodies apothecia which are formed on firm bodies of fungi mycelium, called sclerocia. Carpogenic germination of sclerocia (apothecia formation) occurs from 10 to 25°C with optimum 20°C. The high air or soil humidity is necessary for this process, also [2, 3, 4].

The ascospore dispersal is influenced by changing of temperatures and air humidity during the day [5]. The range of these parameters is used for prediction models of pathogen occurrence [6, 7, 8]. The data from climatological stations measured in 2m are usually used in these models.

Material and Methods

The measurement of soil temperature in and under oilseed rape canopy (variety Sherpa and PX 104) was carried out on Žabčice experimental station of Mendel University in Brno in 2014. This area is located in the floodplain of the river Svratka in altitude of about 184 m in maize production area. The average annual air temperature is 9.2°C and average annual precipitation total is 483 mm. The soil in experimental plot is heavy gleic fluvisol.

Data recording was conducted by means of a mobile meteo-station equipped by digital temperature sensors (Dallas semiconductor, DS18B20 type). The recorders were positioned at two depths (50 and 100 mm under the soil surface). The soil temperatures under grass cover were also measured under grass cover on the near climatological stations by sensors T-107 (10TCRT) at the same depth as for oilseed rape. The spring vegetation period of rape was divided into four stages I. BBCH 30–59 (stem elongation to inflorescence emergence), II. BBCH 60–69 (flowering), III. BBCH 70–79 (development of fruit), IV. BBCH 80–89 (ripening). The regression analysis was carried out to evaluate interrelationships between soil temperatures measured under two types of plant covers (oilseed rape and grass, respectively). The same analysis was done for air temperature on the ground of oilseed rape and soil temperatures. As the course of

temperatures in soil can be delayed, cross correlation were computed for this evaluation, also. These models were tested with the coefficient of determination (R^2).

Results and Discussion

The dependence of soil temperature under oilseed rape canopy on the temperature under grass cover in particular depth was high (Table 1) for the variety Sherpa in depth 50 mm, the coefficient of determination reached values from 0.462 to 0.713. For depths 100 mm determination coefficient for this variety was slightly lower and reached values from 0.135 to 0.465. The regression between course of temperature under grass and PX 104 was not proved, as determination coefficient did not reached value 0.1 in almost all cases, only with some exceptions.

Table 1 Regression relationships of dependence of soil temperature under rape canopy on temperature under grass cover in particular depth

Variety	STAGE	50mm	100mm
Sherpa	I.	y = 0.3408x + 6.9433	y = 0.1421x + 8.7135
		R² = 0.713	R² = 0.319
	II.	y = 0.2871x + 9.6418	y = 0.1085x + 11.669
		R² = 0.462	R² = 0.135
	III.	y = 0.5376x + 7.3181	y = 0.3667x + 9.5832
		R² = 0.659	R² = 0.465
	IV.	y = 0.438x + 12.76	y = 0.1499x + 17.362
		R² = 0.515	R² = 0.159
Px 104	I.	y = 0.08x + 8.2331	y = 0.0484x + 8.377
		R² = 0.147	R² = 0.070
	II.	y = 0.0713x + 10.547	y = 0.0416x + 10.669
		R² = 0.097	R² = 0.039
	III.	y = 0.2456x + 9.3809	y = 0.2144x + 9.5952
		R² = 0.406	R² = 0.352
	IV.	y = 0.0597x + 15.971	y = 0.0203x + 16.435
		R² = 0.095	R² = 0.016

Note: E.g. according to regression equations, 10°C under grass (independent value) means 12.5°C or 11.3°C under Sherpa or PX 104 varieties, respectively (dependent value); depth 50 mm, developmental period II.

As can be seen from the Table 2, the prediction of soil temperature cannot be done from the air temperature in the ground of rape canopy recorded at the same time, because coefficients of determination were usually very low. As it was found out by cross correlation analysis, the best interrelationships between these two variables were achieved in 2 hours delay for the soil temperature in 50 mm and 4 hour delay for 100 mm in variety Sherpa and 6 hours delay for the soil temperature in 50 mm and 7 hour delay for 100 mm in variety PX 104. After the time correction the determination coefficient reached values from 0.85 to 0.89 for 50 mm and 0.66 to 0.79 for 100 mm in variety Sherpa. For variety PX 104 this coefficient reached values from 0.51 to 0.72 in 50 mm depth and from 0.39 to 0.67 in 100 mm depth.

The knowledge concerning soil temperature is inevitable for modelling of some plant growth and development models and it is sometimes used for the prediction of pathogens and pest occurrence. From our results is evident, the relationships between temperatures measured in soil can be influenced by architecture of particular variety of the same crop. Plant architectural traits have been reported to impact pest and disease occurrence and development, because spatial distribution of leaves in space can determines the within plant microclimate and the shoot distribution, topological connections which influence the within plant propagation of attackers [9, 10, 11]. From this point of view, the type of varieties should be included in prediction models.

Table 2 Regression relationships of dependence of soil temperatures in different depths on air temperatures from non-corrected (non) and corrected (corr) data

Variety	Stage	50mm (non)	50mm (corr)	100mm (non)	100mm (corr)
Sherpa	I.	y = 0.3096x + 7.3078 R² = 0.586	y = 0.3787x + 6.6993 R² = 0.851	y = 0.1036x + 9,1157 R² = 0.169	y = 0.2177x + 8.0333 R² = 0.713
	II.	y = 0.3742x + 8.5843 R² = 0.611	y = 0.4434x + 7.6644 R² = 0.865	y = 0.1507x + 11.145 R² = 0.202	y = 0.2687x + 9.5759 R² = 0.657
	III.	y = 0.6965x + 5.1382 R² = 0.824	y = 0.7257x + 4.8217 R² = 0.893	y = 0.4888x + 7.8786 R² = 0.613	y = 0.5554x + 6.9424 R² = 0.790
	IV.	y = 0.572x + 9.8985 R² = 0.729	y = 0.6173x + 9.1475 R² = 0.886	y = 0.2224x + 15.882 R² = 0.290	y = 0.3569x + 13.389 R² = 0.789
Px 104	I.	y = 0.0762x + 8.3578 R² = 0.103	y = 0.1823x + 7.4192 R² = 0.586	y = 0.0455x + 8.4571 R² = 0.048	y = 0.1432x + 7.5964 R² = 0.477
	II.	y = 0.1136x + 10.184 R² = 0.110	y = 0.2376x + 8.7536 R² = 0.514	y = 0.0698x + 10.414 R² = 0.049	y = 0.1897x + 9.0369 R² = 0,393
	III.	y = 0.3479x + 8.3266 R² = 0.530	y = 0.4063x + 7.5293 R² = 0.719	y = 0.3103x + 8.577 R² = 0.480	y = 0.3675x + 7.8021 R² = 0.667
	IV.	y = 0.0432x + 16.278 R² = 0.052	y = 0.1483x + 14.36 R² = 0.634	y = 0.0082x + 16.66 R² = 0.003	y = 0.1109x + 14.783 R² = 0.512

Note: E.g. according to regression equations, ground air temperature 15°C (independent value) means 9.1°C in real time and 14.3°C with 2 hours delay under Sherpa variety (dependent value); depth 50 mm, developmental period II.

Conclusion

As is evident from regression analyses, the course of temperatures can significantly differ in soil under various plant cover and from ones measured in plant stand. The results must be taken in account to precision of prediction models of some harmful agent's occurrence, in models of crop and yield development etc.

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Evaluation of the suitability of grass species for dry conditions

MICHAL KVASNOVSKY, IVA KLUSONOVA, LUCIA HODULIKOVA, PAVEL KNOT
Department of Animal Nutrition and Forage Production
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

michal.kvasnovsky@mendelu.cz

Abstract: The main aim of this study was to evaluate the response of the production types of grasses to stress-induced reduction of normal precipitation in relation to their production characteristics and the structure of biological phytomass. The covers were established by planting of pre-grown plants of the individual grass species in the spring of 2009 in the form of a small-plot experiment in two blocks. Block A - normal precipitation mode, Block B - reduced precipitation mode consisting in roofing of 50% of the experimental area coverage by a special film with a minimum reduction of light conditions in order to drain a half of rainfall out of the area. In the crop year 2011 the annual total Rainfall was relatively lower by 14.0% (632.8 mm) than the long-term average, i.e. 736 mm. In relation to production formation, it is necessary to take into account that in 2011, the precipitation amount for vegetation ranged between 71% and 75% of the annual total. Highest weight of dry matter is in the hybrid Festulolium Fojtan (average 132.6 $g.pc^{-1}$).The effect of reduced precipitation is manifested in a relatively lower weight of the plant Festulolium Fojtan (77.5 $g.pc^{-1}$). The effect of drought on decrease in production and number of pseudostems (offsets) has not been completely proved.

Key-Words: grass, drought, utilization, production, number of offsets.

Introduction

Climate change (rising temperatures, lengthening of the growing season, increasing evaporation) significantly affects agricultural production in traditional production areas, as illustrated by example better results in growing of corn on its northern or upper height limit. Also, the turf can be observed in recent years more and more often occurrence of grass species in group C4 [5]. The important factor that often limits the yield and dry matter grass in drought reflects the significant decrease in soil water content. Water shortage has reflected worse nutrient uptake and thus lower yield [1]. In dry years, yields at higher altitudes are above the average and that compensates short falls in production in major producing areas. These changes of the water balance can significantly influence the possibility of growing crops in our area [6]. However, not only worse nutrient uptake is the cause of reduced yield. Many species respond to drought by maintaining high water potential by reducing water losses or better adsorption. Limitation of water losses can be reduced in the development of water stress by rolling the leaves or fast closing stomata. The plants, however, not only reduce transpiration, but also reduce photosynthesis and thus growth and development [8].

Interaction of drought stress with high temperature has a greater effect than the damaging effects of each stressor separately. There is a loss of water by transpiration required for cooling and thus faster drying [3].

Almost a third of the fresh water that is consumed in Europe is used in agriculture, mostly for irrigation [2]. Although most of the production limits of field crops and grasslands are largely drought [3], the overwhelming majority of agricultural land in the Czech Republic there is not used irrigation. Also grassland in the summer months, when water consumption is highest, on grounds the lack of irrigation possibility is greatly limited. At such situation stands react with deterioration in appearance and functionality. In the Czech Republic during the summer season (April to September), a significant reduction in available water content in the upper soil layer [4]and even in the areas with relatively high rainfall at higher altitudes. Lack of water in the soil is often compounded by the fact that part of the precipitation comes in summer as torrential rains that causes surface runoff, and only part of the total sum increases soil water retention.

Breeding for drought tolerance thus becomes a major objective of breeding programs. Whereas, that

the root system of plants is the organ responsible for the uptake of water and nutrients, in addition to anchoring the plants in the soil. The roots are seen as essential for improving the resistance of plants to drought stress [7].Soil heterogeneity and dynamic availability of nutrients in the soil represents a challenging environment in which the plants efficiently receive nutrients to maintain their internal nutrient homeostasis throughout their growth. They are mathematical models that help to understand the strategy of plant growth and root characteristics associated.

The main aim of the study was to evaluate the response production types of grasses to stress-induced reduction of normal precipitation in relation to their production characteristics and the structure of biological phytomass.

Material and Methods

Characterization of growing locality and experimental design

Experimental studies are conducted at the experimental site of the Mendel University in Brno, in the Fodder Research Station of Vatín. From a geographical point of view it is a potato-growing region, with altitude of 535 m. Weather conditions: average annual temperature 6.9°C of which for vegetation 12.6°Cannual amount of precipitation 736 mm of which for vegetation 440 mm. The covers were established by planting of pre-grown plants of the individual grass species in the spring of 2009 in the form of a small-plot experiment in two blocks. Block A - normal precipitation mode, Block B - reduced precipitation mode consisting in roofing of 50% of the experimental area coverage by a special film with a minimum reduction of light conditions so as to drain a half of rainfall out of the area. The mode of precipitation regulation was applied only in the second year after planting for the reason of allowing the same conditions for initial growth and development of plants.

Growing Variants

The subject matter of monitoring and evaluation was a total of 2 grass species (*Festuca arundinacea* variety Prolate and Festulolium Fojtan) and their suitable varieties for grazing character. Fojtan is the result of a crossing between Italian Ryegrass and Tall Fescue.Each variant consisted of planting 25 pcs of individuals grown in layouts of 200 x 200 mm in triplicate (a, b, c). Planting was carried out in June 2009. In the first year, clearing the covers of weeds was done manually. Harvest of the covers (individual plants) was carried out 2x a year only in the year of establishment. From 2010 was subjected to a "model" 5-fold mowing grazing utilization. Before planting was applied to the surface of the NPK fertilizer (dose of N 50 kg.ha^{-1}). In the next year's crop fertilization was 150 kg N.ha^{-1}, of which 1/3 NPK after hibernation and 2 more doses after mowing LAV 27.5%.

Harvesting of individual plants was performed manually by cutting the whole plant at stubble height of 60 mm. For each harvested plant, its weight in dry state was determined by weighing after drying in a drying room. Also the number of stalk (a leaf) offsets, further the plant height, taken as the maximum length of the stalk, was determined. Rated characteristics:

- number of stalk (a leaf) pseudostems
- Weight in dry matter.

Evaluation of inter-species differences in production and differences in production among the water mode were subjected to the ANOVA test. Results were evaluated with Tukey's test. Differences were declared to be statistically significant when $P \leq 0.05$.

Results and Discussion

Evaluating the Level and Course of Rainfall and Temperature

In crop year of 2011 the annual total Rainfall was relatively lower by 14.0% (632.8 mm) than the long-term average, i.e. 736 mm. In relation to production formation, it is necessary to take into account that in 2011, the precipitation amount for vegetation ranged between 71% and 75% of the annual total. Another unusual feature of the year 2011 is a very low level of precipitation in the period of the major production harvests, i.e. the 1st and 2nd mowing. Their share in the annual total is 15% at the maximum, and in relation to the long-term average in the same season it is lower by 1/3 (Table 5).

From the point of view of temperature, average temperature in 2011 was 7.4 °C, which is above the long-term average (6.9 °C).

Weight of Plants in Dry State

Highest weight of dry matter is in the hybrid Festulolium Fojtan (average 132.6 g.pc^{-1}). A statistically significant difference between two monitored species was the 4th and 5th mowing in reduced precipitation mode (Table 1).

The effect of reduced precipitation is manifested in a relatively lower weight plant. However, the differences are not conclusive in any year. Despite the overall lower fodder production, utilization of multiple mowing may be related to better adaptation

to an uneven course of precipitation during the growing season. Between two precipitation modes, there is significant difference at *Festuca arundinacea* in 3rd mowing and Festulolium in 2nd and 4th mowing (Table 3).

Table 1 Weight of dry matter, difference between two monitored species (g/1 piece)

Species		1st cut	2nd cut	3rd cut	4th cut	5th cut	Average
Festuca arundinacea	R	32.72 a	21.58 a	8.32 a	15.26 b	26.64 b	122.46 b
Festulolium	R	25.48 a	13.82 a	6.2 a	33.2 a	16.7 a	77.46 a
Festuca arundinacea	N	33.26 a	26.7 a	13.54 a	28.44 a	23.42 a	131.54 a
Festulolium	N	35.82 a	28.08 a	13.78 a	31.48 a	29.6 a	132.58 a

N - Normal precipitation mode, R - Reduced precipitation mode

Table 2 Number of stalk (a leaf) pseudostems, difference between two monitored species (pc/1 piece)

Species		1st cut	2nd cut	3rd cut	4th cut	5th cut	Average
Festuca arundinacea	R	91.93 a	58.53 a	38.00 a	107.07 b	68.73 a	72.85 b
Festulolium	R	74.93 a	38.13 a	25.00 a	57.67 a	47.80 a	48.71 a
Festuca arundinacea	N	77.8 a	59.73 a	44.13 a	96.86 a	88.4 a	73.39 a
Festulolium	N	97.40 a	63.47	39.67 a	82.20 a	82.27 a	73.00 a

Table 3 Weight of dry matter, difference between two blocks precipitation mode (g/1 piece)

Species		1st cut	2nd cut	3rd cut	4th cut	5th cut	Average
Festuca arundinacea	N	33.3a	26.7a	13.5b	28.4a	29.6a	131.5a
	R	32.7a	21.6a	8.3a	33.2a	26.6a	122.5a
Festulolium	N	35.8a	28.1b	13.8a	31.5b	23.4a	132.6b
	R	25.5a	13.8a	6.2a	15.3a	16.7a	77.5a

Table 4 Number of stalk (a leaf) pseudostems, difference between two blocks precipitation mode (pc/1 piece)

Species		1st cut	2nd cut	3rd cut	4th cut	5th cut	Average
Festuca arundinacea	N	77.80 a	59.73 a	44.13 a	96.87 a	88.40 a	73.39 a
	R	91.93 a	58.53 a	38.00 a	107.07 a	68.73 a	72.85 a
Festulolium	N	97.40 a	63.47 b	39.67 a	82.20 a	82.27 a	73.00 a
	R	74.93 a	38.13 a	25.00 a	57.67 a	47.80 a	48.71 a

Table 5 Precipitation in year 2011 (distribution in each mowing)

	Spring 1.1.-1.3.	1st mowing	2nd mowing	3rd mowing	4th mowing	5th mowing	Autumn	Total
mm	78.8	47.5	49.5	71.9	190.7	107.4	86.8	623.8
%	12.4	7.5	7.8	11.4	30.1	17	13.7	100

Explanation of the decline in production in the situation of reduced rainfall can be related to the species differences in evapotranspiration of grasses [10] and also to a slowdown in growth of the root system and the associated decrease in production [11].

Number of stalk (a leaf) pseudostems

A statistically significant difference in the number of pseudostems between the monitored species was the 4th mowing in reduced precipitation mode. *Festuca arundinacea* had more pseudostems compared to Festulolium (57.67 pc). At normal precipitation mode there has not been detected significant difference in the number of pseudostems between a species (Table 2).

The effect of drought on number of pseudostems is not conclusive. Only the second mowing at Festulolium is significant difference, between two precipitation mode (Table 4).

The results further confirm a lower fodder production and a higher formation of offsets in all species at the 5-fold mowing utilization of covers. Generally, this fact is expressed in connection with the lower weight and massiveness of the root system. The proof of the results is reported in high-mowed, i.e. less often mowed, and in short-mowed, i.e. very often mowed lawn species [12].

Conclusion

The effect of reduced precipitation is manifested in a relatively lower weight plant. However, the differences are not conclusive in any year. Despite the overall lower fodder production, utilization of multiple mowing may be related to better adaptation to an uneven course of precipitation during the growing season.

The effect of drought on number of pseudostems was not detected statistically significant differences. As it is evident from the results of connection to findings exactly the grazing type variety could be the reason of overall lower production of fodder in grazing utilization on the one hand, but on the other hand, also of a more favorable yield response to drought than in varieties of the meadow character.

The effect of drought on decrease in production and number of pseudostems has not been wholly proved.

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The influence of nitrogen stabilized fertilizers on yield forage of semi-natural grasslands

LUCIE MARSALKOVA, PAVEL RYANT
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

lucie.marsalkova@mendelu.cz

Abstract: The paper deals with the influence of type of nitrogen fertilizers on the yield of dry forage of semi-natural grasslands. The prerequisite is to determine the effect of stabilized fertilizers on semi-natural grasslands because their influence was monitored just on field crops. Particular variants of fertilization were: not fertilized, urea (100 kg.ha^{-1}), ALZON 46 (100 kg.ha^{-1}), UREA stabil (100 kg.ha^{-1}). The growth is used as double-cutting. Experimental habitat is located at Pardubice region. The results show that the effect of classic or stabilized nitrogen fertilizers on yield of dry forage of the primary production was not statistically significant.

Key-Words: grassland, stabilized fertilizers, yield, inhibitor of urease, inhibitor of nitrification

Introduction

Semi-natural grasslands in Central Europe are threatened by intensive fertilization; forestation or leaving the habitat because of changes in agricultural practices [1]. The unharvest areas of semi-natural grasslands leads to loss of species composition and degradation of growth [2]. Their species diversity cannot be maintained without continuous care, namely: regular cutting or grazing and fertilization. In many cases is fertilizing used to increase the quality of grassland which leads on the contrary to reducing species diversity [3].

Nitrogen is the main nutrient affecting the yield [4]. It supports the formation of new offshoots of grasses and elongation of stalks [5]. Without sufficient nitrogen nutrition leaves are yellowing [6]. Semi-natural grasslands are able to take advantage 200 kg.ha^{-1} of nitrogen, depending on sufficient of soil moisture. With other increasing of nitrogen fertilization the yield is not accrue any. With increasing doses of nitrogen is growing yield and quality of dry forage, but species composition of semi-natural grasslands is declining. Increase of yield forage is mainly cause by grass species. More than half of total is harvested during the first cut. In the case of higher doses of nitrogen fertilization should be doses divided. One part should be applicated at the spring and others after first (second) cut [5]. The protection of groundwater is one of the reasons for the application divided doses of fertilizers. Exists condition to protect drinking water quality in the so-called "vulnerable areas", where the content of NO_3 in groundwater should not exceed 50 mg.l^{-1} (Nitrate Directive). This condition can be reached by divided applications of doses of nitrogen fertilizer. It should be used lightly and slowly soluble forms of nitrogen with respect to habitat conditions, vegetation type, intensity of use and by inflow of nutrients from other sources (symbiosis with nodule bacteria, mycorrhiza, atmospheric precipitation, etc.); [4].

For this reason is possible consider using stabilized fertilizers on semi-natural grasslands. The stabilized fertilizers are applied in one dose, to reduce number of crossings and diesel consumption. Another advantage is the slow release of nitrogen and therefore is nitrogen making available plants continually. The higher dose should not be subject to volatilization or be washed up to outside of the root system of plants. The disadvantage is their higher price compared to classic fertilizers [7].

The aim is to evaluate the effect of nitrogen fertilization on the primary production of dry forage of semi-natural grasslands.

Material and Methods

Characterization of habitat, solving of experiment

It's a field study. The experimental habitat is located at Pardubice region, near village Kameničky. Monitored growth is at an altitude of 650 m above

sea level, orientation to the southwest and an inclination 3 °. Data in climate diagram (see Fig. 1) are from weather station in Svratouch, 7 km far away from experiment in Kameničky. The average annual air temperature is 5.8 °C; average annual rainfall is 758.4 mm for the period 1961 - 2000. The second climate diagram (see Fig 2.) is for experimental year 2014 from Svratouch too.

Fig. 1 The course of the average monthly air temperature and monthly precipitation for the period 1961 - 2000

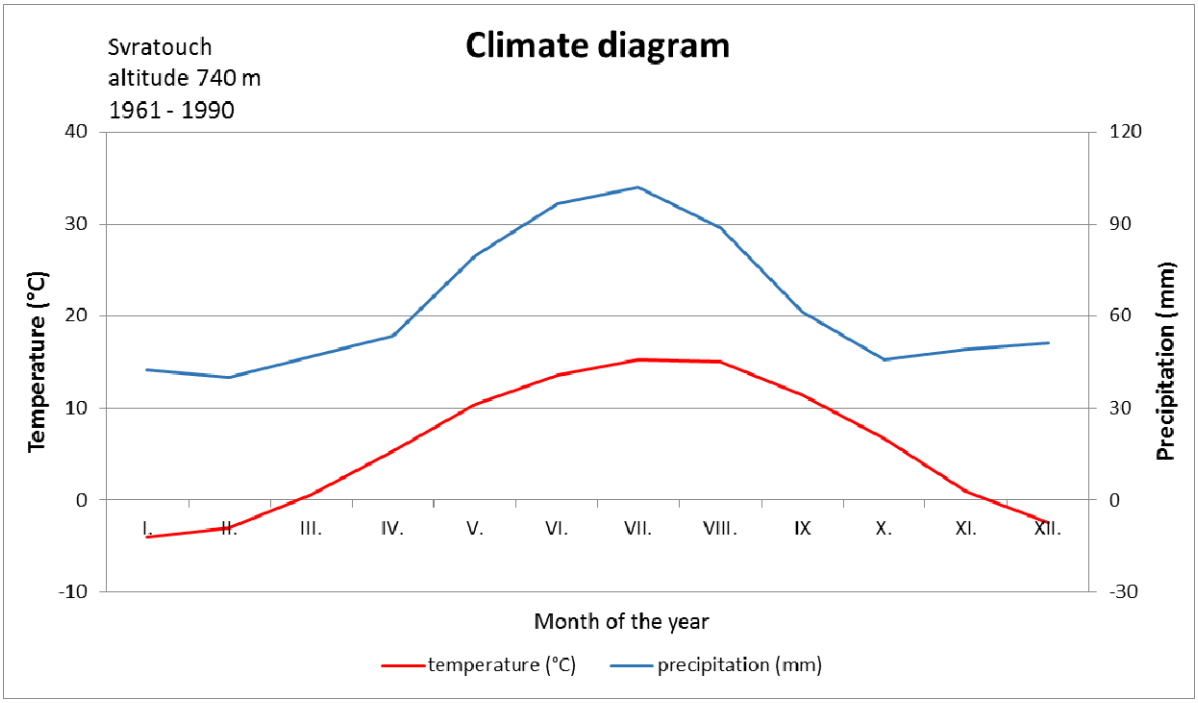
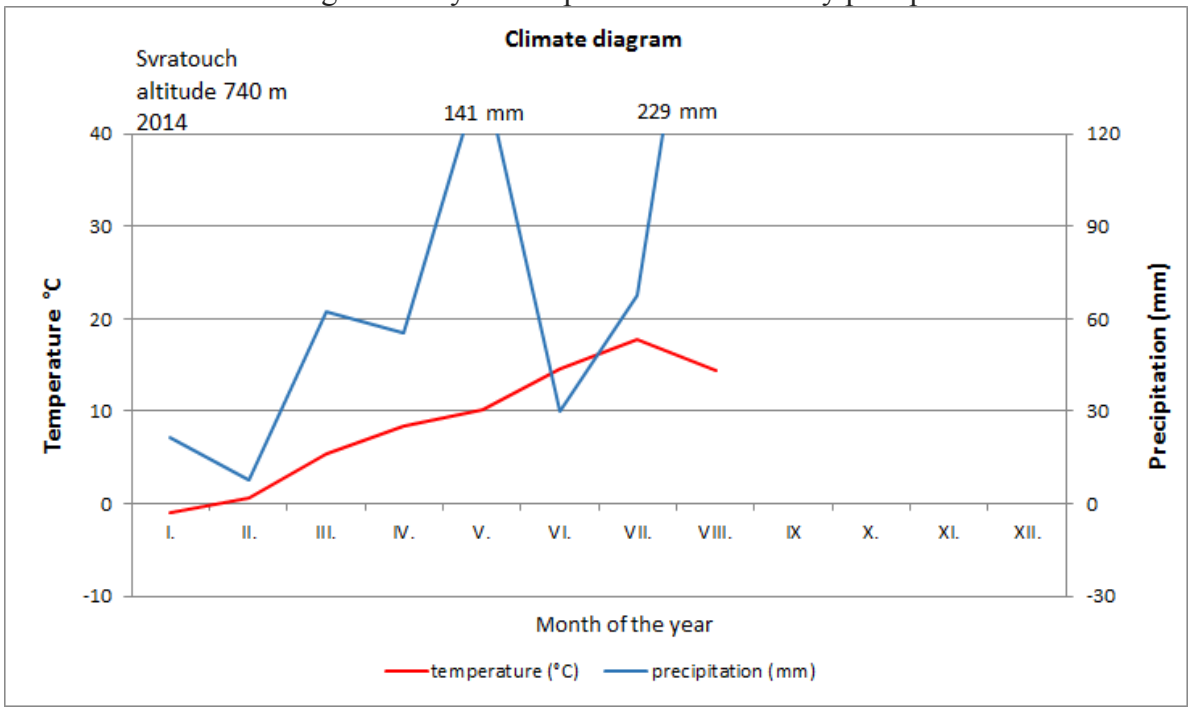


Fig. 2 The course of the average monthly air temperature and monthly precipitation in 2014



The current area of semi-natural grasslands was divided into plots with an area of 15 m^2 (1.5 * 10 m). They were separated according to different types of fertilizers: urea, urea with urease inhibitor

(UREA stabil) and urea with nitrification inhibitor (ALZON 46); (always with eight replications). They were applied before the first cutting in one dose of 100 kg.ha^{-1}. The fertilization was done by mineral fertilizers at spring 2014 (the 8th of April). Also there was not fertilized plots for compare.

Monitored growth was harvested as double-cutting. The first cut was done in June. For cut was used mower MF-70 with width of cutter bar 1.2 m and height of stubble 0.07 m.

Description of fertilizers

Mineral fertilizer urea is the most widely used nitrogen fertilizer. It is thanks to its high nitrogen content (46%) and relatively low production costs. If is not urea insert to the soil mechanically or by rain, shall be subject to high losses by nitrogen volatilization [8]. Urea is the soil surface hydrolytic degradation by the enzyme urease to ammonium carbonate, which is then converted to carbon dioxide and ammonia [9].

Fertilizer UREA stabil works on a temporary blockage of urease inhibitor. A thanks to this accelerates the penetration of nitrogen in the root zone, and it ensures its better availability of nitrogen to the plants. This procedure eliminates the slow effect of urea and is secured faster effect of applied nitrogen. Urease inhibitor eliminates losses of nitrogen by ammonia to the atmosphere.

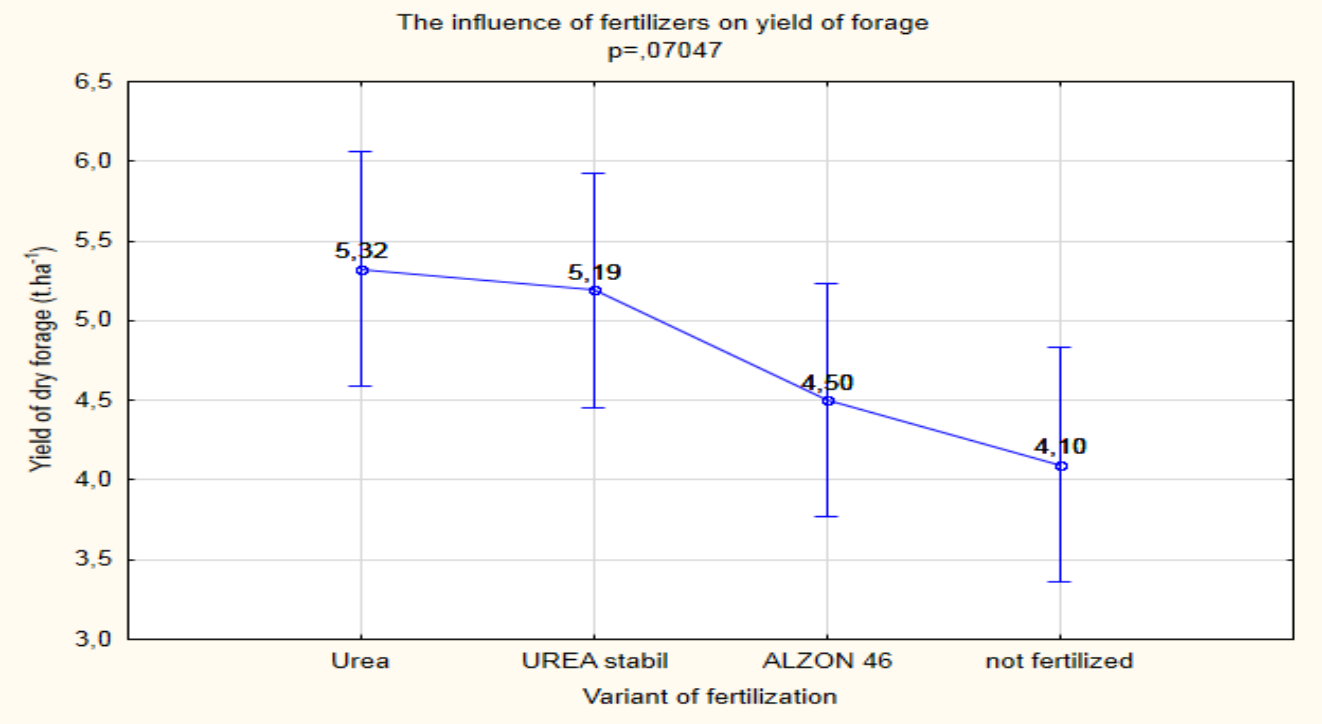
Nitrification inhibitor in fertilizer ALZON 46 slows the conversion of stable ammonium nitrogen on the moving form of nitrate nitrogen, thus allowing better utilization of delivered nutrients and reduces doses of nitrogen by 20%, while maintaining stable yields [7].

The experiment was focus on the primary production. To evaluate the effect of fertilization on the yield was used statistical program Statistica.

Results and Discussion

According to Watson [10] is interested in stabilized nitrogen fertilizers, because of their potential to reduce nitrogen losses to the environment and increase crop yields. With time after application of nitrification inhibitors to the soil, they are degraded. Their influence on yields can be quite variable depending on the crop, soil properties, climatic factors and type of management. Exactly their rate of degradation increases with increasing soil temperature, pH, moisture content and soil organic matter. With temperature below five degrees nitrification inhibitors can be effective up to six months. In case at temperature up to twenty five degrees the inhibitory effect only lasts for a few weeks. With increasing soil moisture nitrification inhibitors are generally less effective. They become unstable and hydrolyse.

Fig. 3 The influence of type of nitrogen fertilizers on the yield of dry forage



The temperature in April and May 2014 was normal, but the precipitation was very unbalanced. After fertilization in April started raining and precipitation in May were more than 140 mm. So not just urea, but the stabilized fertilizers too, could be dissolved earlier than the growth took the nutrients. It could be one of the reasons, why the effect of fertilization on yield of dry forage wasn´t statistically significant (see Fig. 3). Despite the fact that yield of dry forage increase with the doses of nutrients. The highest yield had plots with a one-shot dose (100 $kg.ha^{-1}$) of Urea (5.32 $t.ha^{-1}$). The lowest yield was at: not fertilized plots (4.10 $t.ha^{-1}$). UREA stabil had a higher yield than ALZON 46.

Henning et al. [11], examined the use of stabilized fertilizer on grass species: *Poa pratensis* L. and *Lolium perenne* L. Was concluded that urease inhibitor and nitrification inhibitor in comparison with urea don´t change production, color or quality of the grass species. Even though the efficacy of inhibitors was demonstrated in laboratory and for a number of crops. Likewise other research [12] show that influence of nitrification and urease inhibitors on crop yield is variable, depending on environmental conditions and management of care. Urease inhibitor in alkaline soils showed the highest results. However, the experiment in Kameničky soil has got pH 4.5.

Conclusion

The results don´t support theory about the stabilized fertilizers that slow release of nitrogen making it available for plants continually, because of it increase crop yields. The effect of fertilization on yield of dry forage wasn´t statistically significant. Main reason could be unbalanced precipitation in May (more than 140 mm). Stabilized fertilizers could be dissolved earlier than the growth took the nutrients. Generally are stabilized fertilizers less effective with increasing soil moisture. Urea (100 $kg.ha^{-1}$) had the highest yield (5.32 $t.ha^{-1}$). Not fertilized plots had the lowest yield (4.10 $t.ha^{-1}$).

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Structure of weed species on railway

STANISLAVA NAVRATILOVA, JAN WINKLER
Department of Plant Biology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xnavrat5@node.mendelu.cz

Abstract: The aim of this thesis is to evaluate species structure of weeds on chosen part of railway between Brno-main station and Řečkovice station. Observation was made on twenty localities also on railway embankment and between rails. Vegetation was evaluated by phytocenological relevé during two months (July-August) in the year 2011. Observation was statistically evaluated by DCA and CCA analysis. 41 species of plants was found on the railway. The most cover had species like *Solidago canadensis*, *Urtica dioica*, *Lolium perenne*. *Lolium perenne*, *Aegopodium podagraria*, *Artemisia vulgaris*, *Stellaria media, Achillea millefolium* were the most common species between rails. Species *Urtica dioica*, *Solidago Canadensis, Helianthus tuberosus*, *Fragaria vesca*, *Hypericum perforatum* or *Plantago lanceolata* were the most occurred species on the railway embankment.

Key-Words: weeds, coverage, railway

Introduction

Development of human society requires a significant exchange of material which tend to leave a negative traces in the regions. One of the media which mediates these traces is a transportation. The need of transportation and subsequent road expansion in todays global environment has resulted some changes in landscape and quality of cultural and natural environment [1].

Czech Republic is a landlocked country, that is why the import of raw agricultural materials is high. This goes hand by hand with import foreign expansive weed species mainly via rail transport. The rails in most railways, especially freight railways stations have the largest presence of foreign expansive weed species. These foreign expansive and aggressive weeds species could in the future significantly reduce the fertility of agricultural soils in some areas [2].

Roads and rivers can similarly as railways serve as corridors for spreading of weed species. The concept of corridor then implies that the organisms and plants can move and/or be moved along the longitudinal elements in the landscape [3].

There are three main ways how are these foreign plants brought to the Czech Republic. The most of the plants are brought from North America.

From the south-east are brought plants via road and lastly the eastern route brings weed species commonly known in Mediterranean region. Such weeds are mainly brought with the transportation of cereal [4].

One of the most important reasons for removing the weeds from railway embankments is to preserve the quality and safety of the railways, to ensure a safe movement of staff, who are responsible for track maintenance. The risk of tripping or slipping on weedy track is high, therefore the prevention is very important [5].

If we decide to use herbicides on the railway embankments, it is important to fully understand their behavior and possible impact on the station. Well executed use of herbicides implies the limitation of the weed species as well as the absence of the side effects (e.g. injuries of employees, damage of equipment or environmental damage) [5].

It should be noted that weed species can be found not only in an arable land but also on non-agricultural lands like paths along the roads or railway lines. This paper will therefore address the issue of generic representation of weed species along the railways.

Material and Methods

Characteristic of Experimental Location

The phytocoenological evaluation took place in the area situated between the railways stops Brno, Česká and Řečkovice and follows the same path as previous old monorail connecting Brno and Tišnov called "Old Tišnovka". The train track was officially

opened on July 2, 1885 and extended from Tišnov to Zdar and later to Česká twenty years later. The new railway track was launched in the years 1953 to 1957 and became fully electrified in the years 1964 to 1966.

Řečkovice (GPS 49.250730, 16.589991) is located on north from the Brno city. The altitude varies between 226 to 398 meters a.s.l. While the southern part of the district has more urban character, the eastern part is covered by an extensive forest. Village Česká (GPS 49.284692, 16.566626) is situated in the district Brno-countryside with the altitude of 295 m above the sea level. This area has the highest proportion of black soils, mainly on loess, and than brown soils. The subsoil consists of granodiorite, slope sediments (clay, stones) green shales, arkoses, aplite and pegmatite. Deep magnatiti, unpaved and paved sediments and metamorphites occur here. Meteorological data were used from the Czech Hydrometeorological station Brno - Tuřany. The average temperature and rainfall in the years 1961 to 1990 (per month) are listed in Table 1. The average temperature and precipitation for the months of the year 2011 are presented in Table 2.

Methodology of weed infestation evaluation and statistical processing

The composition of the weeds species on the railways has been observed in the abovementioned rail route Brno - Česká towards to Řečkovice. The route is 5 km long and for the most part is surrounded by forest. 20 habitats differently spaced along the track were selected to evaluate the weed species. Habitats were chosen randomly (eg. in the railway track, on the railway embankment or a few meters from the railway embankment). The weed species were evaluated on the basis of phytosociological relevé in the area of 20 square meters. Each relevé was evaluated separately based on the composition of the weed species as well as its cover which is shown in percentage. The evaluation was out in two periods of the year 2011 (June and August).

Czech and Latin terms of each weed species are used according to Kubat [6].

The obtained data were processed by multivariate analysis of ecological data. Selection of the optimal analysis followed the length of the gradient (*Lengths of Gradient*), which was detected by segment analysis DCA (*Detrended Correspondence Analysis*). A total number of 499 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program called Canoco 4.0 [7].

Table 1 Data of weather conditions from the meteorological station Brno – Tuřany, in period 1961-1990

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	average
The average temperature (°C)	-2.5	-0.3	3.8	9.0	13.9	17.0	18.5	18.1	14.3	9.1	3.5	-0.6	8.7
Precipitation (mm)	24.6	23.8	24.1	31.5	61.0	72.2	63.7	56.2	37.6	30.7	37.4	27.1	490.1

Table 2 Data of weather conditions from the meteorological station Brno – Tuřany, based on data from 2011

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	average
The average temperature (°C)	-0.5	-0.8	5.7	12.5	15.1	19.2	18.9	20.5	17.2	9.5	2.9	2.1	10.2
Precipitation (mm)	17.6	3.0	46.4	24.5	44.2	61.6	92.6	35.6	30.6	18.6	0.0	18.0	392.7

Results and Discussion

41 plant species were observed in total, the highest cover had species as *Solidago canadensis, Urtica dioica* and *Lolium perenne.* For more details reffer tto Table 3.

Statistical evaluation

The obtained data with respect to the frequency and the cover of individual plant species were firstly processed by DCA analysis which calculated the Lengths of Gradient at 4.106, followed by a Canonical Correspondence Analysis (CCA). CCA analysis defines the spatial arrangement of an

individual species and selected monitoring sites. This is then expressed graphically using the ordination diagram. Weed species and different habitats are demonstrated by different shapes and colors. The results of CCA analysis indicate that the influence of the plant frequency and species cover is quiet compelling and significant at the significant level α = 0.050 for all canonical axes. According to the ordination diagram (Fig. 1) plant species can be divided into several groups:

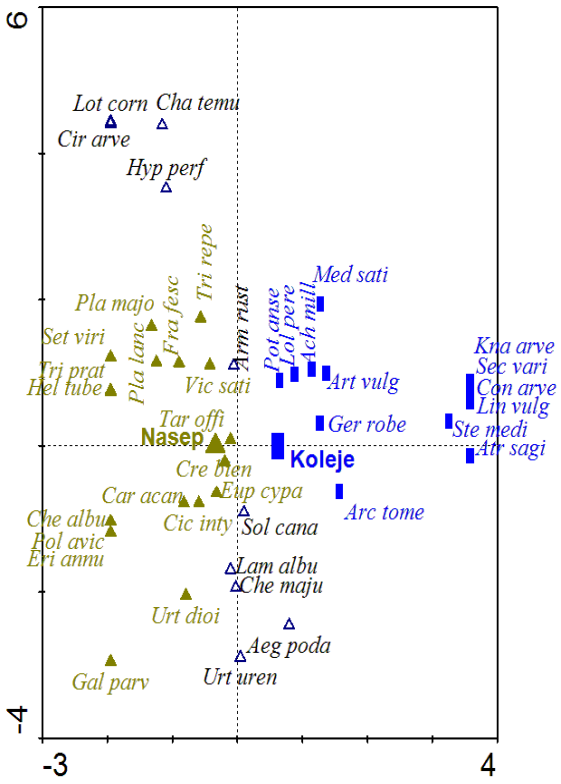
Table 3 The sum of weed species vegetation cover found in the selected section of the railway

Weed species	Stands	
	"Násep"	"Koleje"
Aegopodium podagraria	135	133
Achillea millefolium	67	85
Arctium tomentosum	20	35
Armoratia rusticana	50	26
Artemisia vulgaris	83	124
Atriplex sagittata	0	5
Carduus acanthoides	47	12
Cichorium intybus	40	13
Cirsium arvense	77	0
Convolvulus arvensis	0	3
Crepis biennis	28	13
Erigeron annuus	7	0
Euphorbia cyparissias	48	20
Fragaria fesca	145	34
Galinsoga parviflora	3	0
Geranium robertianum	41	57
Helianthus tuberosus	160	0
Hypericum perforatum	110	20
Chaerophyllum temulentum	30	5
Chelidonium majus	58	31
Chenopodium album	25	0
Knautia arvensis	0	2
Lamium album	50	25
Linaria vulgaris	0	4
Lolium perenne	245	256
Lotus corniculatus	8	0
Medicago sativa	5	7
Plantago lanceolata	89	13
Plantago major	78	10
Polygonum aviculare	5	0
Potentilla anserina	60	53
Securigera varia	0	5
Setaria viridis	10	0
Solidago canadensis	374	220
Stellaria media	6	96
Taraxacum officinale	54	27
Trifolium pratense	40	0
Trifolium repens	84	28
Urtica dioica	408	108
Urtica urens	55	31
Vicia sativa	42	16

Statistical evaluation

The obtained data with respect to the frequency and the cover of individual plant species were firstly processed by DCA analysis which calculated the Lengths of Gradient at 4.106, followed by a Canonical Correspondence Analysis (CCA). CCA analysis defines the spatial arrangement of an individual species and selected monitoring sites. This is then expressed graphically using the ordination diagram. Weed species and different habitats are demonstrated by different shapes and colors. The results of CCA analysis indicate that the influence of the plant frequency and species cover is quiet compelling and significant at the significant level $\alpha = 0.050$ for all canonical axes. According to the ordination diagram (Fig. 1) plant species can be divided into several groups:

Fig. 1 Ordination diagram expressing the weed species composition in both monitoring terms



Legend: railway embankment - a group of phytosociological relevé in the area called "nasep"; train tracks - a group of phytosociological relevé in the area called "Koleje"

Species: *Aeg poda - Aegopodium podagraria, Ach mill - Achillea millefolium, Arct tome – Arctium tomentosum, Arm rusti – Armoratia rusticana, Art vulg - Artemisia vulgaris, Car Acan – Carduus acanthoides, , Cic inty – Cichorium intybus, Cir arve – Cirsium arvense, Con arve – Convolvulus arvensis, Cre bien – Crepis biennis, Eri annu – Erigeron annus, Eup cypa – Euphorbia cyparissias, Fra vesc – Fragaria vesca, Gal parv – Galinsoga parviflora, Ger robe – Geranium robertianum, Hel tube – Helianthus tuberosus, Hyp perf – Hypericum perforatum, Cha temu – Chaerophyllum temulentum, Che albu – Chenopodium album, Che maju – Chelidonium majus, Kna arve – Knautia arvensis, Lam albu – Lamium album, Lin vulg – Linaria vulgarit, Lol pere – Lolium perenne, Lot corn – Lotus corniculatus, Med sati – Medicago sativa, Pla majo – Plantago major, Pol avic – Polygonum aviculare, Pot anse – Potentilla anserina, Sec vari – Securigera varia, Set viri – Setaria viridis, Sol cana – Solidago canadensis, Ste medi – Stellaria media, Tar offi – Taraxacum officinale, Tri prat – Trifolium pretense, Tri repe – Triforium repens, Urt dioi – Urtica dioica, Urt uren – Urtica urens, Vic sati – Vicia sativa.*

The first group of weed species is more often on group-habitat "Násep" and includes: *Aegopodium podagraria, Achillea millefolium, Arctium tomentosum, Armoratia rusticana, Artemisia vulgaris, Carduus acanthoides, Cichorium intybus, Cirsium arvense, Crepis biennis, Erigeron annus, Euphorbia cyparissias, Fragaria vesca, Galinsoga parviflora, Geranium robertianum, Helianthus tuberosus, Hypericum perforatum, Chaerophyllum temulentum, Chelidonium majus, Chenopodium album, Lamium album, Lolium perenne, Lotus corniculatus, Medicago sativa, Plantago major, Polygonum aviculare, Potentilla anserina, Setaria viridis, Solidago canadensis, Stellaria media, Taraxacum officinale, Trifolium pretense, Triforium repens, Urtica dioica, Urtica urenc* and *Vicia sativa.*

On the second group-habitat "Koleje" occurred: *Aegopodium podagraria, Achillea millefolium, Arctium tomentosum, Armoratia rusticana, Artemisia vulgaris, Atriplex sagittata, Carduus acanthoides, Cichorium intybus, Convolvulus arvensis, Crepis biennis, Euphorbia cyparissias, Fragaria vesca, Geranium robertianum, Hypericum perforatum, Chaerophyllum temulentum, Chelidonium majus, Knautia arvensis, Lamium album, Linaria vulgaris, Lolium perenne, Medicago sativa, Plantago major, Potentilla anserina, Securigera varia, Solidago canadensis, Stellaria media, Taraxacum officinale, Triforium repens, Urtica dioica, Urtica urens* and *Vicia sativa*.

The third group consists of weed species occurring in both group-habitat: *Aegopodium podagraria, Armoratia rusticana, Cichorium intybus, Hypericum perforatum, Chaerophyllum temulentum, Chelidonium majus, Lamium album,*

Lotus corniculatus, Solidago canadensis and *Urtica urens.*

According to Torstensson [5] a deep-rooted plants are one of the main reasons for removing the weeds from the railway embankments. Due to significant risk of the weed blocking the hollow spaces of the railway embankment and subsequent raise of water which could freeze in the winter and cause the damages. The risk of tripping or slipping on weedy track is high, therefore the prevention is very important. Additionally, the overgrown track can also be dangerous for the train itself and cause skidding or extend the distance in which the train is capable to stop. Risk of fire due to flying sparks from the trains' wheels surrounded by extremely dry vegetation increased during dry summer.

Conclusion

Number of different weed species were found during the observation of the monitored area on the railway tracks between stops Brno - Řečkovice and Česká.

Cover and occurrence of the weed species differs by localities. The most often weed species in terms of repetition and vegetation cover were: *Solidago canadensis, Urtica dioica, Lolium perenne, Aegopodium podagraria* or *Artemisia vulgaris.* The most occurring weed species on the railway embankment except the already mentioned were: *Helianthus tuberosus, Fragaria vesca, Hypericum perforatum, Plantago lanceolata* and *Cirsium arvense*. On the railway tracks then were for example: *Stelaria media, Achillea millefolium, Geranium robertianum* or *Arctium tmentosum*.

We can represent that the railway tracks have a diverse variety of the weed species which might have negative impact on the safety of the railway line. Weed species that can easily spread and have well-developed root system can be the most dangerous, namely: *Solidago canadensis, Urtica dioica, Artemisia vulgaris, Helianthus tuberosus* and *Cirsium arvense.*

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The effect of fertilization and weather conditions on the yield of forage and species diversity

ADAM NAWRATH[1], SIMON CHMELAR[2], JIRI SKLADANKA[1], ANTONIN KINTL[2]
[1]Department of Animal Nutrition and Forage Production
[2]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xnawrath@node.mendelu.cz

Abstract: The importance of grassland consists not only in forage production but also in non-production functions. Grasslads are particulary important due to high level of biodiversity. For farmers are species rich grasslands less productive and less quality. Nevertheless, these grasslands provide more stable yields and the forage is tastier for livestock. Both agronomists and environmentalists have tackled question whether the productivity of plant communities really depends on the species diversity. The aim of this study was to compare the effect of fertilization and weather conditions on the species diversity and yield of forage. Another goal was to explore if there is a relationship between yield of forage and species diversity. The yields of forage were growing with the increasing intensity of fertilization ($P<0.05$). On the other hand diversity index was statistically significant lower ($P<0.05$) in the variants with added of nitrogen in comparison with PK variant. The results showed that there existed negative relationship between yield of forage and diversity index.

Key-Words: nutrients, Shannon's diversity index, species richness, biomass

Introduction

The origin of grassland in the Czech territory is associated with the production function [1]. Their importance as a source of feed grow with an increasing altitude [2]. The production function in grassland is still crucial, but due to the decline of cattle in the past twenty years [3] are seeking new uses in the form of non-production functions such as soil protection, conservation of water quality, aesthetic and recreational functions [4, 5]. These functions are mainly associated with grassland biodiversity. Meadows and pastures represent a significant source of biodiversity and can host up to two thirds of all species of regional flora [6].

For farmers are species rich grasslands less productive and less quality. Nevertheless, these grasslands provide more stable yields and the forage is tastier for livestock. Both agronomists and environmentalists have tackled question whether the productivity of plant communities really depends on the species diversity [7].

The aim of this study was to compare the effect of fertilization and weather conditions on the species diversity and yield of forage. Another goal was to explore if there is a relationship between yield of forage and species diversity.

Material and Methods

The experimental plot was established in the cadastre of the village Kameničky belonging in the Protected Landscape Area of Žďárské vrchy Hills. Experimental works were launched there in 1992. The presented results include the years from 2009-2013. The site has a SW aspect and is situated on a slope with the gradient of 3°. Mean annual temperature (1951-2000) is 5.8 °C and mean annual precipitation amount is 758.4mm. Soil type is acidic Luvic Stagnosol on the gneiss diluvium. The experiment was designed by using the method of split compartments in four repetitions. The evaluated factor was fertilization (no fertilization, PK fertilization, 90N+PK fertilization and 180N+PK fertilization). The stands were exploited in the system of three cuts. Nitrogen was supplied in the form of ammonium nitrate with limestone (LAV 27%) at a total dose of 90kg.ha^{-1} N resp. 180kg.ha^{-1} N. In the mode of three cuts, the nitrogen dose was applied in three terms (1/3 in spring, 1/3 after 1st cut and 1/3 after 2nd cut). Potassic and phosphoric fertilizers were applied in spring. Phosphorus was applied in the form of superphosphate (26%) at 30 kg.ha^{-1} P and potassium was applied in the form of potassium salt (60%) at 60 kg.ha^{-1} K. The stands were harvested in three terms (early June, early

August and early October). The grass was harvested by the mower Model MF-70 equipped with a cutting bar (engagement 1.2m).

In order to establish the share of individual species in the harvested forage, samples were taken of above-ground biomass from permanently staked plots (0.25m^2). The samples of the above-ground forage biomass were sorted out into individual species and dried at 60 °C. Subsequently, their dry weight was established and the proportions of individual species were expressed as percentages from the total weight of dry forage.

Total precipitation amounts and average temperatures in individual months were obtained from the CHMI meteorological station in Svratouch.

Shannon's diversity index (H) was calculated according to the below formula [17]:

$$H = -\sum_{i=1}^{s} Pi \, (\ln Pi)$$

where H is diversity index and P_i is the share of the i^{th} species in the stand

The determination of the production efficiency of 1 kg supplied nutrients (DPE) in kg of dry matter.1kg nutrients^{-1} was calculated according to the following formula:

$$DPE = \frac{\text{yield of forage no fertilized stand} - \text{yield of forage fertilized stand}}{\text{the amount of added nutrients}}$$

Statistical evaluation was conducted with using Statistica 10 programme by multi-factorial analysis of variance (ANOVA) with Post-Hoc Fischer LSD test. The evaluation of relationship between yield of forage and diversity index was performed with using the correlation analysis.

Results and Discussion

The highest yield of forage was reached in the 180N+PK variant, namely 7.6 t.ha^{-1} while in the unfertilized variant was the average yield of forage lowest, only 3.6 t.ha^{-1} (Table 1). The highest increase in production was recorded by PK fertilizing. This is evidenced by the highest efficiency of nutrient utilization on this variant – there was growth of 27.8 kg.ha^{-1} biomass after supplying 1 kg.ha^{-1} of nutrients. Also REGAL and VESELÁ [8] reached similar values. When dosage of supplied nutrients was increasing, efficiency of nutrient utilization was decreasing to 16.1 kg.ha^{-1} in the variant fertilized with 90N+PK and to 14.8 kg.ha^{-1} in the 180N+PK variant. The fertilizing had significantly positive effect on production of dry matter ($P<0.05$). This conclusion is also consistent with findings of other authors [9, 10, 11]. The yields were affected also by year, namely annual precipitation amounts and average annual temperature, though statistically insignificantly (Table 2). Also HREVUŠOVÁ [12] recorded changes in production due to year. The highest production of dry matter was recorded in 2011, namely 7 t.ha^{-1}. In this year was recorded the highest average annual temperature for the entire observed period (Figure 1). On the other hand the precipitation amounts of this year were below average of long-term precipitation amounts in period 1981-2010 (Figure 2). The lowest dry matter yield was recorded in 2010 (5 t.ha^{-1}). This year, by contrast, was characterized by lower average annual temperature and higher annual precipitation amounts than the long-term averages. DAŇHELKA and HONSOVÁ [13] reported that yields on the site of mesohygrophytic character are more affected by air temperature than total precipitation.

The effect of fertilization was also observed in values of Shannon's diversity index. The highest value of diversity index H=2.4 was recorded in the variant with PK. Conversely both variants with nitrogen showed the lowest value H=2 (Table 1). The difference between these variants and the PK variant was also statistically significant ($P<0.05$). This statement is also supported with results of other authors [14, 15]. The PK variant showed also the most species, namely 23. Significantly ($P<0.05$) the least species, in comparison with PK variant, was found in the 180N+PK variant, namely 19. HOLÚBEK [16] adds that when the doses of fertilizers are higher, the changes in species composition are more pronounced. Although there were average 20 species at 90N+PK variant, which is the same as at the not fertilized variant, there was observed a lower value of diversity index. The explanation for this seems to be the fact that diversity index depends not only on the species richness but also on an equability of the community. At the variants supplied with nitrogen are more supported nitrophilous species that increasingly dominate and equability of this community is declining. According to BEGON et al. [17] the unbalanced species richer community may have even lower diversity index than species-poor community, but well balanced.

Table 1 The influence of fertilization on the diversity index, species richness and a yield of forage

Evaluated characteristic	No fertilization	PK	90N+PK	180N+PK
Shannon's diversity index (H)	2.2^{ab}	2.4^{a}	2^{b}	2^{b}
Number of species	20^{ab}	23^{a}	20^{ab}	19^{b}
Yield of forage t.ha^{-1}	3.6^{a}	6.1^{b}	6.5^{b}	7.6^{c}

Different letters in the rows indicate statistically significant differences at a level of P<0.05

Table 2 The influence of year on the diversity index, species richness and a yield of forage

Evaluated characteristic	2009	2010	2011	2012	2013	Average
Shannon's diversity index (H)	2	2.2	2.1	2.2	2.3	2.2
Number of species	20	19	19	21	22	20
Yield of forage t.ha^{-1}	6	5	7	6.3	5.6	5.9

Also the year had impact on the value of diversity index (Table 2). The highest average diversity index was recorded in 2013 (H=2.3). On the other hand in year 2009 was the diversity index lowest (H=2.0). When comparing values of diversity index with climatic conditions of the particular year (Figure 1, 2) it seems that diversity index is probably more affected by relationships in the community rather than by the weather conditions.

If we compare the averages of diversity index with averages of forage production (Table 2), we can repeatedly observe that in some years with higher diversity indexes were recorded lower dry matter yields. Although there was identified only weak indirect relationship (r = -0.2724). Also ROSE and LEUSCHNER [18] describe the negative impact of species diversity on yield of forage. Conversely, TANG et al. [19] states that biodiversity supports productivity of communities.

Fig. 1 The comparison of average annual temperature with long-term average

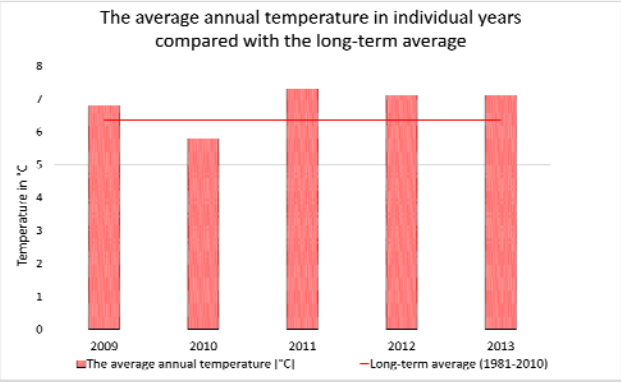
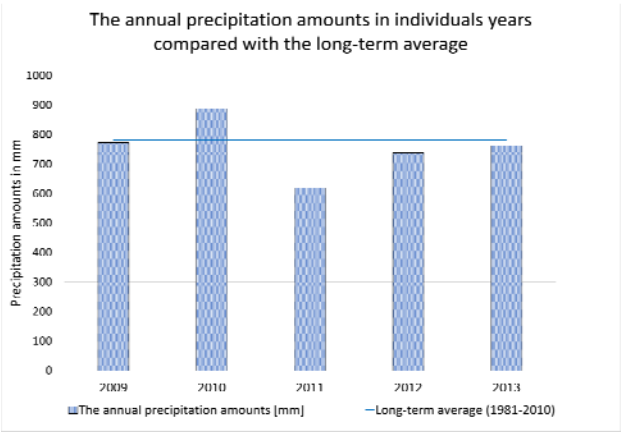


Fig. 2 The comparison of annual precipitation amounts with long-term average



Conclusion

Fertilizing has significant effect (P<0.05) on increase in the forage production. The highest efficiency of nutrient utilization was found at PK variant, namely 27.8 kg.ha^{-1} by dosage 1 kg of nutrients per 1 ha. The year had also influence – higher yields was repeatedly recorded in years with higher average annual temperature and lower annual precipitation amounts. From results it appears that for production is more important annual average temperature than the precipitation amounts, probably due to a sufficiently high level of groundwater throughout the whole year. Excessive rainfall totals can then have a negative impact on the production. The highest species diversity (H=2.4) was found in variants subsidized by PK. On the contrary, the variants subsidized by a nitrogen had the lowest diversity index (H=2). Moreover, this difference was statistically significant (P<0.05).

Acknowledgement
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Rating of soil heterogeneity using by satellite images

JAROSLAV NOVAK, VOJTECH LUKAS, JAN KREN
Department of Agrosystems and Bioclimatology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jaroslavxnovak@gmail.com

Abstract: Knowledge of the level of variability of site conditions is the basis for deciding on deployment of technologies locally targeted farming, known under the term precision agriculture. The aim of this paper is to compare two sets of remote sensing data, acquired between 2012 and 2013, for assessing the variability of arable land. Data are capturing the South Moravien Region with a total area of 1100 km^2 by Rapid Eye (2012) and Landsat 8 (2013) satellites. As the other input data, field boundaries from government database LPIS were used to identify the blocks of arable land. The first step was a selection of arable land through polygons from the LPIS and identification of bare soil by calculation of normalized differential vegetation index (NDVI) from spectral data. An image classification was performed on these grounds in order to create class of information describing the spectrum of surfaces forming the bare soils.
Comparison of both satellite datasets proved difference between the images. Landsat 8 data showed higher error, probably due to the lower spatial resolution of data. (30 m per pixel). In this case Rapid Eye imagery offers higher spatial resolution (5 m per pixel), which seems to be more suitable for identification of soil heterogeneity, especially in smaller fields.

Key-Words: remote sensing, Rapid Eye, Landsat 8, NDVI, soil heterogeneity, coefficient of variation

Introduction

Knowledge of the level of variability of site conditions is the basis for deciding on deployment of technologies locally targeted farming, known under the term precision agriculture. The goal of precision agriculture is to adapt the intensity of cultivation interventions to specific habitat conditions. Determine the heterogeneity of land is therefore first and necessary step. Remote sensing (RS), one of non-invasive mapping methods, is special, very powerful way to mapping soil variability performed by air planes or Satellite carriers of sensors. Its greatest advantage is the possibility of monitoring the spatial variability over time with high resolution and performance. Extensive areas can be mapped in a short time and with high complexity output. By RS can be indicated the soil properties that affect reflectivity, such as organic matter content, soil moisture, soil texture and presence of iron oxides[1]. All above is referred to the upper layer of soil surface[2].

For mapping are used the sensors with several levels of spatial or radiometric resolution. High-resolution (spatial) sensors (e.g., SPOT-HRV, Landsat 8 OLI) with resolutions of approximately 20–30 m can detect objects within the canopy level, which is fine enough to describe the landscape [3]. The using of different variables derived from remote sensing data (e.g., vegetation index, reflectivity of surface, *etc*.) regarding to spatial heterogeneity, however, need to be considered.

For the differentiation of bare arable land in the blocks was used NDVI index in this study. The Normalized Difference Vegetation Index (NDVI) [4] has been widely used for monitoring the biophysical condition and vegetation cover[5]. It is frequently used to describe surface spatial heterogeneity [6, 7]. The using of NDVI may not be always the best choice. The NDVI may be limited to capturing the landscape properties for the saturation of the red or near-infrared bands and the sensitivity to soil [3].

Assessment of soil variability is done by traditional statistical and geostatistical methods.

The aim of this work was to verify the procedure for determining the variability of soil blocks remote sensing data in the selected area of southern Moravia. For these purposes were selected two sets of satellite images – Rapid Eye offered commercially and freely available Landsat 8 LDCM.

Material and Methods

Introduction of used data sets

The RapidEye images are a proper source of information for soil mapping [8, 9]. The Rapid Eye data sets have a spatial resolution of 6.5 m in five spectral bands: Blue (440-510 nm), Green (520-590 nm), Red (630-685 nm), Red Edge (690-730 nm), and Near Infrared (760-850 nm).

Data were purchased as the Level 1B product, which needs to be geometrically processed. The orthorectification was processed by digital elevation model from ASTER satellite and subsequently new bitmap mosaics were created in the Arc GIS 10.1. Landsat 8 data were downloaded from the U.S. Geological Survey website as the free product geometric and radiometric corrected. It consists of eleven bands with a resolution of 15-100 m / pixel. Landsat 8 data set are mentioned for example by [10] in study of soil sealing. Four images (taken in March 2012, April 2012 and September 2012) were used as an input depicting the 1100 km^2 of South Moravian Region by the Rapid Eye satellite. Second part of imagery was completed by Landsat 8 images immediately after Landsat mission launching in April and September 2013. Both sets of data captured approximately the same area of the South Moravian region (Czech Republic). More than 90% of agricultural land in the region, where agricultural subsidies are provided, an arable land, which part is not in the above mentioned periods covered by vegetation. At this time there was an assumption of the largest area of bare soil before the start of the growing season (March and April) and after harvest (September). The rate of soil variability is expressed in this paper by the coefficient of variation (V_x).

The data processing

For selecting of bare soil was also used the data set from Land Parcel Information System (LPIS), managed by Ministry of Agriculture, in form of spatial and descriptive representation of blocks of arable land in SHP format.

For each slide NDVI index was calculated using a Raster calculator and set a limit for arable land based on the values of the index. Through LPIS blocks of bare arable land were selected. However, this method of data selection (combination of intervals of arable land and polygons with arable land) considerably reduced the area which truly represents the arable land. Therefore, the blocks of bare arable land with the number of pixels greater than or equal to 50 % of the original number of pixels of areas of bare soil selected by data from the system iLpis were added to classification. This condition was implemented because of the highest possible selection of the most representative data in the respective periods. After classifying the images were again analysed in an Arc GIS 10.1 to calculate the coefficient of variation. To determine the coefficient of variation and descriptive statistics a tool called Zonall statistic was used. Areas of bare soil were divided, based on the values of the variation coefficient, according to the following clue:

Table 1 The clue for the classification of land according to the coefficient of variation

The value of Cv	Soil variability
0 - 49%	slightly variable soil
50 - 100%	variable soil
More then100%	highly variable soil

This clue is based on the classification of the coefficient of variation based on the statistics.

Results and Discussion

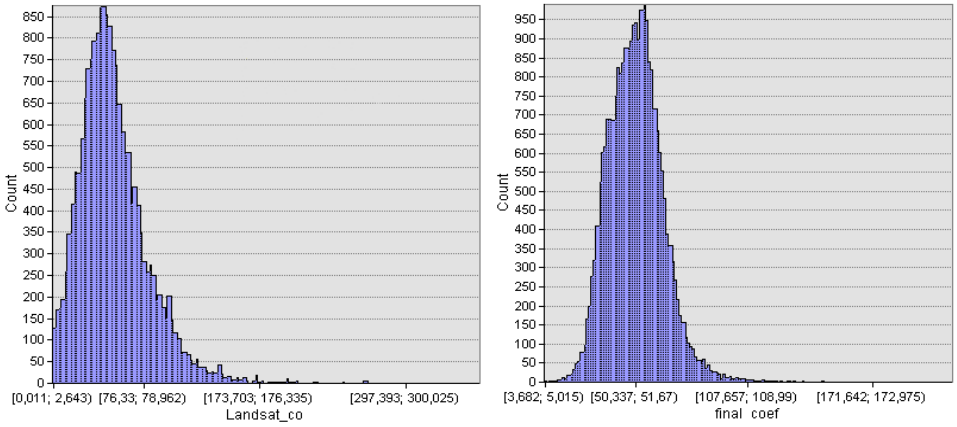
Due to the region of South Moravia, arable land predominates in all scenes and images and therefore provide the ideal material for the study of variability of bare arable land. The trend of reducing of arable land, which participated in the analysis, captures interesting facts. Data taken at the end of March make possible to appreciate the greatest amount of arable land and seem to be from all the scenes information-rich.

Fig. 1 Summary of arable land blocks and blocks satisfying the condition of coverage

Analyzed scenes	Total area of blocks in the scene (ha)	The total area of arable land		The total area of arable land meeting the conditions	
		In ha	% in total area of blocks	In ha	% in total area of blocks
March 25, 2012	209 226	193 445	92,46	129 466	61,88
April 27, 2012	222 529	204 092	91,71	73 913	33,22
Sept 9, 2012	209 100	193 024	92,31	113 221	54,15
Sept 11, 2012	176 805	155 425	87,91	93 360	52,80
April 15, 2013	347 368	314 011	90,39	122 811	32,47
Sept 6, 2013	347 368	314 001	90,39	166 040	47,79

The April term is the smallest of all the scenes of arable land satisfying the condition in both cases. April is thus informative poorest month. The Period at the end of April was not the best choice. The relatively low proportion of bare arable land, satisfying the condition, also shows data taken in September. In both cases, is bare cropland represented in more than half of the image. If we compare the information value of both satellites (the percentage of blocks satisfying the conditions) appear to be data from Landsat 8 overall information less convenient choice. The performance of the coefficient of variation for all four scenes is captured in the following figure:

Fig. 2 Histograms of V_x for each of data set



Both data sets have a sharper division. Median and average data of Rapid eye are virtually a viable. Skewness is close to zero here. Data have almost normal distribution, which spoils the only value of kurtosis. Values are relatively evenly distributed around the center. Data set of Landsat 8 are slightly left of the positively skewed with an excess of small values. Higher value of maximum is probably the result of errors and hangs lower resolution data. Also, a higher standard deviation indicates higher variability data from LANDSAT 8 exact opposite is then the Rapid Eye data.

Fig. 3 Levels of variability captured by Rapid Eye

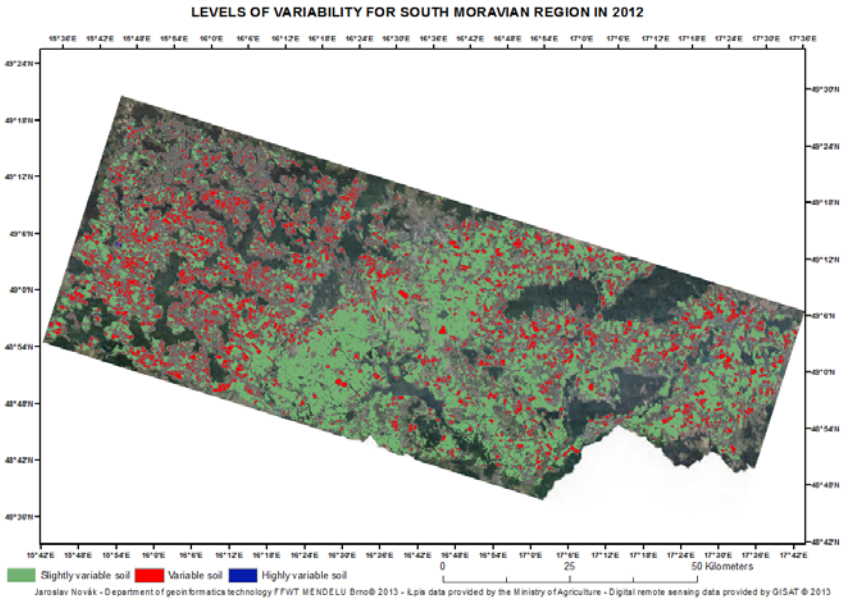


Fig. 3 shows a map with the resulting output variability derived from Rapid Eye data. Most of notably, it seems at first glance the western side of the region overlapping the Highlands, in which is concentrated the largest percentage of variable soil. Rather then, it is a land with a smaller than average specific occupation of the area. Similar interpretation is valid for eastern part of the country and in adjacent areas of the Zlín region too. The central part of the region is an area of slightly

variable soil with occasional occurrence of variable land. The occurrence of variable soil is lower than in the western part.

Fig. 4 Levels of variability captured by Landsat 8

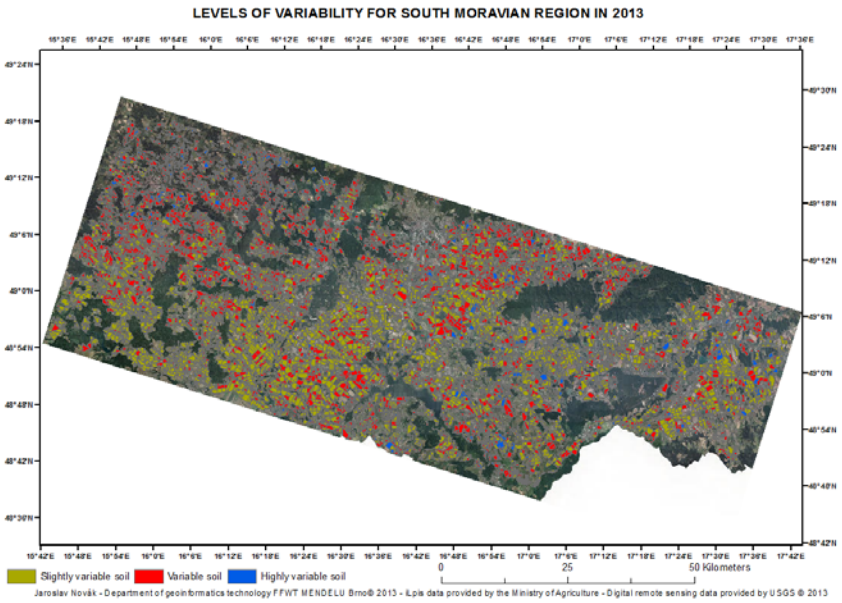


Fig. 4 shows the evaluated data from LANDSAT satellite eighth. Variable soil is here randomly occured throughout almost the whole monitored area and is not correlated with particular places. Quite often is here, in comparison with Rapid Eye data, highly variable soil occured. This exist probably due to the low spatial resolution Landsat 8 data (30 m per pixel) compared with Rapid Eye data (5 m per pixel), which thus appear to be more suitable for the identification of heterogeneity, especially on land with an area less than 10 hectares.

Conclusion

Both data sets can certainly be used as a preview to the variability of soil, however, data Rapid eye seem to be more suitable due to their higher spatial resolution. Their main disadvantage is the cost and the necessity to pre-processing of data sets at this level of quality. Data of Landsat 8 data are free available, but their low resolution can distort the possible variability, especially on land with an area less than 10 ha. Selected process of variability evaluation and classification into three grades are only the first steps in creating of a relevant information source. For more detailed description of spatial variability of soil parameters and their effects on the crop management, more specific soil survey is needed.

Acknowledgement

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The effect of the different vineyard management to composition of weed species

LENKA PORCOVA[1], JAN WIKLER[2]
[1]Department of Agrosystems and Bioclimatology
[2]Department of Plant Biology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xjakabo1@node.mendelu.cz

Abstract: The aim of the study was to define which kinds of weeds were occurred in selected vineyards in Žabčice and evaluated the differences in weed infestation in vineyards with different management. The first vineyard was divided to three parts: grassed space between rows, the part close to the vine trunk and cultivated space between rows. In this vineyard were done common works in growing season (cutting, etc.) and herbicides were applied. In the second vineyard were cultivated spaces between rows left to spontaneous grassing, application of herbicides was limited and common works in growing season weren´t done. The evaluation was taken by phytocenological reléves in years 2013 and 2014. Obtained data were processed by multivariate analysis of ecological data. The most common species in the first vineyard were *Bromus hordeaceus*, *Bromus inermis*, *Digitaria sanguinalis*, *Elytrigia repens*, *Erodium cicutarium*, *Euphorbia cyparissias*, *Fallopia convolvulus*, *Galium aparine*, *Geranium pusillum*, *Geum urbanum*, *Hordeum murinum*, *Chenopodium hybridum*, *Lamium purpureum*, *Lycopsis arvensis*, *Plantago major*, *Robinia pseudoacacia*, *Senecio vulgaris* and *Stellaria media*. The most common species in the second vineyard were *Bromus* sp., *Bromus tectorum*, *Calamagrostis epigejos*, *Convolvulus arvensis*, *Conyza canadensis*, *Cornus sanguinea*, *Echinochloa crus-galli*, *Epilobium ciliatum*, *Chenopodium album*, *Chenopodium pedunculare*, *Lactuca serriola*, *Plantago lanceolata*, *Potentilla argentea*, *Sambucus nigra*, *Taraxacum officinale*, *Tragopogon orientalis*, *Tripleurospermum inodorum* and *Viola arvensis*.

Key-Words: weeds, vineyards, biodiversity

Introduction

Vineyards such as permanent crops are rich to weed spectrum due to management [1]. The occurrence of individual species largely affects geographical, agro-ecological and agro-technical conditions of the vineyard. Many species are in vineyard, annual, perennial, dangerous for vine or harmless. Large numbers of flowering plants enhances the biodiversity, and not least have an aesthetic function. Way of farming in the vineyard, works in growing season and all management have affects at growth and development of plant communities. The aim of this paper is to compare the range of weed species in two vineyards with different ways of management and statistically evaluate the differences. Many authors studied impact of different technologies to weed infestation, f. e. [2, 3, 4, 5]. Possibility of grassing [6] is after years of research in vine growing in the real use [7, 8].

Material and Methods

The aim of this paper was to determine which weeds were occurred in selected vineyards in the area of Žabčice and evaluate the differences in the weeds spectrum in vineyards with different management. GPS coordinates of selected vineyards are 49°00'10.1"N 16°35'25.3"E for intensive managed vineyard and 49°00'25.7"N 16°34'55.5"E for extensive managed vineyard.

Žabčice locates about 25 km south of Brno, in the production area of maize. The altitude of locality is 185 meters above sea level. The average annual temperature is 9.2 °C, which ranks Žabčice to the warmest locations in the Czech Republic. The average rainfall is 480.3 mm. Rainfalls are distributed unevenly during the growing season. Dried winds increase the drought. The first vineyard is kept in intensive management. Grassed strip and cultivated strip are alternated. Space close to the vine trunk is maintained by herbicides. Works in growing season (cutting the vine, chipping of

secundary leaders, trimming of top part of vine plant, defoliation in grapes area, etc.) are making in agronomic terms. The second vineyard is kept in extensive management. The cultivating strips were left spontaneous grassing; application of herbicides in space close to the vine trunk is limited. Works in growing season are not made.

Vegetation was evaluated using the phytocenological reléves in August in 2013 and 2014. Phytocenological reléves were written in part close to the vine trunk, cultivated strip and in grassed strip. In each vineyard were done 10 phytocenological reléves in part close to the vine trunk, 10 from cultivated strip and 10 in grassed strip. Total were made 60 phytocenological reléves in each year. Images were always evaluated in the same place. The abundance of weeds was assessed estimation methods in percentages. Czech and Latin names of each weed species were used in accordance with Kubát [9].

The obtained data were processed by Multivariate Analysis of Ecological Data. The optimal analyze was guided by Length of Gradient, which was obtained by Detrended Correspondence Analysis (DCA). Canonical Correspondence Analysis (CCA) was used for further processing. Data were processed by a computer program CANOCO 4.0. [10]. Significant difference was retrieved by test Monte-Carlo. It was converted 499 permutations.

Results and Discussion

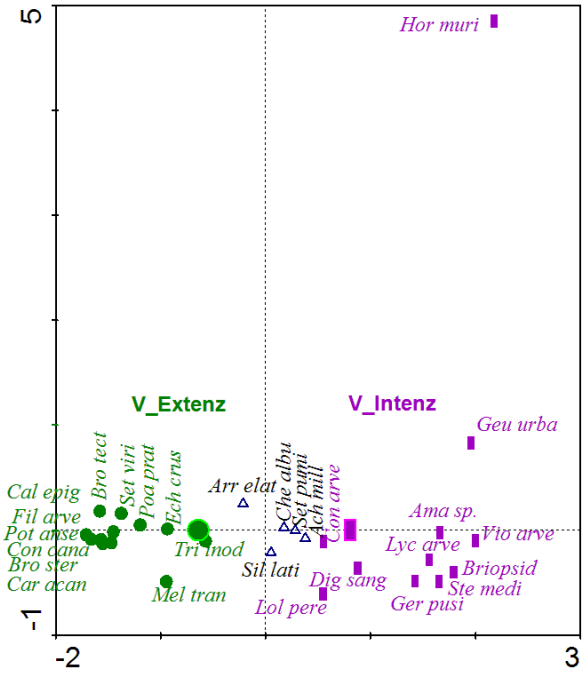
The obtained data of the frequency and coverage of plant species were processed DCA analysis. A length of Gradient was 3.787. Based on this calculation was chosen Canonical Correspondence Analysis for further processing. This analysis defines the spatial arrangement of individual species and selected vineyards. This layout is graphically displayed in the ordination diagram (Fig. 1). Weeds and management of vineyards points are shown with different shape and color. Species and sums of coverage are summarized in Tab. 1.

CCA results show that kind of management have an impact to the frequency and types of coverage in the vineyard. The result is significant at the significance level $\alpha = 0.002$ for all canonical axes. Management mode (intensive/extensive) should have an impact on the species composition of weeds in the vineyards. Therefore, these two different modes of vineyard management were used as factors that influence the occurrence of individual plant species. Purple point shows the factor of intensive management in vineyard, green point shows the factor of extensive management in vineyard. Species are color-divided into three groups in the ordination diagram. Weed species, which were occurred most frequently in vineyard with extensive management, are marked in green colour. When is point of weed species close to the point of factor, that factor affecting their occurrence. The same applies for species marked in purple color. The last group is the types that are marked in black. Those are species which occurrence strongly affects another factor that analysis is not included.

Table 1 The sums of coverage species in intensively and extensively maintained vineyard

Latin name	Kind of management	
	Intensive	Extensive
Achillea millefolium	168	76
Amaranthus sp.	617	335
Arrhenatherum elatius	259	474
Briopsida	122	298
Bromus tectorum		378
Calamagrostis epigejos	205	167
Carduus acanthoides	26	38
Convolvulus arvensis	151	13
Digitaria sanguinalis	521	145
Filago arvensis		1660
Geranium pusillum	219	20
Geum urbanum	164	53
Hordeum murinum	117	13
Chenopodium album	259	177
Lolium perenne	1250	339
Lycopsis arvensis	119	66
Poa pratensis	40	36
Potentilla argentea	79	23
Setaria pumila	315	128
Stellaria media	193	496
Viola arvensis	206	110
Other species	811	1539

Fig. 1 Ordination diagram expressing the impact factor of vineyard management to weed spectrum



Explanatory notes:
Ach mill (*Achillea millefolium*), *Ama sp.* (*Amaranthus* sp.), *Arr elat* (*Arrhenantherum elatius*), *Briopsid* (Briopsida), *Bro ster* (*Bromus sterilis*), *Bro tect* (*Bromus tectorum*), *Cal epig* (Calamagrostis epigejos), *Car acan* (*Carduus acanthoides*), *Con arve* (*Convolvulus arvensis*), *Con cana* (*Conyza canadensis*), *Dig sang* (*Digitaria sanguinalis*), *Ech crus* (*Echinochloa crus-galli*), *Fil arve* (*Filago arvensis*), *Ger pusi* (*Geranium pusillum*), *Geu urba* (*Geum urbanum*), *Hor muri* (*Hordeum murinum*), *Che albu* (*Chenopodium album*), *Lol pere* (*Lolium perenne*), *Lyc arve* (*Lycopsis arvensis*), *Mel tran* (*Melica transsi-lvanica*), *Poa annu* (*Poa annua*), *Pot anse* (*Potentilla argenta*), *Set pumi (Setaria pumila*), *Set viri* (*Setaria viridis*), *Sil lati* (*Silene latifolia*), *Ste medi* (*Stellaria media*), *Tri inod* (*Tripleurospe-rmum inodorum*) and *Vio arve* (*Viola arvensis*).

The most frequent weed species in intensive maintained vineyard were annual species *Amaranthus* sp., *Digitaria sanguinalis*, *Stellaria media*, *Viola arvensis*, perennial species *Convolvulus arvensis*, *Geranium pusillum*, *Geum urbanum*, *Hordeum murinum*, *Lolium perenne* and *Lycopsis arvensis*. Totally were identified 37 annual weed species and 23 perennial weed species.

Crabgrass (*Digitaria sanguinalis*) was species with the most coverage percentage in intensive maintained vineyard. It occurs mainly on sandy soils, not only in fields, but also in vineyards. We can found it in the unconnected stands where there is enough space for growth and development. Its competitiveness is increasing with abundance. Great coverage this kind is likely related with high production of seeds [11].

Another abundant species in the reporting vineyard was small-flowered cranesbill (*Geranium pusillum*). This species is relatively undiscerning to soil alkalinity. It is occupying acidic and alkaline positions with enough of rainfall and sunshine.
Chickweed (*Stellaria media*) is an annual creeping herb. It is often found on disturbed positions. It is a kind, which is typical for repeated mechanically cultivated soil [2].

The most frequent weed species in extensive maintained vineyard were annual species *Bromus sterilis*, *Bromus tectorum*, *Conyza canadensis*, *Echinochloa crus-galli*, *Filago arvensis*, *Setaria viridis* and *Tripleurospermum inodorum*, perennial species *Carduus acanthoides*, *Calamagrostis epigejos*, *Melica transsilvanica*, *Poa annua* and *Potentilla argentea*. Overall, it was identified 32 annual species and 20 perennial species in this vineyard. In general, vineyards with extensive maintained are richer in plant and weed spectrum [12, 13]. After time, there is competing fight between weed species. Annual species are disappearing over time and abundance of dominant weed species is increasing [14, 15].

Extensive management of the vineyard and spontaneous greening originally cultivated strips could have under certain conditions a negative impact on the culture. In the Žabčice are frequent low summer rainfall and dry winds, which in conjunction with excessive greening can cause drought stress, thereby, reducing not only the quantity but also the quality of grapes and wine [16]. Bushgrass (*Calamagrostis epigejos*) was species with the most coverage percentage in extensive maintained vineyard. It is a perennial grass with thick rhizomes, which is ecologically very plastic. Grass is able to suppress competitively weaker species in a relatively short time in the low-tech management [17, 18]. This invasive species produces large quantities of biomass [19, 20, 21] and seeds [22]. Another abundant species in this vineyard was Silverleaf Cinquefoil (*Potentilla argentea*). It is a permanent type, with creeper root bines. It has a high competitive ability; it quickly fills the free space [23]. This species hates repeated processing of soil and regular mowing [24]. This kind of management suits its growth in this vineyard.

Barnyardgrass (*Echinochloa crus-galli*) is annual grass, which is occurring in whole Czech Republic, from plains to hilly country. Together with other annuals weeds is located at the beginning of

uninterrupted succession, then it is extruded via more dominant species [25].

Scentless Mayweed (*Tripleurospermum inodorum*) abounded not only in the field but also in other synanthrophic areas. This species produces large quantities of seeds [26], but it can occupy only unconnected stands. At a greater involvement of the crop, seedlings not able to get ahead [23].

Wood Avens (*Geum urbanum*) is a ruderal species, which is found on all positions, which affects a person by their activities.

Annual species are dominant in intensive maintained vineyard, for example *Amaranthus* sp., *Digitaria sanguinalis*, *Stellaria media* or *Viola arvensis*. Perennial species have greater abundance under extensive management in vineyard, for example *Calamagrostis epigejos*, *Melica transsilvanica* or *Potentilla argentea*. Similar results have many authors in their research [2, 28, 29, 30].

Conclusion

Spectrum of weed species was evaluated in two vineyards with a different way management in the area Žabčice in 2013 and 2014. Total were identified 61 weed species in the vineyard with intensive management. More coverage had 37 annual species, 23 perennial species had lower coverage. Further, it was identified 52 weed species in a vineyard with extensive management. This number including 32 annual species and 20 perennial species. Perennial species had higher coverage. Competitively stronger species gradually displace species with low competitive ability.

This study proves that the way in vineyard management significantly influences the composition of the species spectrum of weeds. The results not only contribute to the monitoring of the occurrence of weeds in vineyards, but also allows look into the course of succession on abandoned areas.

Acknowledgement

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Interactive effects of elevated CO_2 concentration, nitrogen nutrition and UV-exclusion on yield, aboveground biomass and root development in winter wheat and spring barley

WUTTHIDA RATTANAPICHAI[1], KAREL KLEM[1,2]
[1]Department of Agrosystems and Bioclimatology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Global Change Research Centre AS CR v.v.i.
BElidla 986/4a. 603 00 Brno.
CZECH REPUBLIC

xrattana@node.mendelu.cz

Abstract: Within the manipulation experiment in open top chambers (Domanínek near Bystřice nad Pernštejnem) that allow simulation of elevated concentration of CO_2 ($[CO_2]$) (expected by the end of this century – 700 μmol mol^{-1}) and exclusion of solar UV radiation, the interactive effects of these environmental drivers together with nitrogen application were studied. Aboveground biomass at the time of harvest, grain yield and root area were studied in winter wheat (variety Bohemia) and spring barley (variety Bojos).From three replications of each treatment were the roots of four plants dug out from the soil and gently washed. Photos of roots taken using the digital camera were analysed by image processing software Image J (plugin SmartRoot). The result showed that the elevation of $[CO_2]$ increased the above ground biomass and grain yield. High level of nitrogen increased the stimulatory effect of $[CO_2]$ on the above-ground biomass and grain yield. Also UV exclusion stimulated the effect of the evaluated $[CO_2]$ on above-ground biomass and grain yield. UV exclusion resulted in higher root area than the treatment of non-exclusion of UV.

Key-Words: winter wheat, spring barley, elevated CO_2 concentration, nitrogen nutrition, UV exclusion, root area

Introduction

Atmospheric carbon dioxide concentration ($[CO_2]$) has increased about 30% since pre-industrial times and during this century $[CO_2]$ levels could double or triple compared to pre-industrial levels [1]. Numerous experiments have demonstrated that in many plant high atmospheric $[CO_2]$ leads to increases in photosynthetic rate, whole-plant growth and water use efficiency (WUE) and decreases in transpiration. It is known that CO_2 enrichment causes partial stomatal closure and reduces stomatal conductance [2]. With elevation of $[CO_2]$, stomata do not appear to limit photosynthesis any more than they do at normal ambient $[CO_2]$, even though stomatal conductance usually decreases under these conditions [3]. But reduced stomatal conductance potentially leads to a decreased transpiration rate on a leaf area basis [3][4][5]. Elevated $[CO_2]$ directly affects photosynthesis and has been shown to enhance growth and yield of crop plants under suitable conditions [6][7]. Moreover the effect of elevated CO_2 on growth is strongly modulated by N supply. When nitrogen is high, elevated $[CO_2]$ leads to a sustained stimulation of photosynthesis and growth [8]. Plants grown at elevation of $[CO_2]$ also increase in root:shoot ratios that are symptomatic of N limited plants [9]. Elevation of $[CO_2]$ cause reduction of nitrogen in plant [10]. Reductions of nitrogen concentrations in tissue under high $[CO_2]$ grown plants might indicate physiological changes in the efficiency with which plants use nitrogen to gain biomass (i.e. increased nitrogen use efficiency).

The effects of $[CO_2]$ on R/S ratio are contradictory due to complexity in accurate underground biomass estimation under diverse crops and conditions. Roots become more numerous, longer, thicker, and faster growing in crops exposed to high $[CO_2]$ with increased root length in many plant species. Branching and extension of roots under elevated $[CO_2]$ may lead to altered root architecture and ability of roots to acquire water and nutrients from the soil profile

with exploration of the soil volume. Root growth under elevated atmospheric [CO_2] results in proportionally higher C allocation belowground, and increasing R/S ratios [11]. Many studies showed increased R/S [12, 13, 14, 15], while other studies showed decreased R/S [16, 17]. The direct effect of elevation [CO_2] on R/S has no clearly defined conclusion [18, 19]. The underlying assumption that a larger proportion of dry matter produced under [CO_2] enrichment is preferentially allocated to roots and increased under limiting water and nutrient supply needs to be evaluated [12].

Effects of ultraviolet (UV) irradiation on biological matter became an important issue over the past few decades since the man-made changes in atmosphere affected ozone layer which covers and protects the earth's surface from harmful ultraviolet radiation. Exposure to high UV-B radiation alters photosynthetic enzyme activities and disrupts PSII reaction centres [20]. Many researchers have reported reduction in biomass accumulation due to UV-B exposure in several trees [21, 22] and crop species [23]. Exclusion of UV significantly increased the total chlorophyll while Chl a/b ratio decreased. The efficiency of PS II (Fv/Fm), rate of photosynthesis and stomatal conductance significantly enhanced along with a remarkable increase in Carbonic anhydrase, PEP carboxylase and total soluble proteins. Thus UV excluded plants have higher reducing power and increased CO_2 fixation [24] Moreover, exclusion of solar UV also enhanced root growth [25].

Material and Methods

The experiment was conducted in experimental station Domanínek, near Bystřice nad Pernštejnem in Bohemian-Moravian highlands (Czech Republic, 49°521'N, 16°235'E, altitude 575 m a. s. l.). The soil type is modal cambisol, with geological bedrock weathered gneiss in depth 60-90 cm. Soil texture is sandy loam (45-60% sand and up to 16% clay) and pH(KCl) is between 4-5. This region is characterized as rain-fed area with mean annual precipitation 610 mm and mean annual temperature 7.2°C. The experiment consists of 24 open-top chambers, which allows manipulation of [CO_2] and precipitation (Fig. 1). UV radiation was excluded by using the non-transparent plastic roof. Spring Barley variety Bojos was sown on 19th March 2014 in the chambers with density 4 MGS (millions of germinating seeds). Winter wheat variety Bohemia with bread quality A was sown on 9th October 2013 in the chambers with density 4 MGS (millions of germinating seeds). Fumigation with elevated [CO_2] (EC; 700 μmol mol^{-1}) started at the beginning of stem elongation (middle of May). The plots inside chamber were divided to two subplots and one of them fertilized with calcium nitrate in a dose 200 kgN ha^{-1} for winter wheat and dose 100 kgN ha^{-1} for spring barley at the growth stage end of tillering. The second subplot remained unfertilized with nitrogen. Each combination of factors was three times replicated.

The aboveground biomass was harvested manually at full ripening and weighted. This was followed by threshing of grain using a small plot harvester. The roots of four plants were dug out from the soil by excavation of soil around the root system in depth about 20 cm and gently washed them on 0.05 mm mesh sieve. Photos of roots taken using the digital camera were analysed by image processing software Image J (Fig. 2).

Fig. 1 Experimental site with 24 open-top chambers, which allows manipulation of [CO_2] and precipitation

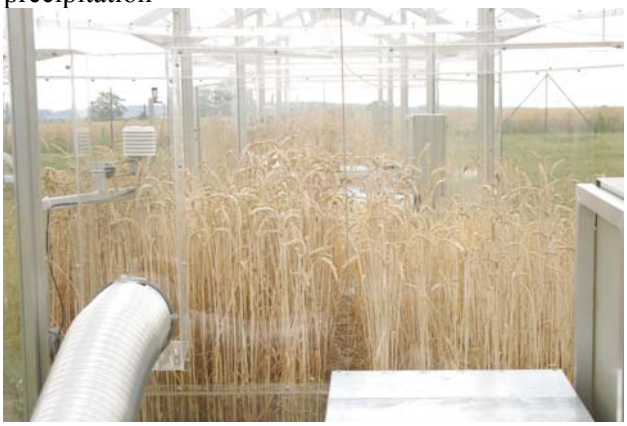
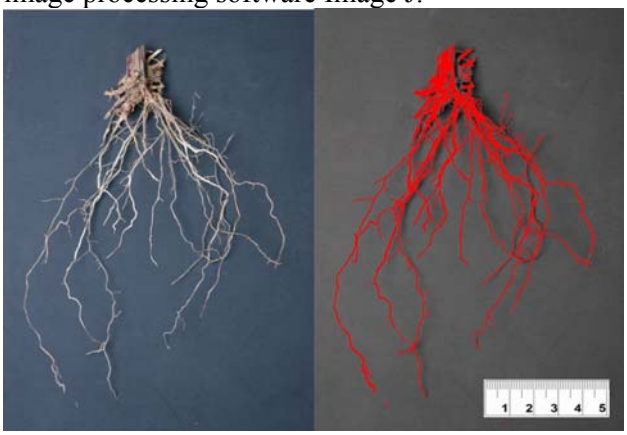


Fig. 2 Photos of roots of winter wheat were taken by digital camera and the root which were analysed by image processing software Image J.



Results and Discussion

The assessment of the effect of elevated [CO_2] in the interaction with the effect of nitrogen and UV exclusion reveals similar results for above-ground

biomass and grain yield for both of spring barley and winter wheat (Fig. 3, 4, 5 and 6). The results indicated that elevated [CO_2] increased the above ground biomass and grain yield for both, with and without nitrogen fertilizer. In case of the UV exclusion (UV-), the elevated [CO_2] had higher effect to above-ground biomass and grain yield than non-exclusion of UV (UV+) probably because exclusion of UV increased rate of photosynthesis and CO_2 fixation [24]. However, the treatment without nitrogen fertilizer in conditions with present UV radiation (UV+) slightly decreased the above-ground biomass and grain yield of winter wheat. It was probably due to decrease of nitrogen uptake caused by UV radiation. Therefore UV exclusion combined with application of nitrogen fertilizer resulted in the highest above-ground biomass and grain yield when compared with the others treatment for both spring barley and winter wheat.

Fig. 3 Effect of CO_2 concentration, nitrogen and UV exclusion on above-ground biomass of winter wheat. AC (ambient CO_2 concentration; 390 µmol mol^{-1}), EC (elevated CO2 concentration; 700 µmol mol^{-1}), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 200 kg ha^{-1}), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.

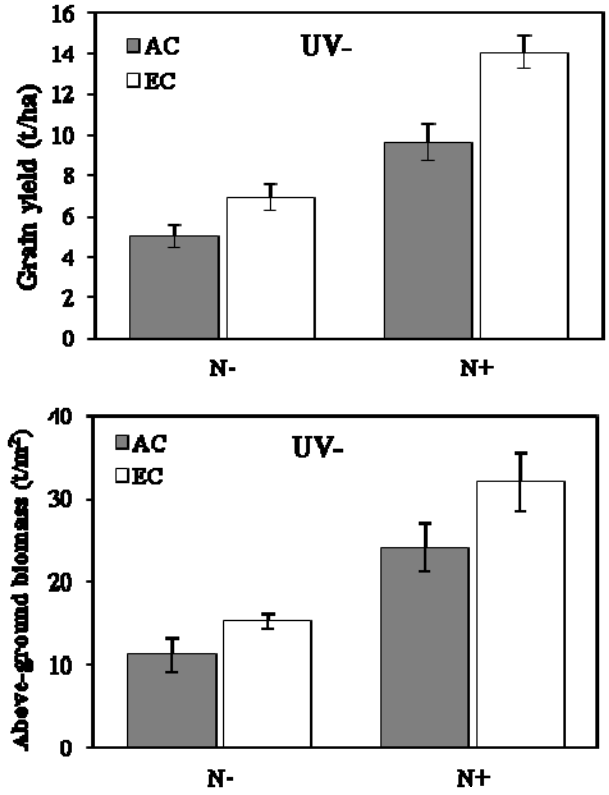


Fig. 4 Effect of CO_2 concentration, nitrogen and UV exclusion on grain yield of winter wheat. AC (ambient CO_2 concentration; 390 µmol mol^{-1}), EC (elevated CO2 concentration; 700 µmol mol^{-1}), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 200 kg ha^{-1}), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.

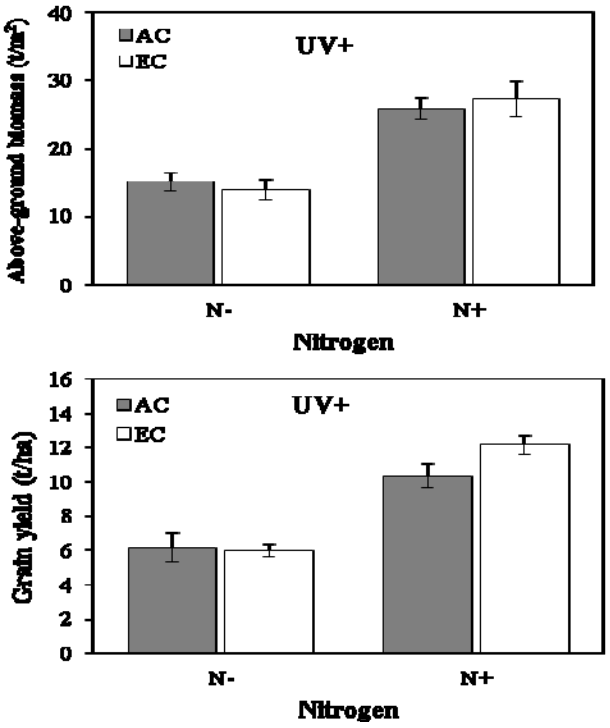
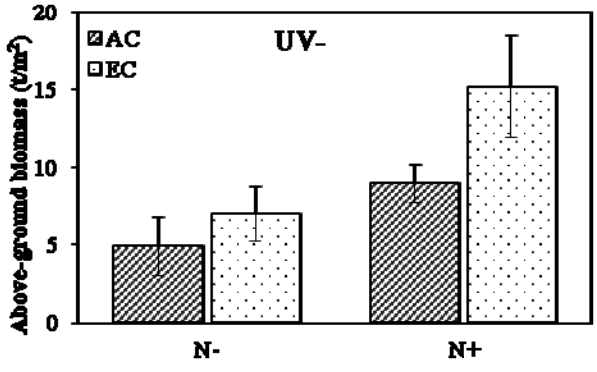


Fig. 5 Effect of CO_2 concentration, nitrogen and UV exclusion on above-ground biomass of spring barley. AC (ambient CO_2 concentration; 390 µmol mol^{-1}), EC (elevated CO2 concentration; 700 µmol mol^{-1}), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 100 kg ha^{-1}), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.



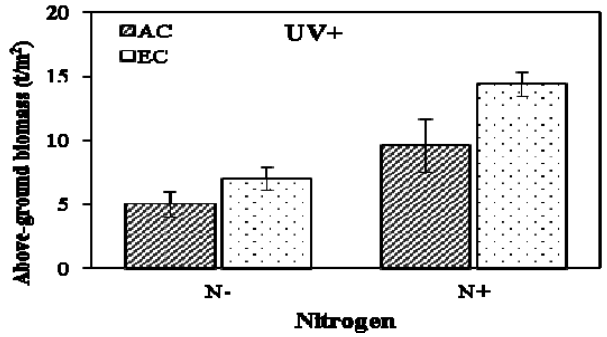
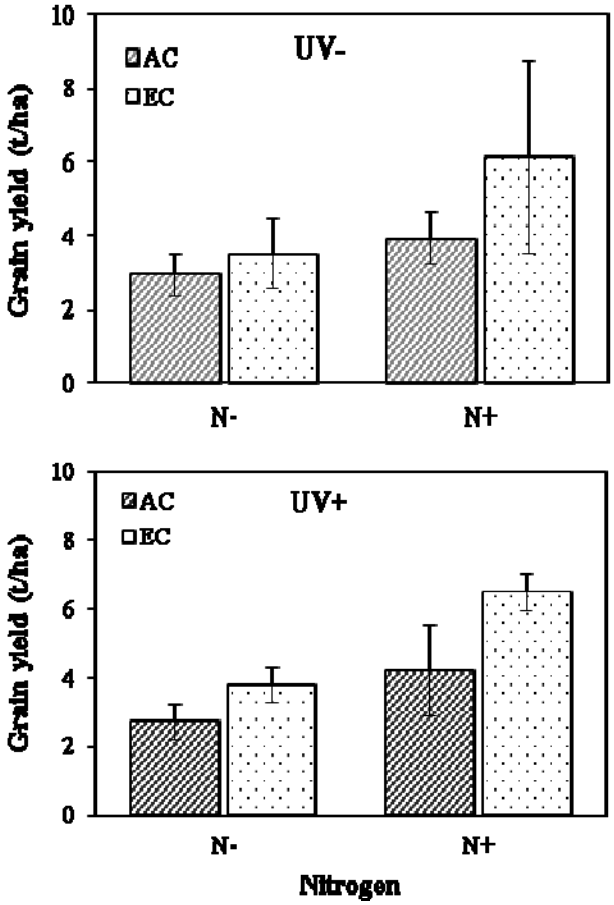


Fig. 6 Effect of CO_2 concentration, nitrogen and UV exclusion on grain yield of spring barley. AC (ambient CO_2 concentration; 390 μmol mol^{-1}), EC (elevated CO2 concentration; 700 μmol mol^{-1}), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 100 kg ha^{-1}), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.



High level of nitrogen increased the stimulatory effect of CO_2 on the above-ground biomass and grain yield. Nitrogen supply strongly interact with elevated [CO_2] effect influencing plant metabolism and grain yield [10]. When nitrogen nutrition is high, elevated [CO_2] leads to a sustained stimulation of photosynthesis and growth [8].

The effects of [CO_2], nitrogen nutrition and UV-exclusion on root area was generally smaller compare to effects on aboveground biomass and grain yield. UV exclusion resulted in higher root area than the treatment of non-exclusion of UV (Fig. 7 and 8).

Fig. 7 Effect of CO_2 concentration, nitrogen and UV exclusion on root area of winter wheat. AC (ambient CO_2 concentration; 390 μmol mol^{-1}), EC (elevated CO2 concentration; 700 μmol mol^{-1}), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 200 kg ha^{-1}), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.

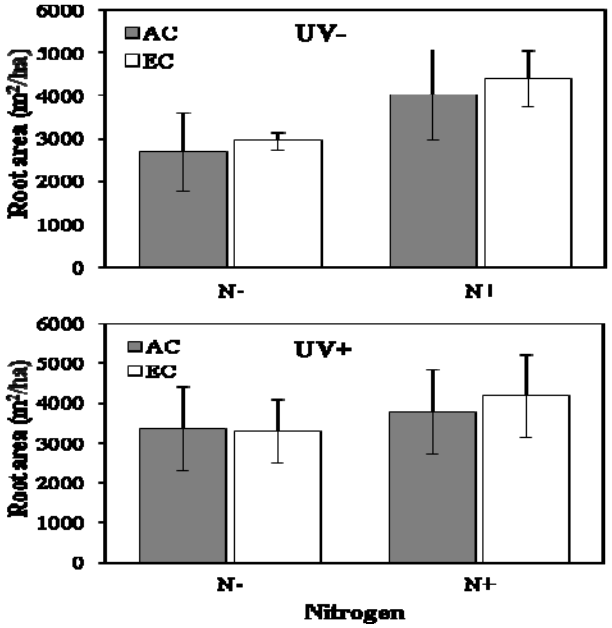
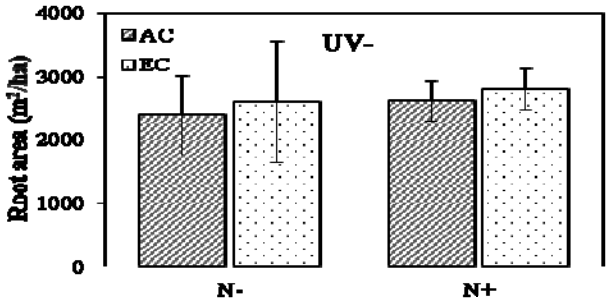


Fig. 8 Effect of CO_2 concentration, nitrogen and UV exclusion on root area of spring barley. AC (ambient CO_2 concentration; 390 μmol mol^{-1}), EC (elevated CO2 concentration; 700 μmol mol^{-1}), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 100 kg ha^{-1}), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.



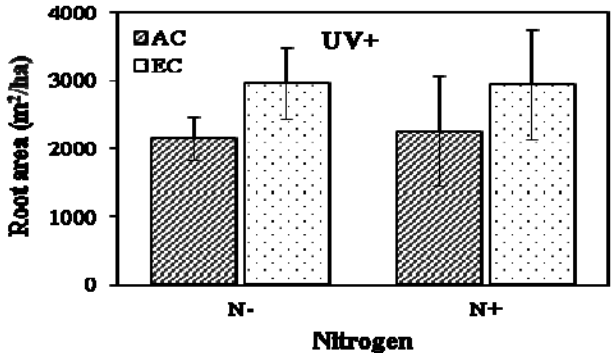


Fig. 9 The relationship between the above-ground biomass and root area of winter wheat from the effect of elevated [CO_2] in the interaction with the effect of nitrogen and UV exclusion.

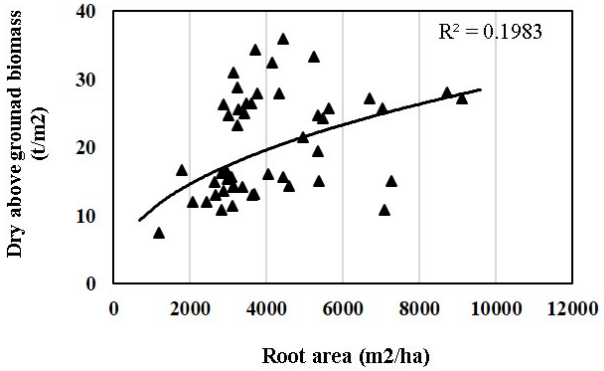
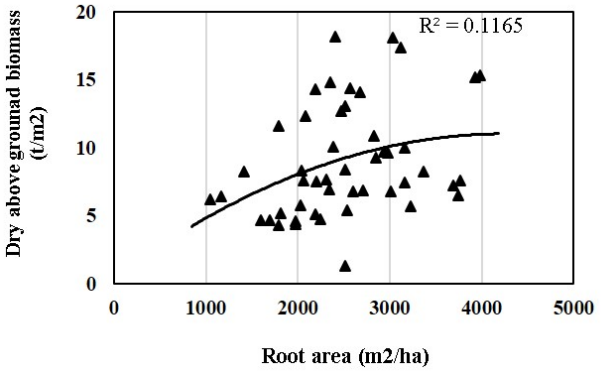


Fig. 10 The relationship between the above-ground biomass and root area of spring barley from the effect of elevated [CO_2] in the interaction with the effect of nitrogen and UV exclusion.



The figure 9 and 10 showed the relationship between the above-ground biomass and root area. The results show that increase of root area is related to increase of aboveground biomass.

Table 1 The F-value from ANOVA test. Significant values was indicated: **, P<0.01; *, P<0.05.

Source	Biomass		Grain yield		Root areas	
	Winter wheat	Spring barley	Winter wheat	Spring barley	Winter wheat	Spring barley
CO_2 concentration	34.067**	61.467**	104.610**	21.238**	0.658^{ns}	7.203*
Nitrogen	651.625**	157.071**	803.937**	34.140**	12.231**	0.239^{ns}
UV exclusion	0.052^{ns}	0.000^{ns}	1.661^{ns}	0.225^{ns}	0.177^{ns}	2.156^{ns}
UV * N	7.132*	0.019^{ns}	3.340^{ns}	0.193^{ns}	1.537^{ns}	3.373^{ns}
UV * CO2	30.339**	0.610^{ns}	36.617**	0.209^{ns}	0.084^{ns}	1.923^{ns}
N * CO2	9.197**	13.117**	33.728**	4.642*	0.276^{ns}	0.317^{ns}
UV * N * CO2	0.273^{ns}	0.522^{ns}	0.366^{ns}	0.0956^{ns}	0.063^{ns}	0.249^{ns}
REP	11.505**	4.283*	2.283^{ns}	1.982^{ns}	0.548^{ns}	0.041^{ns}

Conclusion

The effect of elevated [CO_2] in the interaction with the effect of nitrogen and UV exclusion reveals similar results for above-ground biomass and grain yield for both spring barley and winter wheat. The elevation of [CO_2] increased the yield of above ground biomass and grain yield. High level of nitrogen increased the stimulatory effect of CO_2 on the above-ground biomass and grain yield. UV exclusion stimulated the effect of the elevated [CO_2] on above-ground biomass and grain yield. The effect of elevated [CO_2] in the interaction with the nitrogen application and UV radiation on root area is smaller compare to aboveground biomass and grain yield.

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Monitoring of San Jose Scale (*Diaspidiotus perniciosus*) Occurrence and Comparison of Temperature Models

KATERINA RYCHLA
Department of Crop Science, Breeding and Plant Medicine
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

katerina.rychla@mendelu.cz

Abstract: The San Jose scale (*Diaspidiotus perniciosus*) is a wide spread pest all over the world. Its harmfulness is increasing over the last years in the Czech Republic (especially in South Moravia), where our experiment was based. Firstly we were testing different pheromone traps (Wing Trap, Delta Trap, open trap) suitable for monitoring of the San Jose scale male occurrence in two different orchards. The second area of our research was to compare different temperature models with our measured effective temperature sums that were set mainly for biofix (occurrence of first male in a trap) and occurrence of crawlers. Delta Trap appeared as the best to catch the San Jose scale male. Temperature model according to Buhroo et al. [1] is the most suitable in prediction of males and crawlers in the Czech Republic. Nevertheless it is necessary to observe the occurrence of this pest also directly on trees.

Key-Words: San Jose scale, pheromone, trap, crawler, effective temperature sum, degree day

Introduction

The San Jose scale (*Diaspidiotus perniciosus*) is known all over the world and it is widely distributed. This pest is important especially in fruit production and it is often intercepted in quarantine mainly on oranges and tangerines [2]. The European Union deleted it from the list of quarantine pests because of its extension in almost all European states. Significant damages are recorded on apples, pears, peaches, plums, currants and many other plants. The scale develops on vegetative organs, blossoms and fruits. It occurs the most on bark of strain and branches. Due to suction of plant saps the trees may die [3]. The population of the San Jose scale started to graduate at the end of the 90s because of elimination of nonselective pesticides in the integrated pest management in the Czech Republic [4].

The San Jose scale is almost all its life protected by cover that makes a protection against this pest very difficult. The applied pesticides can not get to any sensitive part of its body. Consequently they have none or very low effect. The only stage that is sensitive to treatments is larva (crawler) that crawl out of female cover after 33 to 40 days after fertilization. When the crawlers find a suitable place to suck they settle down and start to make their own cover. It can happen even after two hours if the conditions are appropriate. Therefore it is necessary to manage the application of pesticides in time [2]. The emergence of males is observed using pheromone traps.

Modeling of effective temperature sum can be used to predict the emergence of males and crawlers. The crawlers from overwintering generation (the first generation) need less than 500 day degrees (DD) to develop, crawlers from the second generation need 770 DD (for the lower developmental threshold 7.3°C) [5].

The aim of this research was to find the suitable combination of a pheromone trap and a model of effective temperature sum for exact determination of time to apply the treatments against the crawlers of the San Jose scale.

Material and Methods

The experiment was done in 2014 in pear orchard (using biological control) in Kobyli and in apple

orchard and peach orchard (using integrated pest management) in Tesetice.

We cut 20 two-year branches (20 cm long) on March 10th in Kobyli and on March 14th in Tesetice in each orchard. Then the overwintering scales were counted using a microscope and it was determined the average counts of scales per one meter of branch. The total attack of trees was defined.

The presumed terms of emergence of males and crawlers were set according to Alston et al. [6] and using of data from online meteorological station in our localities.

The pheromone traps for observing amount of males were installed on April 18th on three places (A, B, C) in each orchard. We used three types of traps on each place. The small open trap was constructed from white sticky board and wire (Fig. 1). Then we tested common Delta Trap (Fig. 2) and Wing Trap (Fig. 3). The parameters of the traps are written in the following table 1.

Sexual pheromone of San Jose scale female from International Pheromone Systems Ltd was used in all traps.

Table 1 Types of pheromone traps

Type of trap	Size (cm)	Sticky surface (cm^2)	Producer
Open trap	12 × 14	168	White sticky board by Biocont Laboratory
Delta Trap	12 × 20	240	Csalomon
Wing Trap	17.5 × 22.5	393.75	Gemplers

The traps had been observing daily since April 20th to determinate the exact term of biofix (the first male catch in a trap). Then the control had been doing twice a week till the end of male flying. The sticky boards in the traps had been changing regularly depending on other insects that get on sticky board. Males were counted using microscope in laboratory. The results are in the table 2.

Occurrence of crawlers was determined using double site sticky tape on branches. They were checked every day in expected period of crawler emergence.

The effective temperature sums were recorded from data from meteorological stations (operated by AMET – association Litschmann & Suchy) in orchards for biofix and time of crawler emergence. These effective temperature sums were compared with temperature models according to Alston et al. [6], Buhroo et al. [1], Jorgensen et al. [7] and Rice et al. [8]. The observing was done in two generations of San Jose scale. The pheromone was changed on July 10th.

Results and Discussion

Comparison of different type of pheromone traps

We determined the attack of San Jose scale. There were 44.5 overwintering scales per meter of branch in Kobyli and 267.75 overwintering scales per meter of branch in Tesetice. The male counts in different traps are written in the tables 2 and 3 for the location Kobyli and Tesetice respectively.

In Kobyli the biggest amounts of males were found in Wing Traps in two places and in Delta Trap in one place. In Tesetice the biggest amounts of males were found in Delta Traps in two places and in open trap in one place. The males from overwintering generation were not register in Tesetice at all.

Table 2 Comparison of different types of pheromone traps in Kobyli

	A		B		C	
	2nd generation	1st generation	2nd generation	1st generation	2nd generation	1st generation
Open trap	58	16	27	20	12	25
Delta trap	**66**	**44**	31	33	13	49
Wing trap	28	30	**270**	**38**	**34**	**101**

Legend: 2nd generation – males from the overwintering generation; 1st generation – males from the summer generation

Table 3 Comparison of different types of pheromone traps in Tesetice

	A		B		C	
	2nd generation	1st generation	2nd generation	1st generation	2nd generation	1st generation
Open trap	0	9	0	14	0	**67**
Delta trap	0	**12**	0	**28**	0	40
Wing trap	0	2	0	10	0	40

Legend: 2nd generation – males from the overwintering generation; 1st generation – males from the summer generation.

Efficiency of different types of traps for San Jose scale males had been checked in 1978 – 1980 in California, Washington and Oregon. They used two basic types of traps, open traps and closed traps similar to Delta Traps. The open trap was established as the most effective [9], but it has not been proved in our experiment.

In practical point of view the Delta traps appeared as the most convenient. It was difficult to count San Jose scale males from the Wing Trap due to big amount of other insects and results could be inexact.

Comparison of effective temperature sums

The effective temperature sums (with lower developmental threshold 10.6°C and upper developmental threshold 32.2°C) were calculated in terms of installation of the traps, biofix and emergence of crawlers in both generations and the length of generation in both localities (table 4). They can be compared with effective temperature sums set by Alston et al. [6], Buhroo et al. [1], Jorgensen et al. [7] and Rice et al. [8] in table 5.

The absence of males from overwintering generation was the main problem in Tesetice in comparison with Kobyli. It could be caused by different systems of pest management or climatic conditions.

San Jose scale males mate with the females immediately after eclosing, and then die after mating [9]. If they eclose in close proximity to the female, they can register her pheromone rather ten pheromone in the trap. The reason can be also small amount of males in the population, even though males should greatly outnumber females in the overwintering generation [2].

Temperature models that are based on biofix do not allow counting of next terms in case of biofix absence. Big differences are evident by comparison of our measured effective temperature sums and sums in temperature models. Therefore it is not possible to rely only on this prediction systems but it is necessary to observe the San Jose scale development by eye and using a microscope.

Table 4 Measured effective temperature sums in Kobyli and Tesetice

	Instalation of pheromone traps (DD)	Biofix 1 (DD)	Crawlers 1 (DD)	Biofix 2 (DD)	Crawlers 2 (DD)	Lenght of a generation (DD)
Kobyli	49	135	324 (189 since biofix 1)	726	1021	697
Tesetice	20	-	259	556	886	627

Legend: Biofix 1 – the first male catch from the overwintering generation; Crawlers 1 – crawlers from the first (summer) generation; DD – day degree; Biofix 2 – the first male catch from the summer generation; Crawlers 2 – crawlers from the second (overwintering) generation.

Table 5 Degree-day accumulation according to Alston et al. (2011), Buhroo et al. (2001), Jorgensen et al. (2000) and Rice et al. (1982)

	Instalation of pheromone traps (DD)	Biofix 1 (DD)	Crawlers 1 (DD)	Biofix 2 (DD)	Crawlers 2 (DD)	Lenght of a generation (DD)
Alston et al. (2011)	102	135	207 since biofix 1			
Buhroo et al. (2001)		141	279	692	736	457
Jorgensen et al. (2000)	111		128 since biofix 1			583
Rice et al. (1982)			225			583

Legend: Biofix 1 – the first male catch from the overwintering generation; Crawlers 1 – crawlers from the first (summer) generation; DD – day degree; Biofix 2 – the first male catch from the summer generation; Crawlers 2 – crawlers from the second (overwintering) generation.

Conclusion

The biggest amount of males was determined in the Wing Traps in Kobyli and in Delta Trap in Tesetice where was the problem to catch males from the overwintering generation. From practical point of view the Delta Trap is the most suitable.

The optimal temperature model for using in the Czech Republic is according to Buhroo et al. [1] due to the problems to set the first biofix. Although it is necessary to control the pest directly on plants and we can not rely only on the prediction system.

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Fig. 1 Open trap

Fig. 2 Delta trap

Fig. 3 Wing trap

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The influence of nitrogen fertilization on quality of winter wheat grain

SARKA SHEJBALOVA[1], JINDRICH CERNY[1], KATARZYNA MITURA[2], KAROLINA JOANNA LIPINSKA[2], JAKUB KOVARIK[1], JIRI BALIK[1]
[1]Department of Agro-Environmental Chemistry and Plant Nutrition
Faculty of Agrobiology, Food and Natural Resources
Czech University of Life Sciences Prague
Kamycka 129, 165 21 Prague - Suchdol
CZECH REPUBLIC
[2]Department of Environmental Chemistry
Institute of Agricultural Chemistry
Faculty of Agriculture and Biotechnology
University of Technology and Life Sciences in Bydgoszcz
Seminaryjna 5, 85-326, Bydgoszcz
POLAND

shejbalova@af.czu.cz

Abstract: The aim of this study was to evaluate winter wheat grain quality after the application of different organic and mineral fertilizers at two sites in the Czech Republic (S1 - Červený Újezd, S2 - Prague-Suchdol). For the purpose of this paper were determined dry matter yield (DM, t ha^{-1}), bulk density (BD, kg hl^{-1}), thousand kernel weight (TKW, g) and grain crude protein content (CP, %) with regard to the requirements for food quality of wheat. The highest average yields were obtained after the use of mineral nitrogen fertilizers, at S1 6.9 t ha^{-1} and at S2 6.5 t ha^{-1}. In the first year of experiment was at S1 the highest average BD for treatments with mineral nitrogen (76.8 kg hl^{-1}), but in the second year for organic fertilized treatments (79.9 kg hl^{-1}), probably due to different weather conditions. At S2 was the highest average BD for treatments with mineral nitrogen fertilizers (78.6 kg hl^{-1}) in both years. At S1 was the highest average value of TKW found for farmyard manure (51.4 g), at S2 for mineral nitrogen treatment (56 g). The highest values of CP were determined after application of nitrogen in mineral form at both sites (12.3 %). At S2 was found high correlation between parameters, better quality and higher DM yield after the use of nitrogen in mineral form compare to the organic fertilizers. At S1 was high variability between years and treatments, but values of DM yield and CP were higher for mineral nitrogen fertilizers than for organic.

Key-Words: quality, nitrogen, wheat, fertilizer, protein concentration

Introduction

Nitrogen is the most important element to achieve stable high grain yields [1, 2] and is essential for improving grain quality of wheat [3, 4, 5]. Wheat is our most grown crop, in 2013 it was grown at 31.8 % of the total sown area of the Czech Republic [6]. Both high yield and good and stable quality are important features in today´s wheat market. To reach these goals N fertilizer rates, split N application and timing of application have been the major strategies recommended to increased protein content and improved alveograph parameters [7, 8]. Wheat quality and yield is influenced by the interaction of a number of factors, including cultivar, soil, climate, cropping practices and grain storage conditions. The performance of many quality characteristics depends greatly on environmental conditions, which result in differential expression of grain quality from site to [9, 10, 11]. Predictions of grain quality for the prospective wheat harvest would be of considerable value to grain buyers in aiding purchasing decisions, and to farmers to help optimize late-season agronomy [12]. The objective of this paper was to evaluate influence of different organic and mineral fertilizers on dry matter yield, bulk density, thousand kernel weight and crude protein content in winter wheat grain.

Material and Methods

In the experiment was examined the effect of different fertilizers on selected parameters of wheat grain quality. Experiments were established at two sites of the Czech Republic with different soil and

climatic conditions: S1 (Červený Újezd), S2 (Praha-Suchdol) (table 1). A simple crop rotation contained: maize (at S1)/potatoes (at S2), winter wheat and spring barley. Treatments of fertilization were repeated in three blocks allow for growing all crops every year.

For the purpose of this paper were evaluated six treatments with winter wheat: Control, Sewage sludge (SS), Farmyard manure (FYM), N in mineral fertilizers (N), NPK and N in mineral fertilizers + spring barley straw (N+ST). For these treatments was determined: dry matter yield (DM, t ha $^{-1}$), bulk density (BD, kg hl^{-1}), thousand kernel weight (TKW, g), and grain crude protein content (CP, %). All the quality traits were assessed using two random samples from each treatment.The whole experiment was based on the same nitrogen rate 330 kg N ha^{-1} to the crop rotation (of which 140 kg N ha^{-1} to the winter wheat) except the Control treatment, which was not fertilized (table 2). For mineral nitrogen fertilization was used Calcium ammonium nitrate. The dose of nitrogen was for winter wheat divided into two doses (such as regeneration and production fertilization). Organic fertilizers were applied only on the potatoes (S1)/silage maize (S2) in the crop rotation. The amount of applied nutrients is described in Table 2. The areas of experimental plots were 60 m^2, respectively 80 m^2 on the Červený Újezd site. The grown variety of winter wheat was Alana, which is characterized as a late variety with baking quality A.

For determination of thousand kernel weight was calculated 2 x 500 seeds using the electronic calculator and weighted the seeds with a prescribed accuracy. Bulk density was determined on laboratory meter model 1938 (Meopta, Czech Republic). Grain samples for determination of total nitrogen were homogenized on a laboratory cutting mill SM 100 (Retsch, Germany) and measured following the Kjeldahl method on the device VAPODEST 50s (Gerhardt GmbH & Co. KG, Germany). The observed nitrogen content was multiplied by a coefficient (for baking wheat 5.7) to obtain a crude protein content in grain [13].

Statistical evaluation of the results was performed between treatments with data over 2 years in the STATISTICA 12.0 program (StatSoft, Tulsa, USA) with the Main effects ANOVA followed by the Tukey´s test at the level of significance $P < 0.05$. Coefficients of correlation (r) between assessed traits are presented in correlation matrix for both sites and years.

Table 1 Characteristic of experimental sites

Site	S1	S2
Location	50°4'22''N, 14°10'19''E	50°7'40''N, 14°22'33''E
Altitude (m a.s.l.)	400	286
Average annual temperature (°C)	7.7	9.1
Average annual precipitation (mm)	493	495
Soil type	haplic Luvisol	haplic Chernozem
Soil texture	silt loam	silt loam

Table 2 Rates of nutrients N-P-K (kg/ha) during crop rotation cycle

	Potatoes/Silage maize	Winter wheat	Spring barley
Control	-	-	-
SS[1]	330-207-44	-	-
FYM[1]	330-102-307	-	-
N[2]	120-0-0	140-0-0	70-0-0
NPK[2]	120-30-100	140-30-100	70-30-100
N[2]+ST[1,3]	138-6-47	140-0-0	70-0-0

[1] P and K in organic fertilizers - average dose taking into account nutrient content in organic fertilizers; [2] mineral fertilizers: N - calcium ammonium nitrate (27 % N), P - triple super phosphate (21 % P), K - potassium chloride (50 % K); [3] 5 t/ha spring barley straw

Results and Discussion

Results were collected in years 2013 and 2014. Average values of DM yield were at both sites for Control treatment about 4 t ha $^{-1}$. After organic fertilizers increased average values to 6.1 t ha $^{-1}$ at S1 and 5.5 t ha $^{-1}$ at S2. Mineral fertilization increased average yields to 6.9 t ha $^{-1}$ at S1 and 6.5 t ha $^{-1}$ at S2. Yields were highly influenced by interannual variability. According to the standard ČSN 461100-2 is the limit of bulk density for food wheat 76.0 kg hl^{-1}. Required limit was not reached in year 2013 at S1 in Control, SS and FYM treatment. In this year and site were found the lowest values in our experiment. In the year 2014 all variants at S1 exceeded the required limit.

For all fertilized treatments at S2 values of BD were higher than 76.0 kg hl^{-1} in both experimental years. The significantly highest values were observed after mineral nitrogen fertilization at both sites, except year 2014 at S1, where the highest bulk density was for treatments with organic fertilizers. Values of BD were higher in the year 2014 compare to the year 2013 at both sites (see table 3). Bulk density is highly determined by weather conditions, particularly by high temperature during the final phase of grain filling [8, 10], which probably caused differences between years.

Thousand kernel weight is one of the yield parameter. The lowest values of TKW were at S1 site observed after application of mineral nitrogen and in combination with straw. In the contrary at S2 reached these treatments the highest values of TKW (table 4). At S2 were achieved higher values compare to S1. At S1 were the highest values found for FYM and NPK treatments, at S2 for N and NPK treatments. There were lower differences between years for TKW than for BD.

In the standard ČSN 461100-2 is the minimal requirement of CP for food wheat 11.5 %. This limit was not achieved by all Control and FYM treatments at both locations and by SS treatment at S1 and NPK at S1, but only in 2013. The highest values of CP were determined after application of nitrogen in mineral form at both sites (table 5). Kozlovský et al. [14] achieved similar high values of CP after application of 150 kg N ha^{-1} as in our experiment. At S2 was recorded significant correlation between all parameters. At S1 was correlation significant only between DMY and CP and DMY and BD. According to Kučerová [15] is between CP and DM yield very strong correlation, which was confirmed in our experiment (table 6).

Table 3 Bulk density (BD, kg hl^{-1})

	S1		S2	
	2013	2014	2013	2014
Control	74.66^{a}	78.25^{a}	75.58^{a}	75.95^{a}
SS	75.71^{b}	80.76^{d}	76.94^{b}	78.04^{b}
FYM	75.47^{ab}	80.61^{d}	76.40^{ab}	78.18^{b}
N	76.82^{c}	79.89^{c}	78.38^{c}	79.10^{c}
NPK	77.01^{c}	78.92^{b}	77.88^{c}	79.24^{c}
N+ST	76.44^{bc}	78.82^{ab}	77.83^{c}	79.13^{c}

Values of BD lower than 76.0 kg hl^{-1} are marked red. Values in the column with the same letter were not significantly different at P < 0.05.

Table 4 Thousand kernel weight (TKW, g)

	S1		S2	
	2013	2014	2013	2014
Control	48.70^{ab}	49.92^{ab}	51.36^{ab}	51.19^{a}
SS	48.44^{ab}	50.86^{b}	53.86^{bc}	54.34^{b}
FYM	50.20^{a}	52.54^{c}	53.72^{bc}	54.69^{b}
N	46.01^{b}	47.03^{d}	56.01^{c}	55.93^{b}
NPK	50.22^{a}	49.25^{a}	54.97^{c}	56.04^{b}
N+ST	46.26^{b}	46.27^{d}	54.22^{abc}	55.22^{b}

Values in the column with the same letter were not significantly different at P < 0.05.

Table 5 Protein content (CP, %)

	S1		S2	
	2013	2014	2013	2014
Control	8.55[a]	8.11[a]	9.90[a]	9.47[a]
SS	9.18[b]	9.58[b]	12.00[bc]	11.49[b]
FYM	9.31[b]	9.60[b]	10.28[a]	10.84[b]
N	11.89[c]	12.94[c]	11.87[b]	12.44[c]
NPK	11.38[d]	12.86[c]	12.39[bc]	12.40[c]
N+ST	11.92[c]	12.65[c]	12.56[c]	12.28[c]

Values of CP lower than 11.5 % are marked red. Values in the column with the same letter were not significantly different at P < 0.05.

Table 6 Trait correlation

Site	Trait	DMY	BD	TKW
S1 2013	BD	0.95	-	-
	TKW	-0.30	-0.29	-
	CP	0.89	0.92	-0.54
S1 2014	BD	0.28	-	-
	TKW	-0.43	0.49	-
	CP	0.83	-0.11	-0.73
S2 2013	BD	0.96	-	-
	TKW	0.94	0.94	-
	CP	0.88	0.87	0.72
S2 2014	BD	0.98	-	-
	TKW	0.93	0.99	-
	CP	0.98	0.97	0.95

The highest coefficients of correlation (r) indicated in red were significant at P < 0.05

Conclusion

The highest DM yields by up to almost 3 t ha $^{-1}$ in treatments with mineral nitrogen were achieved. Values of bulk density were highly determined by weather conditions. Higher values of BD after mineral nitrogen fertilizing compare to the organic fertilizing were observed, except of S1 in year 2014. At S1 were higher values of TKW found for SS and FYM treatments, at S2 for treatments with mineral nitrogen. Sufficient values of CP were in N, NPK and N+ST treatments, at S2 even in FYM treatment. At S2 was found high correlation between parameters.

For mineral nitrogen treatments higher values of DM yield, BD, TKW and CP compare to organic fertilized treatments at S2 were achieved. At S1 were values of DM yield and CP in both years and BD in year 2013 higher than for organic treatments.

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Effect of increasing doses of boron on oil production of oilseed rape (*Brassica napus* L.)

MARIA VARENYIOVA, LADISLAV DUCSAY
Department of Agrochemistry and Plant Nutrition
Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2, 949 76 Nitra
SLOVAK REPUBLIC

xvarenyiova@uniag.sk

Abstract: The aim of the experiment was monitoring importance of boron in nutrition of oilseed rape (*Brassica napus* L.) as well as the effect of rising doses of boron on oiliness of rapeseed. The small plot field experiments were based at the Research – Breeding Station Vígľaš – Pstruša. The block method of experimental plot size of 10 m^2 in quadruplicate was used and hybrid Baldur was seeded and fertilized by the same doses of nitrogen and sulfur (183 kg $N.ha^{-1}$, 46.5 kg $S.ha^{-1}$) and different doses of boron (200 g $B.ha^{-1}$, 400 g $B.ha^{-1}$, 800 g $B.ha^{-1}$). The highest oil content in seed was observed at unfertilized control treatment 41.61 % and the lowest 37.49 % at the treatment, where only nitrogen and sulfur was applied. The increasing oil content in the seed was also observed. The oil content was by 3.96 % higher at the treatment fertilized by the highest dose of boron than at the treatment fertilized only by nitrogen and sulfur. From this it follows that the boron nutrition positively influences the oil content in seeds of oilseed rape (*Brassica napus* L.).

Key-Words: oilseed rape, boron, oil content

Introduction

Oilseed rape (*Brassica napus* L.) has really significant position in the global cultivation of oilseed crops. Its share in the total production of oil reached about 10.5 to 12.5% in recent years. Oilseed rape (*Brassica napus* L.) and the sunflower (*Helianthus annuus*) belong to the main oilseed crops in the Slovak Republic. The seed of oilseed rape (*Brassica napus* L.) is mostly processed in food industry to produce oil. The oilseed rape´s seed contains on average 45 to 47% oil, 28-30% protein, 3% fiber, nitrogen-free extract (NFE) and ash. About 20% of the total weight of the seed of oilseed rape consists of the testa, which contains 15% oil, 15 protein and 30% fiber [1]. Especially the content of the saturated and unsaturated fatty acids is the decisive factor of the quality of the obtained fat. The oil obtained from the seed is used also in industry to produce varnish, detergents and cleaning products, soaps, glycerin, cosmetics and in the leather and rubber industry, too. In addition to the use of oilseed rape (*Brassica napus* L.) in food industry, seed of oilseed rape (*Brassica napus* L.) is used to produce a biodiesel in recent years. This type of biodiesel is called rape methyl ester, which is used as a renewable source of energy instead of oil and fossil fuels [2]. Besides the nutrition and fertilization the content of oil in oilseed rape´s seeds is affected also by following factors: crop year (0.5%), variety (1 – 4%), soil compaction (0.5%), post-harvest treatment (0.5 – 1%) and some agrotechnical influence [3].

Dicotyledonous plants of the crucifer family demand a high level of boron. Especially oilseed rape (*Brassica napus* L.) is sensitive to boron deficiency [4]. The collapse of growth cone and roots are symptoms of boron deficiency. Strong growth of side shoots which usually collapse shortly is also observed. Chlorosis of upper leaves is sometimes noticed. Plants flower fewer, loose their flowers, produce only a few seeds or even any seeds. Pods and seeds are deformated. Symptoms of boron deciciency may be confused with symptoms of potassium deficiency [5]. At present, boron fertilization of oilseed rape is often discussed theme [6, 7]. The specificy of boron is its extremely narrow margin between lack and surplus (toxicity) [8]. For this reason, there are different opinions on boron fertilization methods, application rates and economics (profitability) of oilseed rape´s growing. Foliar application of boron in conditions of its limited availability may be more effective than the application of boron to the soil [6, 7]. Appropriate time for foliar nutrition is from a phase of extension growth to a phase of start flowering [2]. Boron

fertilization has a significant impact not only on yield seeds, but also on the oil content in seeds. Boron fertilization increases yield seeds and partially improves the quality of the oil [9, 10].

We focused on monitoring the effect of increasing foliar doses of boron on oiliness of oilseed rape (*Brassica napus* L.), in this paper. We explored its influence on the content of oil in oilseed rape´s seeds.

Material and methods

Small plot field nutritionists experiments were based at Research – Breeding Station Vígľaš – Pstruša. These experiments were focused on oilseed rape´s nitrogen, sulfur and boron nutrition. There was used block method of experimental plots with plot size 10 m^2 tested in quadruplicate. Hybrid Baldur was seeded. Quantity of seeds was 0.5 million germinable seeds per 1 ha. The oat (*Avena sativa* L.) was a previous crop. Research – Breeding Station Vígľaš - Pstruša belongs to the potato growing region at an altitude of 375 m. Soil type is podzolic brown soil. Climatic region is warm, slightly damp with cold winters. The average annual temperature during the growing season is 14°C . Average annual rainfall is 666 mm, with prevailing north winds. The amount of rainfall in autumn fluctuated in the long-term standard, in years of cultivation (see Table 1).

Also there was good conditions for emergence and rooting of oilseed rape. Temperatures were relatively steady during the years of cultivation (see Table 2).

In small plot field experiment was studied the effect of increasing foliar boron doses on oiliness of oilseed rape´s seeds. Before setting up a coppice was applied 100 kg of ammonium nitrate (27 kg $N.kg^{-1}$), 100 kg of potassium salt (50 kg $K.ha^{-1}$) and 100 kg of ammophos (12 kg $N.ha^{-1}$, 23 kg $P.ha^{-1}$). On the basis of soil agrochemical analysis, spring regeneration fertilizing of oilseed rape (*Brassica napus* L.) by nitrogen and sulfur in the form of ANAS was realized in growth phase BBCH 19-20. The production and qualitative foliar fertilization of UAN by Folibor (4% B) in growth phases BBCH 29 – 30 and BBCH 59 – 60. Doses of nitrogen, sulfur and boron are stated in the Table 3.

Soil analysis were performed by routin analytical methods (Mehlich III). The impact of treatments of fertilization on the content of oil in oilseed rape´s seed was monitored after the harvesting. The oil content was performed according to the standard STN 4610111-28. The determination was realized by the extraction for assistance to petroleum ether (50/70). The apparatus DET-GRAS N (P Selecta) was used for this determination. A superfluous extractant was distilled after the extraction. An obtained oil was drained and weighed. For the calculation of oil content in oilseed rape´s seed was used this formula:

$W = m_1/m_2 * 100$

m_1 = the amount of extracted oil (g)

m_2 = mass of the test sample (g)

Table 1 The average monthly precipitation in 2007-2009 (the evaluation of month precipitation normality according to the long-term average of 1961 – 2001)

Month	Long-term average	2007		2008		2009	
		Percipi-tation (mm)	Evaluation of normality	Percipi-tation (mm)	Evaluation of normality	Percipi-tation (mm)	Evaluation of normality
I.	28.1	-	-	29.9	normal	39.2	wet
II.	28.5	-	-	19.9	normal	40.2	wet
III.	29.8	-	-	49.6	wet	49.4	wet
IV.	46.7	-	-	36.3	normal	11.0	very dry
V.	63.9	-	-	64.2	normal	62.8	normal
VI.	85.2	-	-	59.4	dry	96.4	normal
VII.	75.6	-	-	117.5	wet	34.2	dry
VIII.	61.6	-	-	35.9	normal	35.6	normal
IX.	49.5	49.6	normal	40.9	normal	-	-
X.	45.7	38.9	normal	49.8	normal	-	-
XI.	53.5	18.6	very dry	35.8	normal	-	-
XII.	41.8	32.8	normal	83.5	very wet	-	-

Table 2 The average monthly temperatures in 2007-2009 (the evaluation of month air temperature normality according to the long-term average of 1961 – 2001)

Month	Long-term average	2007		2008		2009	
		Tempera-ture (ºC)	Evaluation of normality	Tempera-ture (°C)	Evaluation of normality	Tempera-ture (ºC)	Evaluation of normality
I.	-3.8	-	-	0.1	very warm	-4.4	normal
II.	-1.5	-	-	1.8	very warm	-1.2	normal
III.	2.8	-	-	4.0	normal	3.4	normal
IV.	8.4	-	-	9.7	normal	11.5	very warm
V.	13.1	-	-	14.5	normal	14.4	normal
VI.	16.3	-	-	18.4	very warm	16.1	normal
VII.	17.8	-	-	18.7	normal	19.8	very warm
VIII.	17.3	-	-	18.1	normal	19.5	very warm
IX.	13.2	11.8	cold	12.8	normal	-	-
X.	8.1	8.0	normal	10.0	warm	-	-
XI.	3.0	2.3	normal	4.9	very warm	-	-
XII.	-1.6	-2.6	normal	1.5	very warm	-	-

Table 3 Variants of oilseed rape nutrition, hybrid Baldur, Vígľaš

Treatment	Fertilization level						The total spring dose of N ($kg.ha^{-1}$) and B ($g.ha^{-1}$)	
	Regenerative fertilization		Production fertilization		Qualitative fertilization			
	BBCH (19 - 20)		BBCH (29 - 30)		BBCH (59 - 60)			
	N ($kg.ha^{-1}$)	S ($kg.ha^{-1}$)	N ($kg.ha^{-1}$)	B ($g.ha^{-1}$)	N ($kg.ha^{-1}$)	B ($g.ha^{-1}$)	N total	B total
1	0	0	0	0	0	0	0	0
2	93	46.5	60	0	30	0	183	0
3	93	46.5	60	200	30	0	183	200
4	93	46.5	60	400	30	0	183	400
5	93	46.5	60	400	30	400	183	800

Results and Discussion

The average yield of seeds of oilseed rape (*Brassica napus* L.) fluctuated from 2.15 to 3.19 $t.ha^{-1}$. The lowest average yield of rapeseed was found out in unfertilized control treatment 1 (2.15 $t.ha^{-1}$). Statistically significant differences compared to the unfertilized treatments 3, 4 and 5, where were applied different doses of boron (200 g, 400 g, 800 $g.ha^{-1}$). It is increase by 30.7%, 42.3% and 48.4%, in percentage term. The highest yield of seeds of oilseed rape (*Brassica napus* L.) 3.19 $t.ha^{-1}$ was found out where was to nitrogen and sulfur fertilization added boron at dose 800 $g.ha^{-1}$ [11]. The average oil content of variants moved a range from 37.49% to 41.61%. The highest average oil content was found at the first control (unfertilized) treatment, where the oil content was 41.61%. The lowest average oil content 37.49% was observed at the second treatment, where only nitrogen and sulfur was applied. The oil content at this treatment was statistically significantly lower by 9.9% compared to the control treatment. The averege oil content 41.45% was at the fifth experimental treatment where the highest boron dose 800 $g.ha^{-1}$ was applied. The oil content at the fifth treatment was statistically significant by 3.96% higher compared to the treatment fertilized only by nitrogen and sulfur.

From the following findings it follows that by application of increasing boron doses at different treatments was observed increasing oil content compared to treatment fertilized only by nitrogen and sulfur (see Table 4, 5).

Table 4 Effect of increasing doses of boron on oil content in oilseed rape seed (hybrid Baldur) in 2007-2008 and 2008-2009

Treatment	Oil content in seed %			
	2007 - 2008	2008 - 2009	Average 2007-2008 and 2008-2009 in %	Relatively in %
1	41.64	41.57	41.61 ± 1,45 Cb	100
2	37.31	37.66	37.49 ± 1,45 Aa	90.10
3	38.02	37.65	37.84 ± 1,45 ABa	90.94
4	38.91	42.46	40.69 ± 1,45 BCb	97.79
5	39.88	43.01	41.45 ± 1,45 Cb	99.62
LSD $_{0,05}$ treatment			2.25	
LSD $_{0,01}$ treatment			3.12	

Averages indicated by different letters are statistically significantly different on the significance level of α = 0.05 (small letters) and α = 0.01 (capital letters)

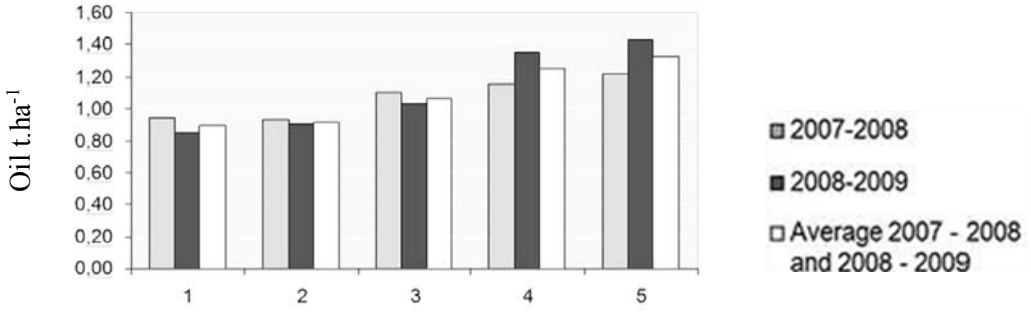
Table 5 Statistical evaluation of oil content in oilseed rape seed (hybrid Baldur) in experimental years (average of treatments)

Year	Oil content %	LSD test $_{0.05}$	LSD test $_{0.01}$
2007 – 2008	39.15	1.42	1.98
2008 – 2009	40.47		

Averages indicated by different letters are statistically significantly different on the significance level of α = 0.05 (small letters) and α = 0.01 (capital letters)

In other experiments was oilseed rape fertilized by nitrogen doses 0, 80, 160 and 240 kg N.ha^{-1}. There was found out the lowest oil content in seeds (43.8 – 44.1%) at treatments fertilized by nitrogen doses 160 and 240 kg N.ha^{-1}. The highest oil content 47.25% was at treatment, where wasn´t any nitrogen dose [12]. A large number of thesis clearly demonstrate the connection of boron with complex of processes of plant mineral nutrition [2]. In experiments with oilseed rape fertilized by 120 kg N.ha^{-1}, 20 kg P.ha^{-1}, 56.8 kg K.ha^{-1} and 300 g B.ha^{-1} was found out the oil content in seeds 42.5%. Compared to unfertilized treatment (41.9% oil), oil content increased by 0.6%, at the treatment fertilized by N, P, K and B [13]. The variety of oilseed rape Huashuang 4 was fertilized by 150 kg N.ha^{-1} a 250 g B.ha^{-1}. The oil content was 37.8%. It was by 3% higher compared to the control (unfertilized) treatment (34.8%) [10]. The highest average yield of oil 1.33 t.ha^{-1} was reached at fifth treatment, where was applied the highest boron dose. Compared to the unfertilized treatment, where was the highest average percentage oil content in dry mater, oil content at fifth treatment was by 0.43 t.ha^{-1} higher. The lowest average oil content 0.90 t.ha^{-1} was achieved at the unfertilizered control treatment (see Fig. 1). These findings indicate that boron is an essential nutrient for oilseed rape (*Brassica napus* L.) and boron nutrition positively influences the oil content in seeds of oilseed rape (*Brassica napus* L.). However, it is necessary to take into account the fact that oilseed rape (*Brassica napus* L.) is sensitive to boron, when the dose of boron is determinated.

Fig. 1 Effect of increasing doses of boron on the oilseed rape oil yield (hybrid Baldur) in 2007 – 2008 and 2008 – 2009.



Conclusion

The effect of the increasing foliar boron doses on the oiliness of rapeseed during the spring vegetation was monitored in the small field experiments. The highest average oil content was found out at the first control unfertilized treatment, where the oil content was 41.61%. The highest boron dose 800 g.ha^{-1} was applied at the fifth treatment. There was found out average oil content 41.45%, at this treatment.

The oil content at this treatment was statistically significantly higher by 3.96% compared to the treatment fertilized only by nitrogen and sulfur. The oil content in seeds of oilsed rape (*Brassica napus* L.) was stabilized by boron application. There was applied, besides nitrogen and sulfur, also boron 400 g.ha^{-1} and 800 g.ha^{-1}, at these treatments. From this it follows that, boron application in fertilization of oilseed rape (*Brassica napus* L.) is well-founded.

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Possibility of selection for higher seed vigour of barley

EVA VINTRLIKOVA, TOMAS STREDA
Department of Crop Science, Breeding and Plant Medicine
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xvintrl2@mendelu.cz

Abstract: High seed vigour is a precondition for fast and homogenous field emergence and good malting quality in barley. The aim of this study was to (i) assess the possibility of selection for greater vitality of barley seeds (*Hordeum vulgare* L.) in conditions of drought and cold, (ii) to quantify the relationship between the size of the root system of the parental plants and seed vigour of progeny. The root system size was measured by its electrical capacitance directly in field. The seed vigour of four spring varieties of malting barley and their mutual 12 combinations was assessed in three variants (without drought stress, under drought stress of -0.2 MPa and -0.5 MPa), in two years (2012 and 2013) and on the two locations (Hrubčice and Želešice). The vigour of 12 combinations from two locations was compared with vigour of their parents. Highly significant correlation was found in 2012 between the vigour of the mothers and their progenies ($r = 0.832$; $P = 0.01$), between that of fathers and of their progenies ($r = 0.882$; $P = 0.01$) and between the vigour of both parents and their progenies ($r = 0.894$; $P = 0.01$). The correlation between seed vigour combinations in F_3 generation (harvest 2012) and in F_4 generation (harvest 2013) was $r = 0.886$. This is another evidence for potential effective breeding for the seed vigour.

Key-Words: barley, root system size, seed vigour, stress conditions, drought escape

Introduction

The development of seed quality testing has become increasingly important due to global climate change. Laboratory testing of seed is usually carried out in ideal conditions (optimal temperature and air humidity). However, for practical purposes it is necessary to know the real behavior of seeds outside the optimum conditions [1]. Environmental factors affecting germination process significantly [2]. Seed quality is often understood as the vigour and viability of seeds [3; 4; 5; 6]. The most-used method of assessing the quality of seeds is testing of seed germination (realized under laboratory conditions). This characteristic of seeds indicates the ability to germinate in ideal conditions (ie. potential of seeds). Nevertheless, as noted above, for detecting the properties of the seeds is also important to test seed germination under field conditions or in conditions close to the situation in the field. It is in a situation where the plant is exposed to a stressful to temperatures, lack of water or nutrients [1]. At this point comes to the fore vigour of seeds, or the ability of seeds to germinate under stressful conditions. Vigour tests are according to [1] divided into three groups: tests based on germination, physiological and biochemical tests and multifactor tests. Most of them are based on the principle of simulation stress conditions. In any case, the ability of repeatability and reproducibility of methods are important.

The root system was due to lack of appropriate methods in breeding programs neglected. But recently is proved as successful the method of measuring the size of the root system by electric capacity [7]. The root system is cited as the most sensitive organs of the plant. Due to physiological and morphological characteristics responds the root system to the external environment sensitively than the aboveground part. In the absence of water is also affected by the passage of nutrients through membranes, and up to 40% [8]. In case of stress caused by lack of water react roots by growth of small diameter roots. This helps to enlargement of surface, through which is perhaps the absorption of water from the soil. During stress period may come up to

growth of roots into the deeper soil layers [9]. Important is whether grain vigour affects the rate of growth of the root system – whether is correlated with the size of the root system. It would make sense for higher resistance to drought („drought escape“). The roots have a certain degree of "memory" [10]. This feature is important in plant breeding. Successful selection focused on vigour increase may lead to tolerance of progeny to drought. The more vital seeds are then able to avoid any dryness in a period of stand establishment. In a case that it soon creates a sufficiently large root system it will be more resistant to drought and vegetation will be better emanate.

The purpose of this study was to obtain information about seed vigour of different genotypes of spring barley (in the case of stress incurred cool and dry); the ability to transfer the character to posterity; and interconnection of the root system with the grain vigour.

Material and methods

The spring barley malting varieties *Diplom*, *Jersey*, *Prestige* and *Saloon* were mutually crossed in 2010 in a diallel manner; i.e., each variety was crossed with all others, including reciprocally (as both mothers and fathers). The resulting 12 combinations (mark as U1 – U12; Parentage of the combinations:

U1 – Prestige × Saloon; U2 – Prestige × Jersey; U3 – Prestige × Diplom; U4 – Saloon × Prestige; U5 – Saloon × Jersey; U6 – Saloon × Diplom; U7 – Jersey × Prestige; U8 – Jersey × Saloon; U9 – Jersey × Diplom; U10 – Diplom × Prestige; U11 – Diplom × Saloon; U12 – Diplom × Jersey) were reproduced in winter 2010/2011 in a glasshouse. F_2 and F_3 generation was sown on 2012 and 2013 in a field at the Hrubčice Plant Breeding Station in the Czech republic (49°26'41.160"N, 17°11'54.940"E) in a fertile lowland (elevation 210 m; long-term annual average temperature, 8.5°C; long-term total annual rainfall, 578 mm; soil type, Haplic Chernozem with 38–39% clay particles) and at the Želešice Plant Breeding Station (49°07'10.390"N, 16°35'35.057"E) in a fertile lowland (elevation 205 m; long-term annual average temperature, 9.3°C; long-term total annual rainfall, 511 mm; soil type, Haplic Luvisol with 37% clay particles). Each combination was sown in four replicates between the two parents in rows consisting of 12 plants spaced at 0.1 × 0.1 m.

The root system size was measured in nanofarads [11]. One wire of the capacitance meter was connected using a clamp to all the basal parts of the plant of equal height. This height was near the soil but not in contact with it. The second wire, which leads the measuring current to the soil, was grounded in the spacing, midway between the measured plants. An impedance bridge ESCORT ELC-131D LCR meter (Escort Instruments Corporation, Taiwan) was used and set to parallel measured capacitance at a frequency of 1 kHz.

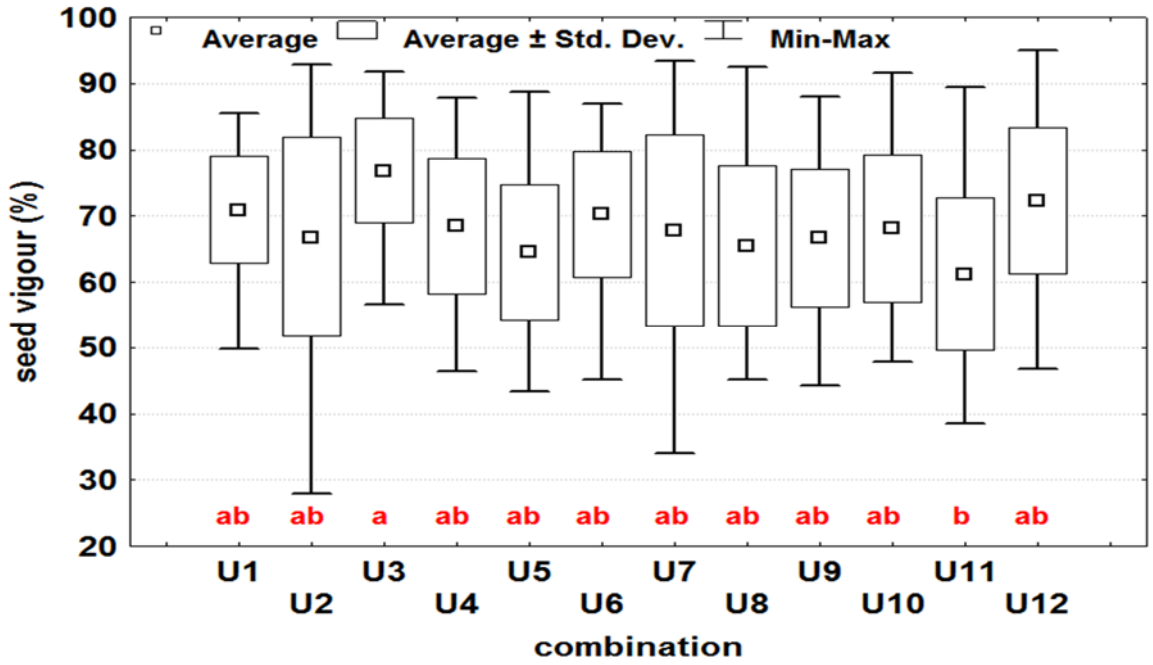
After dormancy, i.e., after approximately 100 days after harvest, the seed vigour was evaluated. The vigour of grains of the 12 combinations and their parents was evaluated at a low air temperature of 10°C and under a drought stress of -0.2 MPa and -0.5 MPa using a water solution of polyethylene glycol (PEG) 6000 [11;12] and without drought stress using of distilled water (control variant).

Each combination and parents was evaluated for 4 samples (2 locations × 2 years). The grains were placed between filter paper moistened with the PEG solution on stainless surfaces coated with plastic to prevent evaporation, which could change the solution concentration, in climate boxes (Q-CELL ST5/B/40) for 14 days. Vital, i.e., normal, seeds were regarded as those producing at least three roots when the hypocotyl attained at least one half of the grain length and were without mould.

Results and discussion

Evaluation of the average vigour of the combinations without regard to drought intensity is shown in Fig. 1. Significant differences are marked by different letters; different were only combinations U3 (Prestige × Diplom) and U11 (Diplom × Saloon). Average vigour of seed (12 combinations and their parents) was in 2012 when drought stress -0.2 MPa 85.6%, during stress -0.5 MPa 52.9%. Effects of drought stress level and locality on vigour of seeds were statistically significant (P = 0.01).

Fig. 1 Seed vigour of 12 resulting combinations (2012)



From the F_3 generation were selected combinations with highest vigour (U12 – Želešice – vigour 84%; U3 – Želešice – vigour 89%; U2 – Želešice – vigour 81%) and with the lowest vigour (U11 – Želešice – vigour 61%; U5 – Hrubčice – vigour 45%; U9 – Hrubčice – vigour 53%). These combinations and their parents were sown in experimental locations Hrubčice and Želešice the spring of 2013. In 2013, the average vigour of seeds (diameter of combinations and also of their parents) in the variant without drought stress 94.5% in a period of drought stress 94.7% -0.2 MPa and during stress by drought -0.5 MPa 66.2%.

The correlation between vigour of seeds by combinations in F_3 generation (harvest 2012) and in the F_4 generation (harvest 2013) was 0.886. The results showed that barley seed vigour is a trait affecting the field emergence and malting quality. The increased vigour can be successfully achieved with traditional breeding methods [13].

Testing the relationship between the size of the root system and vigour of seeds yielded no conclusive correlation (Tab. 1). However, a closer correlation can be expected only in dry years.

Table 1 Relationship between root system size (RSS) measurements and grain vigour (expressed by the correlation coefficients)

Treatment	Locality	RSS stem elongation	RSS heading	RSS grain filling	RSS average of 3 measurements
Control	Hrubčice	-0.239	0.061	-0.140	-0.166
	Želešice	-0.333	0.247	-0.082	-0.209
	Average	-0.088	0.110	-0.078	-0.076
Low stress	Hrubčice	-0.171	0.076	-0.043	-0.105
	Želešice	0.031	0.272	0.182	0.198
	Average	0.119	0.234	-0.006	0.151
Medium stress	Hrubčice	-0.047	0.279	0.102	0.057
	Želešice	-0.121	-0.064	-0.139	-0.199
	Average	0.064	0.144	-0.087	0.069

Conclusion

It can be seen that the vigour of parents was significantly correlated with vigour of their progeny. This is another evidence for potential effective breeding for the vigour. A higher response to selection can be expected for the grain vigour.

Our results indicate the possibility of the successful selection for higher seed vigour as an indicator of agronomic and malting quality, even in favourable weather years (the highest vigour during both years in average ranged between 85–94.5 % during drought stress 0.2 a 52.9–66.2% during drought stress 0.5). The results show, that barley seed vigour is a polygenic trait with importance for field emergence and also for malting quality, with good prospects of improvement by traditional breeding methods.

Acknowledgements

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The impact of *Fusarium* infection on the content of selected basic nutrients in the barley grain

MARTA ZAVRELOVA[1], KATERINA VACULOVA[1], IRENA SEDLACKOVA[1], MILENA ZACHARIASOVA[2], ZDENA VEPRIKOVA[2], PETRA SLAVIKOVA[2], PAVLINA SMUTNA[3], PETR ELZNER[3]
[1]Agrotest Fyto, s.r.o.,
Havlickova 2787/121, 767 01 Kromeriz,
CZECH REPUBLIC
[2]Institute of Chemical Technology, Prague,
Technicka 5, 166 28 Praha 6-Dejvice,
CZECH REPUBLIC
[3]Department of Crop Science, Breeding and Plant Medicine
Mendel University in Brno,
Zemedelska 1, 613 00 Brno,
CZECH REPUBLIC

zavrelova@vukrom.cz

Abstract: Economic quality of agricultural products is considerably influenced by a health status of crops. In the recent past, a greater attention has been paid to infections of cereals caused by pathogens of the genus *Fusarium*, which in addition to grain yield and quality decrease exhibit health risks to consumers, especially due to the presence of mycotoxins, their toxic secondary metabolites. A set of 28 cultivars and genetic resources of spring barley was grown in 2011–2013 at two locations (Kromeriz and Zabcice) under natural infection and inoculation with a selected strain of *Fusarium culmorum*. Barley materials differed in selected morphological traits and chemical characteristics. Contents of basic nutrients (N-substances, starch, beta-glucans, fat in %) and levels of selected mycotoxins (trichothecenes B, trichothecenes A, zearalenone + zearalenols and "emerging" mycotoxins in $\mu g.kg^{-1}$) in grain were determined. The differences due to the infection were evaluated both within the examined groups of barley and on the level of relationships between analysed nutrients and detected mycotoxins.

Key-Words: mycotoxins, genetic resources, spring barley, natural infection, inoculation, *Fusarium culmorum*

Introduction

Cereal diseases associated with infections of the pathogens from genus *Fusarium* are linked to reduced grain yield and quality and can result in substantial economic loss due to contamination of production with mycotoxins [1], their secondary metabolites. In addition, *Fusarium* mycotoxins, which are present in plants and products derived from them might pose significant health risks for humans and farm animals. Most common diseases which can be related to mycotoxicoses are nephropathy, various types of cancer, alimentary toxic aleukia, hepatic diseases, various hemorrhagic syndromes, and immune and neurological disorders [2].

Much attention is paid to the occurrence of these toxicogenic pathogens worldwide, however, prediction of the infection severity and level of mycotoxins in production still remains very difficult [3] because the extent of the occurrence and degree of infection of cereals are affected by a variety of external and internal factors (weather course, preceding crop, choice of cultivars and others). The spectrum of detected mycotoxins and information on their toxicity are broadened owing to the development of detection methods, instrumentation and a size of analysed samples. Until now, tens of mycotoxins have been known but a maximum acceptable level has been regulated by law for a low number of them (deoxynivalenol, zearalenone, ochratoxin A, fumonisins B1 and B2, and aflatoxins B1, B2, G1 and G2; recommendations for monitoring toxins T-2 and HT-2 in cereals and cereal products have been issued).

All these mycotoxins have been investigated for several decades and much information on their occurrence and toxic effects has been available.

Along with the development of instrumentation and detection methods, other *Fusarium* mycotoxins (so-called "emerging" mycotoxins, for example, enniatins, beauvericin, moniliformin and fusaproliferin and others [4]) have begun to occur, but little is known about their toxicity and especially about joint effects with other groups of mycotoxins.

Changes in the spectrum of mycotoxins are also encouraged by climatic changes when the occurrence of early less spread *Fusarium* spp. is recorded [5].

Infection of barley grain by pathogens of the genus *Fusarium* has an impact on the yields. It is also reported that germination ability, 1000-kernel weight, kernel plumpness, the content of N-substances and beta-glucans, and other malting parameters are affected [6, 7].

The objective of the paper was to examine the occurrence of basic groups of mycotoxins and changes in the content of basic nutrients in grain harvested from naturally and artificially infected stands of various cultivars, breeding lines and genetic resources of spring barley.

Material and Methods

Material, crop management practice Selected cultivars and genetic resources of spring barley (a total of 28 – Table 1), registered in the Czech Republic or maintained in the Genebank), with differences in the type of grain, ear and starch were grown in the years 2011–2013 at the locations Kromeriz (49°17‘N, 17°22‘E, 235 m a.s.l.; average annual temperature 8.7 °C, average annual precipitation 559 mm.) and Zabcice (49°01'N, 16°37´E, 184 m a.s.l.; average annual temperature 9.2 °C, average annual precipitation 480 mm) following standard preceding crops (plot area was 2.5-4.5 m^2), under conditions of both natural and artificial infection with *F. culmorum.* The inoculation with *F. culmorum* (W. G. Sm.) Sacc. strain KM16902; DON chemotype) was carried out according to Tvaruzek et al. [8] by spraying at an appropriate growth stage (BBCH 61-64), when 50 % of plants are at the beginning of anthesis (concentration of 0.5 million conidia of *F. culmorum* in 1 ml of inoculum, spray dose of 200 l.ha^{-1}).

Table 1: List of cultivars and their characteristics of the grain (Kromeriz and Zabcice, 2011–2013)

Cultivar	Caryop.[1)]	Typ of ear	Type of starch[2)]	Cultivar	Caryop.[1)]	Typ of ear	Type of starch[2)]
6NDRFG-1	cov	6-row	stand.	KM 2551	n	2-row	waxy
AC Klinck	cov	6-row	stand.	Kompakt	cov	2-row	stand.
AF Lucius	n	2-row	stand.	Krasnodarskij 95	cov	2-row	stand.
Amulet	cov	2-row	stand.	Madeira	cov	2-row	stand.
Annabell	cov	2-row	stand.	Merlin	n	2-row	waxy
Arra	cov	6-row	stand.	Nitran	cov	2-row	stand.
CDC Rattan	n	2-row	waxy	Nordus	cov	2-row	stand.
Cebada Capa	cov	6-row	stand.	Pejas	cov	2-row	stand.
Diplom	cov	2-row	stand.	Primus	cov	2-row	stand.
Druvis	cov	6-row	stand.	Prosa	cov	2-row	stand.
Henrike	cov	2-row	stand.	Ricardo	cov	6-row	stand.
Chevron	cov	6-row	stand.	Rolfi	cov	6-row	stand.
KM 1057	n	2-row	stand.	Taiga	n	2-row	stand.
KM 2460	cov	2-row	waxy	Waggon	cov	2-row	stand.

[1)] - huskinness of caryopsis: cov = covered, n = naked (hulless) ; [2)] - type of starch: stand. = standard starch with ca 25% of amylose and 75% of amylopectin, waxy = starch with low level of amylose

Analysis of quality parameters Moisture content of flours was determined according to Method CSN ISO 712 (2003). Protein content was determined by Method ICC STANDARD No. 167 (2000) using the conversion factor 6.25, starch content by CSN EN ISO 10520 and fat content by Javorsky [9]. Beta-glucans were determined enzymatically using the beta-glucan enzymatic assay kit (Megazyme Ireland International, Ltd., Wicklow, Ireland) following the procedure of Megazyme (ICC Nr. 166).

Analysis of mycotoxins Sample preparation for mycotoxins analysis was performed by means of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure, *i.e.* acetonitrile/water extraction followed by phases partition induced by inorganic salts addition, where

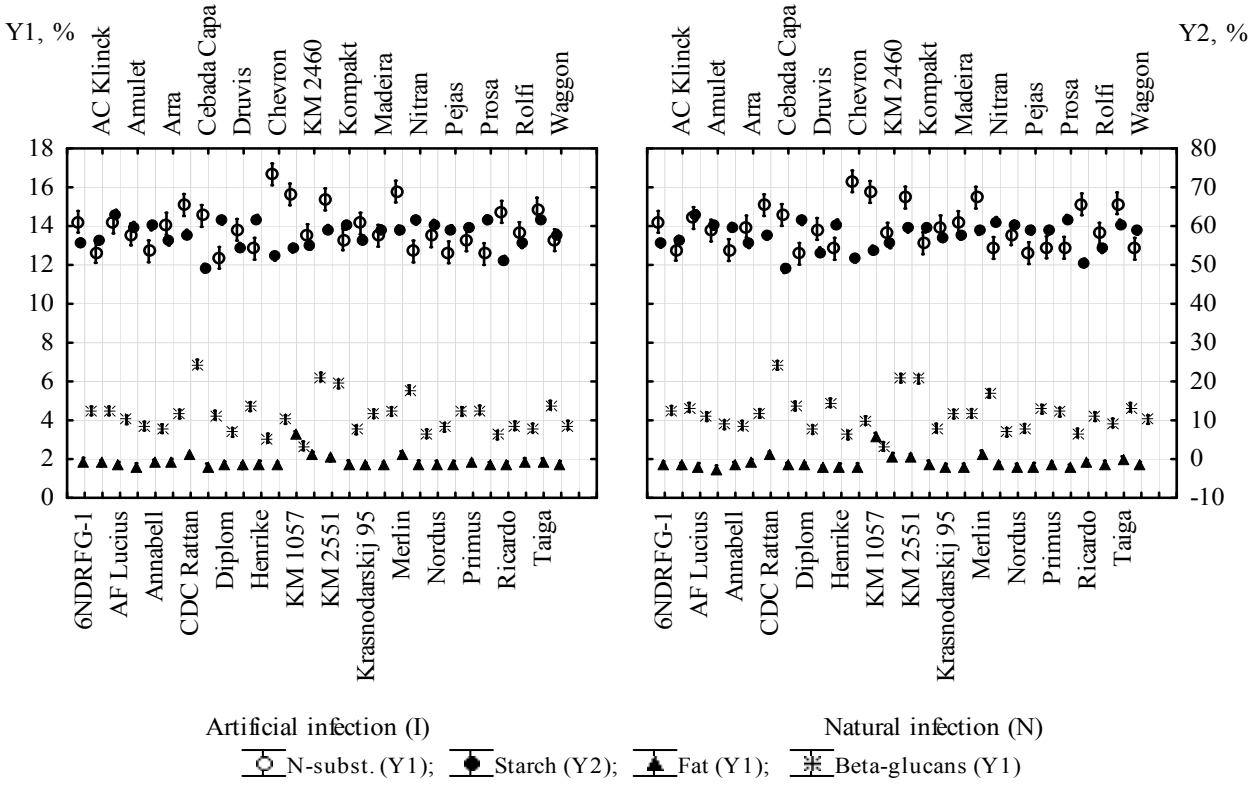
mycotoxins are salted out in the upper acetonitrile phase, and polar matrix co-extracts remain in aqueous phase. For separation and target mycotoxins detection, ultra-high performance liquid chromatography (analytical column HSS T3 - 100 x 2.1 mm; 1.8 µm) coupled with tandem mass spectrometry (QTRAP 5500, AB Sciex) was used. Quantification of mycotoxins was realized by the external matrix-matched calibration method, results were corrected for recovery determined based on spikes. Summary concentrations of four groups of mycotoxins (all in $\mu g.kg^{-1}$) were determined: trichothecenes B: nivalenol, deoxynivalenol, deoxynivalenol-3-glucoside, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon X; trichothecenes A: T-2 toxin, HT-2

Table 2: Summary concentrations of mycotoxin groups ($\mu g.kg^{-1}$) in natural and inoculated variants

Factor /	Trichothecenes B		Trichothecenes A		ZEA + ZOLs		„emerging“	
MXT/infection[1)]	N[1)]	I[1)]	N	I	N	I	N	I
naked grain	204.2 a	8 541.0 a	70.7 a	**96.0 b**	5.5 a	181.6 a	589.5 a	463.4 a
covered grain	193.8 a	**13 678.8 b**	40.8 a	45.5 a	4.2 a	406.6 a	520.9 a	331.8 a
standard starch	191.1 a	13 083.0 a	45.9 a	51.9 a	3.9 a	378.4 a	550.9 a	359.5 a
waxy starch	225.5 a	9 546.9 a	55.1 a	82.7 a	8.0 a	238.2 a	443.7 a	362.8 a
2-row	218.9 a	10 697.2 a	51.2 a	**67.9 b**	5.8 a	249.1 a	518.1 a	343.7 a
6-row	139.0 a	**17 279.5 b**	37.2 a	27.5 a	1.2 a	**631.6 b**	579.3 a	366.5 a
location: Kromeriz	136.5 a	**22 475.7 b**	11.3 a	17.5 a	0.7 a	**713.8 b**	218.2 a	151.2 a
location: Zabcice	255.5 a	2 680.0 a	83.2 a	**95.2 b**	8.3 a	2.9 a	**853.0 b**	**568.8 b**
year: 2011	170.3 a	**19 255.6 c**	16.4 a	18.1 a	12.3 a	**990.1 b**	479.4 a	95.3 a
year: 2012	248.3 a	12 597.5 b	**58.1 b**	**94.5 c**	0.6 a	77.7 a	547.3 a	**483.2 b**
year: 2013	169.5 a	5 880.5 a	**67.2 b**	56.4 b	0.5 a	7.2 a	580.2 a	**501.5 b**

[1)] - N – variant with natural infection, I – variant with F. culmorum inoculation ; [2)] – different letters in the column indicate significant difference at P0.05, bold indicates statistically significant difference

Fig. 1 – Average contents and variability of basic nutrients in spring barley stands after inoculation with *F. culmorum* and under natural infection (Kromeriz and Zabcice, 2011–2013)



toxin, diacetoxyscirpenol, neosolaninol; zearalenone + zearalenols (ZEA+ZOLs): zearalenone, α-zearalenol, β-zearalenol; "emerging" mycotoxins: beauvericin, enniatin A, enniatin A1, enniatin B, enniatin B1.

Data analysis: Experimental data were assessed using STATISTICA software, version 12.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and Discussion

Basic nutrients

Individual cultivars responded to inoculation by their chemical composition in a different way (Fig. 1). In a whole set of genotypes at least one of the examined nutrients decreased, but in naked line KM 2551 with waxy starch the decrease was found in all of them. In contrast, some materials increased the content in the inoculated variant on average of all years. The content of N-substances was increased most (+0.44%) whereas starch content decreased most (-0.97%) in cv. Waggon. In cv. Madeira, however, an opposite effect of inoculation was observed (N-substances -0.71%, starch +1.51%). The content of beta-glucans decreased in most cases due to strong infection pressure. The highest reduction in beta-glucan content was assessed in 6-row cvs. Ricardo and Cebada Capa (both -0.47%).

Concentrations of mycotoxins were different in naturally and artificially infected variants and ranged on average from 0.5 to 1065.6 $\mu g.kg^{-1}$ (natural infection) and from 7.7 to 31144.2 $\mu g.kg^{-1}$ (inoculation with *F. culmorum*). Statistically significant differences were found in concentrations of individual groups of mycotoxins between the natural and inoculated variant (data not shown) and also between groups of barley genotypes, experimental locations and years (Table 2). However, over-limit contents of mycotoxins monitored in foods and feeds were not determined in any sample from the naturally infected variant.

Trichothecenes B

The average values of summary concentrations of all mycotoxin groups are in Table 2. In particular years, the content of trichothecenes B significantly differed only in the inoculated variant. The highest level was assessed in 2011, which considerably differed from the years 2012 and 2013 (19255.6, 12597.5 and 5880.5 $\mu g.kg^{-1}$, respectively). Considerable differences were also recorded between locations; significantly higher content of trichothecenes B was in Kromeriz (22475.7 *vs.* 2680 $\mu g.kg^{-1}$, respectively). In the inoculated variant the highest total contents of trichothecenes B were determined in 6-row cultivars (17279.5 $\mu g.kg^{-1}$), which is consistent with the results by Legzdina, Buerstmayr [10]. An exception was 6-row cv. Chevron which had lower concentrations of all studied groups of mycotoxins in the inoculated variant (data not shown). This cultivar was resistant to *Fusarium* spp. infection already in earlier experiments [10, 11, 12].

Under higher infection pressure, a significantly lower content of trichothecenes B was determined in naked genotypes in comparison with covered ones (8541 $\mu g.kg^{-1}$ *vs.*13678.8 $\mu g.kg^{-1}$). Similar findings are also reported by foreign authors [10, 13 and others] who found that a major portion of trichothecenes was accumuluted in the hulls of barley. Correlations between summary contents of the examined mycotoxin groups and individual nutrients are given in Table 3. Positive and in most cases significant correlations were calculated between the content of trichothecenes B and the content of starch and fat in the inoculated variant in all groups of genotypes (r = 0.16-0.56**). The increased concentration of trichothecenes B reduced mainly the content of N-substances and beta-glucans when the correlations were stronger under inoculation. At the infection of barley kernels, some authors detected higher levels of proteolytic and cytolytic enzymes that are attributed to both defensive reaction of the genotype and higher enzymatic activity of the pathogen [14, 15, 16]. In the naturally infected variant, a significant negative correlation was found between the presence of trichothecenes B (but as well as ZEA + ZOLs and "emerging" mycotoxins) in grain and the content of beta-glucans in the group with waxy starch. Given that the beta-glucans are building blocks of the cell walls, it can be concluded that it is a mechanical barrier to the penetration of the pathogen into the cells.

Trichothecenes A

The toxicity of these *Fusarium* mycotoxins is very high, which is documented by tolerable daily intake (TDI) of 100 $\mu g.kg^{-1}$ b.w. for the sum of T-2 and HT-2 toxins [17]. In Zabcice, the content of trichothecenes A in the inoculated variant was significantly higher than in the naturally infected one (95.2 *vs.*17.5 $\mu g.kg^{-1}$, respectively). Average contents of trichothecenes A were significantly higher in 2-row and naked genotypes and non-significantly higher in genotypes with waxy starch.

Table 3: Correlations between summary contents of the examined mycotoxin groups and nutrients

		Trichoth. B		Trichoth. A		ZEA + ZOLs		„emerging“	
Nutrient	Factor	N[1)]	I[1)]	N	I	N	I	N	I
N-subst.	naked	0.12	-0.25	0.44**	0.50**	-0.09	-0.37*	0.39*	0.42*
	covered	-0.07	-0.25**	0.24**	0.16	-0.18*	-0.22*	0.08	0.31***
	standard	-0.06	-0.28**	0.31***	0.26**	-0.17*	-0.22**	0.20*	0.34***
	waxy	0.15	-0.14	0.43	0.55**	-0.08	-0.37	0.21	0.46*
	2-row	-0.04	-0.25**	0.34***	0.38***	-0.16	-0.26**	0.18	0.36***
	6-row	0.20	-0.42**	0.32*	0.31*	-0.25	-0.32*	0.19	0.35*
Starch	naked	-0.11	0.44**	-0.41*	-0.43**	0.06	0.46**	-0.40*	-0.30
	covered	0.06	0.24**	-0.21*	-0.13	0.12	0.18*	-0.22*	-0.32***
	standard	0.02	0.21**	-0.24**	-0.17*	0.11	0.16	-0.28**	-0.28**
	waxy	0.03	0.56**	-0.25	-0.31	0.16	0.49*	-0.19	-0.28
	2-row	-0.03	0.41***	-0.34***	-0.35***	0.11	0.32***	-0.28**	-0.27**
	6-row	-0.33*	0.46**	-0.39**	-0.48**	0.29*	0.38**	-0.35	-0.55***
BG[2)]	naked	-0.13	-0.01	-0.15	-0.02	-0.18	-0.02	-0.33	-0.06
	covered	-0.07	-0.26**	-0.07	-0.06	-0.10	-0.15	-0.08	-0.03
	standard	-0.07	-0.23**	-0.10	-0.06	-0.09	-0.15	-0.12	0.03
	waxy	-0.50*	-0.35	-0.17	-0.08	-0.67***	-0.21	-0.47*	-0.21
	2-row	-0.08	-0.19*	-0.03	0.03	-0.10	-0.09	-0.19*	-0.02
	6-row	0.04	-0.44**	-0.17	0.05	-0.15	-0.37**	-0.12	0.07
Fat	naked	0.33*	0.28	0.26	0.08	0.18	0.33*	0.34*	-0.15
	covered	0.20*	0.30***	0.13	0.09	0.08	0.27**	0.06	-0.01
	standard	0.16	0.16*	0.28**	0.21*	0.04	0.15	0.25**	-0.02
	waxy	0.46*	0.56**	-0.03	-0.06	0.41*	0.67***	0.18	-0.13
	2-row	0.19*	0.20*	0.27**	0.18*	0.09	0.20*	0.23**	-0.02
	6-row	0.12	0.32*	0.05	-0.27	-0.26	0.31*	0.08	-0.13

[1)] N – variant with natural infection, I – variant with F. culmorum inoculation; [2)] BG – beta-glucans

The high level of mycotoxins of this group in naked barley cv. Merlin was also detected by Malachova et al. [18]. As in the case of trichothecenes B, high resistance to the accumulation of this mycotoxin group was detected in cv. Chevron (data not shown). In contrast to the results obtained with trichothecenes B, there was no significant relationship between the content of beta-glucans and trichothecenes A. Positive correlations were calculated between summary concentration of trichothecenes A and the content of N-substances (r = 0.16-0.55**) and partly fat content, and on the contrary, negative correlations with starch content.

ZEA + ZOLs

This group of mycotoxins in barley grain was present in lowest concentrations but, as reported by Zinedine et al. [19], their toxicity for humans and animals is higher than in trichothecenes B. The highest average levels were measured in the inoculated variant in 2011 (990.1 μg.kg^{-1}) and in Kromeriz (713.8 μg.kg^{-1}), and in 6-row genotypes (631.6 μg.kg^{-1}). Consistent with the results observed for trichothecenes B, in this mycotoxin group there were also significant correlations with starch and fat content and negative with the content of N-substances in the inoculated variant. In the naturally infected variant, these relationships were also found, nevertheless, they were not always significant. No considerable relationships between the content of beta-glucans and ZEA+ZOLs, except genotypes with waxy starch, were found.

"Emerging" mycotoxins

The significantly higher (3.7 to 3.9 times) content of this group of mycotoxins was determined in samples from Zabcice in both infection variants. Average contents of these mycotoxins (especially enniatins B – data not shown) were lower in the inoculated variant where pathogen *F. culmorum* obviously predominated due to inoculation and which, as reported by Stepien [20], produces mainly trichothecenes B and zearalenone. In the variant with a natural spectrum of *Fusarium* pathogens, especially in Zabcice, *F. poae* [21] was prevalent ranking among producers of mycotoxins falling in the group so-called "emerging". The relationships determined between contents of basic nutrients and these mycotoxins were similar to those for trichothecenes A. A significant negative correlation was calculated between the accumulation of "emerging" mycotoxins and the content of beta-glucans in waxy genotypes and 2-row barleys in samples from the naturally infected variant.

Conclusion

In 2011–2013, levels of four groups of *Fusarium* mycotoxins (trichothecenes B, trichothecenes A,

zeralenone+zearalenols and "emerging" mycotoxins) were investigated in different cultivars, breeding lines and genetic resources of spring barley. The genotypes were grown at two locations (Kromeriz and Zabcice) in two variants, natural infection and inoculation of stands with *F. culmorum*, and the content of basic nutrients in samples from both variants was determined. Strong pressure of the pathogen due to the inoculation was most apparent in 2011 and in Kromeriz where the major contaminants were groups of trichothecenes B and ZEA + ZOLs. In 2012 and 2013 and in Zabcice, higher levels of trichothecenes A and "emerging" mycotoxines were detected not only in the inoculated but especially in the naturally infected variant. The inoculation of stands resulted in the decrease in at least one of the examined nutrients, differently depending on a particular cultivar/line or genetic resource and decrease in beta-glucan content in nearly all barley materials. Dividing the set of examined barley genotypes according to kernel, starch or ear row type affected the level and direction of interrelationships between mycotoxin groups and basic grain nutrients.

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Section – Animal Production

The quality of eggs of gene reserve Czech Golden Spotted Hens kept in backyard under different external conditions

VOJTECH ANDERLE, MARTINA LICHOVNIKOVA, ALENA PRZYWAROVA, ELIŠKA DRACKOVA
Mendel University in Brno
Faculty of Agronomy
Department of Animal Breeding
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

vojtaanderle@seznam.cz

Abstract: In Czech Republic, there are two national poultry breeds, Czech Goose and Czech Golden Spotted Hen (CZH), which are under governmental project of gene reserves. In 2012 there were less than 220 CZH both males and females in this program. The aim of this study was to evaluate the egg quality of CZHs included in the project of gene reserves kept by six different breeders in backyard under different both nutrition and environmental conditions. The hens were the same age. Eggs for measurement their quality were collected three times during three months. The egg weight ranged from 51.7g to 60.7g ($P<0.05$). The eggshell strength was, except one flock, higher than 30.0N, and the differences among the flocks were no significant ($P>0.05$). There was high variability in eggshell color expressed as SCI (shell color index) ($P<0.05$). But there were not significant differences in yolk weight. The weight of yolk was higher than 18g in all flocks. The yolk proportion was significantly ($P<0.05$) different only between two flocks (36.6% versus 33.4%). Although the hens were at the same age and representatives of relatively small population the egg quality was very unbalanced. The main reason of the variability is probably different nutrition among the breeders. Advantage of the eggs is high weight of yolk and consequently yolk proportion.

Key-Words: egg weight, eggshell color, yolk proportion, Czech Golden Spotted Hen

Introduction

During the last 50 years of the 20th century, about 20% of livestock and poultry breeds have become extinct, and the remainder is at risk. This erosion of unique biodiversity is due to changes in farm practices developed in the West that involve mono-breed, intensive farming systems that are unsustainable. The close symbiotic relationship of *Homo sapiens* and domestic animals and birds over millennia is changing, resulting in a lost understanding of sustainability among urban communities (Hodges, 2006). In European countries, various governmental, non-governmental, and private organizations try to preserve genetic diversity of livestock in situ (e.g., by stimulating the use of indigenous, rare breeds by farmers; in nature reserves; or in noncommercial farms). In the case of poultry, maintaining in situ populations of the noncommercial (fancy) breeds largely relies on hobby farmers. In addition to in situ conservation, gene banks are being established for ex situ conservation (Wiekders et al, 2006). In Czech Republic, there are two national poultry breeds, Czech Goose and Czech Golden Spotted Hen (CZH), which are under governmental project of gene reserves. Despite the financial support for the breeders the numbers of CZHs included in the gene reserves project was no more than 220 both males and females together in 2012 and they were kept by six breeders. The breeders focus their attention mainly on exterior characteristics to the detriment of performance. The laying intensity ranges from 58 to 110 eggs per hen and the egg weight from 51.5 to 56.5 g in years 2007-2012. The aim of this study was to evaluate egg quality of CZHs, included in the project of gene reserves, kept in backyard by six different breeders under different both nutrition and environmental conditions.

Material and methods

All CZHs were the same age and they were kept by six breeders under different nutrition and environmental condition in backyards. All breeders were under the gene reserves project. They kept different number of hens in their flocks with

different sex ratio and different diets. Eggs for measurement egg quality were collected three times in three months; 20 eggs every time per each flock, in 70, 78, 84 weeks of hens age. Followed characteristics were evaluated: egg weight, yolk weight, proportion and color, eggshell thickness, weight and proportion, eggshell strength, eggshell color, albumen weight, proportion and Haugh units.

The shell breaking strength was determined on the vertical axis using Egg Force Reader. The color of the yolk was measured using the DSM Yolk Color Fan. The albumen height was measured using a digital micrometer (TSS EQS, England). Haugh units (HU) were calculated as indicated by Haugh (1937). Shell weight and shell thickness were determined after washing and drying of shells. The shell thickness was evaluated using the micrometer expressed as mean of thickness on equator and both poles. Eggshell color was determined by the L*a*b* Color System using a spectrophotometer (CM-2600d, Konica Minolta, Osaka).

- L*: lightness (value between 0= black and 100=white)
- a*: hue as a function of the red-green scale (<0 = green, >0 = red)
- b*: hue as a function of the blue-yellow scale (<0 = blue, >0 = yellow)

Color was expressed by shell color index (SCI) based on the three color parameters calculated with the formula SCI = L* - a* - b*, lower values indicating darker shell color (Cavero et al., 2012). Standard conditions for the SCI method were set: measurement gap 8mm, 10° standard observer, standard illuminant D65 – average daylight including ultraviolet wavelength region. The color of eggshell was measured at the equatorial area.
Data obtained from this experiment were analyzed using the single factor analysis of variation. Data were followed by LSD test using the software package Unistat 5.1 (UNISTAT Ltd, ENGLAND).

Results and discussion

Egg weight, eggshell strength and eggshell color (SCI) are shown in table 1. The egg weight was very unbalanced among the flocks (P<0.05). The highest difference was 9g. Except two flocks, the egg weight was higher than Stanishevkaya and Toritsina (2007) reported for local unselected breed (55g). Zanon et al. (2006) also published lower egg weight for local light Italian breeds Modenese and Romagnolo (53.7g and 54.0g).Turkish breeds (Benizli and Gerze) also lay eggs with lower weight than CZHs (53.94 and 54.30g, Sarica et al., 2006). The main reason for the higher egg weight in comparison with other authors is also probably the higher age of the CZHs.

The eggshell strength was sufficient, except one flock (28.6N) it was higher than 30.0N, and the differences among the flocks were no significant (P>0.05). The significantly darkest eggshell color expressed as SCI was found in fifth flock in comparison with the others (P<0.05). It means that there were also eggs with brown eggshell but this color is not in agreement with breed standard. The eggshell should be cream-colored. On the other side there were eggs with white eggshell too.

Table 1 Egg quality of CZHs – weight, eggshell strength and eggshell color

Flock	*Egg weight (g)*	*Eggshell strength (N)*	*SCI*
	mean ± SE	mean ± SE	mean ± SE
1	54.8 ± 0.63^{ab}	28.6 ± 0.91^{a}	75.3 ± 1.14^{cd}
2	51.7 ± 1.23^{a}	32.4 ± 1.01^{a}	68.9 ± 1.51^{bc}
3	57.8 ± 0.85^{bc}	32.7 ± 1.54^{a}	76.3 ± 0.80^{d}
4	55.5 ± 0.79^{ab}	33.2 ± 1.04^{a}	65.9 ± 1.76^{ab}
5	60.7 ± 0.95^{c}	32.1 ± 1.77^{a}	60.9 ± 1.48^{a}
6	56.7 ± 0.67^{bc}	30.2 ± 1.69^{a}	75.8 ± 1.14^{cd}

a, b *indicate statistical significant difference between groups (P<0.05) for the same characteristics*

Eggshell weight, proportion and thickness are shown in table 2. There was no significant difference among the flocks in eggshell thickness. There were significant differences in eggshell weight only between three flocks (P<0.05). The proportion of eggshell from the egg weight ranged

from 7.8 to 9.3% and the thickness form 0.325 to 0.347mm. Zanon et al. (2006) reported higher eggshell proportion for both local Italian breeds (12.88 and 13.29%) but the eggshell thickness were similar to CZHs (0.337 and 0.369mm).

Table 2 Eggshell quality of CZHs

Flock	*Eggshell weight (g)*	*Eggshell proportion (%)*	*Eggshell thickness (mm)*
	mean ± SE	mean ± SE	mean ± SE
1	4.67 ± 0.099^{ab}	8.5 ± 0.13^{ab}	0.325 ± 0.50^{a}
2	4.82 ± 0.141^{ab}	9.3 ± 0.13^{c}	0.343 ± 0.61^{a}
3	5.04 ± 0.102^{b}	8.7 ± 0.16^{bc}	0.347 ± 0.69^{a}
4	5.03 ± 0.112^{b}	9.1 ± 0.17^{bc}	0.344 ± 0.82^{a}
5	4.96 ± 0.109^{ab}	8.2 ± 0.21^{ab}	0.339 ± 0.54^{a}
6	4.42 ± 0.149^{a}	7.8 ± 0.28^{a}	0.346 ± 0.71^{a}

a, b *indicate statistical significant difference between groups (P<0.05) for the same characteristics*

Yolk quality is shown in table 3. There were no significant differences in yolk weight (P>0.05). The weight of yolk was higher than 18g in all flocks.

Concerning yolk proportion there was significant difference only between two flocks (P<0.05). Very high variability was found in yolk color (P<0.05). As the diet has the highest effect on egg weight and consequently yolk weight and yolk color too, there were significant differences, because all hens fed the different diets. If the diet is the same to all layers, the color of the egg yolk of laying Czech hens is well balanced (Anderle et al., 2014).

Anyway the yolk weight of CZHs is relatively high. Stahishevskaya and Toritisna (2007) published weight of yolk in local breeds 18.2g, in Rhode Island 18.3g and in Leghorns 17.4g. Comparison of local and modern breeds revealed, that enlargement of egg weight for 8-10g was caused by increase of portion of albumen in eggs (Stahishevskaya and Toritisna, 2007). On the other hand it is necessary remind relatively high age of the CZHs in this experiment.

The yolk proportion was significantly (P<0.05) lower in fifth flock (33.4%) in comparison with the first flocks (36.6%). Zanon et al. (2006) found similar yolk proportion in one of the local Italian breeds; 34.69% in Modenese and lower proportion 32.35% in Romagnolo in comparison with CZHs. On the other side in commercial white hybrids these authors reported 30.8% of yolk and even only 26.2% in commercial brown laying hens. However Turkish breeds lay eggs with comparable weight of yolk (19.38 and 18.94g), and consequently with higher yolk proportion (35.94 and 34.90 %). Other authors also reported significantly (P<0.05) higher yolk weight of local breed in comparison with commercial layers (Rizzi and Chiericato, 2005).

In albumen quality significant difference were found among the flocks (table 4), except albumen proportion. There was significant difference in Haugh units only between two flocks (P<0.05).

Albumen weight ranged from 28.5 to 36.9g (P<0.05).

At feeding the same diets to CZHs under the same housing system and environmental condition, there were no significant differences among the flocks in majority of observed characteristics (Anderle et al., 2014). Despite the high variability in egg weight the advantage of the CZHs eggs is high weight of yolk and consequently yolk proportion, which is higher than in commercial hybrids.

Table 3 Yolk quality of CZHs

Flock	*Yolk weight (g)*	*Yolk proportion (%)*	*Yolk color*
	mean ± SE	mean ± SE	mean ± SE
1	20.1 ± 0.38^{a}	36.6 ± 0.55^{a}	10.5 ± 0.23^{bc}
2	18.3 ± 0.75^{a}	35.0 ± 0.87^{ab}	9.2 ± 0.26^{a}
3	19.7 ± 0.37^{a}	34.2 ± 0.57^{ab}	9.8 ± 0.23^{abc}
4	19.1 ± 0.35^{a}	34.3 ± 0.62^{ab}	10.1 ± 0.33^{abc}
5	20.3 ± 0.37^{a}	33.4 ± 0.53^{b}	11.1 ± 0.23^{c}
6	19.8 ± 0.48^{a}	34.9 ± 0.73^{ab}	9.6 ± 0.35^{ab}

a, b *indicate statistical significant difference between groups (P<0.05) for the same characteristics*

Table 4 Albumen quality of CZHs

Flock	*Albumen weight (g)*	*Albumen proportion (%)*	*Haugh units*
	mean ± SE	mean ± SE	mean ± SE
1	32.8 ± 1.10^{ab}	59.9 ± 1.89^{a}	58.8 ± 1.39^{a}
2	28.5 ± 0.68^{a}	55.6 ± 0.86^{a}	68.8 ± 1.65^{b}
3	34.7 ± 1.42^{b}	59.9 ± 1.72^{a}	62.8 ± 1.94^{ab}
4	33.1 ± 0.97^{ab}	59.7 ± 1.69^{a}	60.9 ± 1.87^{ab}
5	36.9 ± 1.22^{b}	60.7 ± 1.77^{a}	63.8 ± 1.85^{ab}
6	33.1 ± 0.86^{ab}	58.5 ± 1.45^{a}	61.4 ± 1.77^{ab}

a, b *indicate statistical significant difference between groups (P<0.05) for the same characteristics*

Conclusion

In conclusion, as both diet and environmental conditions have the highest impact of external factors on eggs quality the egg quality of Czech Golden Spotted hens kept by six breeders in backyards was very unbalanced, despite all breeders were included in the project of gene reserves. There were found significant differences among the flocks in followed characteristics (P<0.05): egg weight, yolk color and its proportion, albumen weight, Haugh units, eggshell weight, proportion and its color.

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The effect of milk yield on conversion coefficients between lactations of Czech Fleckvieh cows

JIRI ANDRYSEK, GUSTAV CHLADEK, JANA JAVOROVA, MILENA VELECKA, MILAN VECERA, DANIEL FALTA
Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jiri.andrysek@mendelu.cz

Abstract: The number of lactations of dairy cows has a high influence on milk production, especially in first three lactations. The observation was carried out in the group of 8144 cows with 75-100% proportion of Czech Fleckvieh cattle in genotype. All cows finished the first three lactations. The total mean milk yield was 6154.45 kg in the first lactation, 7208.32 kg in the second lactation and 7506.72 kg in the third lactation. The coefficient for calculation of the milk yield for 1st, 2nd and 3rd lactation were 1.17; 1.22 and 1.04 respectively. For detailed evaluation of the influence of the estimation coefficients between first three lactations the herd was divided into five groups according to the milk yield in the first lactation (up to 4000 kg, 4001-5000 kg, 5001-6000 kg, 6001-7000 kg and over 7000 kg). For each group the estimation coefficients were calculated. The estimation coefficients between lactations decreased with increasing yield in the first lactation. It was in all cases statistically highly significant.

Key-Words: Czech Fleckvieh, milk yield, conversion coefficients

Introduction

The number of productive lactation has significant decreasing tendency in last years. The influence of number of lactation is unquestionable, therefore the main interests of the farmers are focused on determination the relationships between first three lactations. For the estimation of milk yield for following lactations the estimation coefficients are used. These coefficients are based on relationships between lactations of dairy cows which already was the completed [1]. Already in 1969 [7] the yield in the first five lactations of Czech Fleckvieh cattle was studied. The study from [7] indicates the production in 1st, 2nd, 3rd, 4th and 5th lactation. The yield was 2544.4 kg; 3037.1 kg; 3354.1 kg; 3371.1 kg and 3332.9 kg respectively. The estimation coefficients in this group for 1st, 2nd and 3rd lactation were 1.32; 1.11 and 1.01 respectively.

New estimation coefficients of milk yield were not published for many years. Milk yield between lactations was studied by many authors but the studied group consisted of different dairy cows species with different number of lactations. These calculated estimation coefficients between lactations were influenced by selection of dairy cows with higher number of lactations and by their lower breeding value which is caused by their later date of birth. [4] also studied milk production in first three lactations of Slovak Pied (3486.6 kg of milk, 4086.8 kg of milk and 4292.0 kg of milk). In Czech republic [6] studied milk yield of Czech Fleckvieh dairy cows in first three lactations (3642 kg, 4106 kg and 4328 kg), [5] declare 3963 kg, 4541 kg and 4739 kg of milk yield in first three lactations of this breed. All authors say that the value of the yield in first lactation influences negatively the prediction of estimation coefficients between all lactations.

As the yield of dairy cows significantly increases, the new question how this increase influences the value of currently used estimation coefficients appears.

The aim of this study was to recalculate estimation coefficients of milk yield in first three lactations in the group of Czech Fleckvieh dairy cows and to determine how the milk yield in the first lactation effects these coefficients.

Material and Methods

The data on the milk yield of 8144 cows were analyzed. The cows were born in the period from 2000-2006 and the fourth lactation had to elapse. All dairy cows were more than 75% Czech Pied breed. The average yield for first, second and third lactation for the whole group was calculated. The

estimation coefficient between first and second lactation, first and third lactation and second and third lactation were calculated. The group was divided into five groups according to milk yield in the first lactation - group A (up to 4000 kg), group B (4001-5000 kg), group C (5001-6000 kg), group D (6001-7000 kg) and group E (over 7001 kg).

The estimation coefficient between first and second lactation, first and third lactation and second and third lactation were calculated for each group as a ratio of milk yield in later lactation to milk yield in earlier lactation. The estimation coefficient and results of milk yield of Czech Pied breed were compared with results of other authors. Estimation coefficients in groups (milk yield) were calculated as simple mean values of individual coefficients. Main statistical parameters mean ($\bar{x}$), standard deviation (s_x) and coefficient of variation (Vx) of the observed traits and phenotypic correlations (r) between them were calculated. The significance of differences was tested by STATISTICA 10.

Results and Discussion

The main parameters of milk production in the first three lactations of observed cows are shown in Table1. The total milk yield in the 1st, 2nd and 3rd lactation was 6154.45 kg; 7208.32 kg and 7506.72 kg respectively. The difference between all lactations was statistically highly significant. The standard deviation (s*x*) ranged from 1257.61 in the first lactation to 1524.87 in third lactation. The variation coefficient ranged from 20.31 in the third lactation to 20.67 in the second lactation. The estimation coefficients for all dairy cows in the 1st, 2nd and 3rd lactations were 1.17; 1.22 and 1.04 respectively.

Table 1 Milk yield of dairy cows at different lactation and their estimated coefficients (n = 8144)

lactation	$\bar{x}$	s_x	Vx	conversion coefficients
first lactation	6154.45^{A}	1257.61	20.43	1.17
second lactation	7208.32^{B}	1489.97	20.67	1.22
third lactation	7506.72^{C}	1524.87	20.31	1.04

The values in the same column marked with different letters (A, B, C) differ highly significantly (P < 0.01)

Table 2 The values of the estimated coefficients between the first three lactations of dairy cows Czech Fleckvieh breed at different performance on first lactation

	The level of milk production on first lactation				
estimated coefficient for the relationship	A up to 4000 kg n = 302	B 4001 to 5000 kg n = 1123	C 5001 to 6000 kg n = 2396	D 6001 to 7000 kg n = 2372	E over 7001 kg n = 1951
1. and 2. lactation	1.46^{A}	1.28^{A}	1.20^{A}	1.16^{A}	1.10^{A}
1. and 3. lactation	1.60^{B}	1.38^{B}	1.26^{B}	1.20^{B}	1.12^{B}
2. and 3. lactation	1.10^{C}	1.07^{C}	1.05^{C}	1.04^{C}	1.02^{C}

The values in the same column marked with different letters (A, B, C) differ highly significantly (P < 0.01)

Table 3 Comparison of values of milk production and estimation of coefficients in Czech Fleckvieh cattle between authors

	Mikšík and Poul (1969)		Chládek and Kučera (2003)		Andrýsek et al. (2014)	
lactation	$\bar{x}$	conversion coefficients	$\bar{x}$	conversion coefficients	$\bar{x}$	conversion coefficients
1. and 2. lactation	2544.40	1.19	3799.50	1.14	6154.45	1.17
1. and 3. lactation	3037.70	1.32	4348.50	1.23	7208.32	1.22
2. and 3. lactation	3354.10	1.10	4676.70	1.08	7506.72	1.04

The influence of milk yield were described by [7]. [8] say that dairy cows still grow up to third lactation and much energy is consumed for the growth. Therefore [3, 5, 6] say that the milk yield increases with number of lactations.

The estimation coefficients for dairy cows which were divided into groups according to milk yield in first lactation are shown in Table 2. The estimation coefficients in group A (milk yield up to 4000kg in the first lactation) between first and second lactation, first and third lactation and second and third lactation were 1.46; 1.60 and 1.10 respectively. The estimation coefficients in group B (milk yield from 4001 kg to 5000 kg in the first lactation) between first and second lactation, first and third lactation and second and third lactation were 1.28; 1.38 and 1.07 respectively. The estimation coefficients in group C (milk yield from 5001 kg to 6000 kg) between first and second lactation, first and third lactation and second and third lactation were 1.20; 1.26 and 1.05 respectively. The estimation coefficients in group D (milk yield from 6001 kg to 7000 kg) between first and second lactation, first and third lactation and second and third lactation were 1.16; 1.20 and 1.04 respectively. The estimation coefficients in group E (milk yield over 7001 kg) between first and second lactation, first and third lactation and second and third lactation were 1.10; 1.12 and 1.02 respectively. The highest values of estimation coefficients were calculated for the relationship between first and third lactation. This relationship was not influenced by the value of the yield in the first lactation. This fact is highly significant. As the results show, it is highly significant that with the increase of milk yield in first lactation the value of estimation coefficients decrease.

When comparing our results with results of [7] the yield was higher in first lactation by 4000 kg. Due to this difference the value of estimation coefficients are different. The same conclusion is confirmed by a study of [2] who say that the most significant was in relationship between first and third lactation where the estimation coefficient was the highest. The least significant relationship, as shown in our study, was calculated when comparing second and third lactation. Also the lowest estimation coefficient was calculated. [1] focused on relationships of milk yield of Holstein dairy cows. The estimation coefficient between first and second lactation, first and third lactation and second and third lactation was 1.175; 1.297 and 1.119 respectively. [2] say, that the study was negatively influenced by milk production in first lactation. The positive relationship was determined by relationship between first and second lactation and first and third lactation. On the other hand the relationship between second and third lactation was negative. The same results were published by [4, 5, 6].

According to [7] in Table 3, the milk yield in first, second and third lactation was 2544.4 kg; 3037.7 kg and 3354.54 kg respectively and also values of estimation coefficient between first and second, first and third and second and third were 1.19; 1.32 and 1.10 respectively. [2] determined the milk yield of 75 and more % Czech Fleckvieh cattle in first, second and third is 3799.5 kg; 4348.5 kg and 4676.7 kg respectively. Values of estimation coefficients between first and second lactation, first and third lactation and second and third lactation were 1.14; 1.23 and 1.08 respectively.

The milk yield of our monitored group in first, second and third lactation was 6154.45 kg; 7208.32 kg and 7506.72 kg respectively. Estimation coefficients between first and second, first and third and second and third were 1.17; 1.22 and 1.04 respectively.

Comparing the data with [7] the of Czech Fleckvieh dairy cows increased the milk yield in first lactation by 3619.05 kg, in second lactation by 4170.62 kg and in third lactation by 4152.62 kg. Comparing the data with average yield determined by [2] the milk yield increased by 2354.95 kg in first lactation, by 2895.82 kg in second lactation and by 2830.02 kg in third lactation. There is a significant decrease of estimation coefficient in relation between first and second lactation by 0.02 in comparison to [7] and increase of estimation coefficient by 0.03 in comparison to [2]. The estimation coefficient in the relationship between first and third lactation decreased in comparison to [7] and are comparable with [2] where the difference was 0.01. The relationship between second and third lactation had the lowest estimation coefficient in our study as well as in study of [7] where the difference was 0.06 and also in study of [2] where the difference was 0.04.

Conclusion

This study declares that the increase of the milk yield in first lactation is accompanied by decrease of estimation coefficients between all three lactations. This relationship is declared by high significantly of measured data. We assume that dairy cows with high milk yield in first lactation will have only a small yield increase in second and third lactation. The dairy cow with low milk yield in the first lactation is assumed to increase significantly the yield in second and third lactation.

The lowest numbers of estimation coefficients achieved between second and third lactation. Higher values of estimation coefficients between first and second lactation and more significant relationship between first and third lactation reveals a significant ability to compensate the yield in first three lactations. It means that dairy cows with high increase of milk yield after first lactation will probably not repeat this increase after starting second and third lactation. The results show that the difference between average yield in second and third lactation was not highly significant and the yield stagnated. We assume that dairy cows with small increase of milk after first lactation will highly increase the yield after second lactation. Interesting results appeared after comparing our results with previous studies. The estimation coefficients between three lactations of the same dairy cows are relatively stable. However the yield of monitored dairy cows was significantly higher than in previous studies, the values of estimation coefficients are conform and have the same tendency. In concrete population the dairy cows above average yield in first lactation have lower values of estimation coefficient and the dairy cows with average yield in first lactation have higher values of estimation coefficient between other lactations.

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The effect of bee pollen consumption on functional morphology of small intestine of rats

ZUZANA HAJKOVA[1], ROBERT TOMAN[1], BRANISLAV GALIK[2], MONIKA MARTINIAKOVA[3]
[1]Department of Veterinary Disciplines
[2]Department of Animal Nutrition
Slovak University of Agriculture in Nitra
Trieda A. Hlinku 2, 949 76 Nitra
SLOVAK REPUBLIC
[3]Department of Zoology and Antropology
Constantin the Philospoher University
Nabrezie mladeze 91, 949 74 Nitra
SLOVAK REPUBLIC

zuzana.hajkova01@gmail.com

Abstract: In our experiment, the effects of bee pollen addition in diet on the functional morphology of small intestine in rats were investigated. The microscopic changes in the small intestine after administration of the pollen addition were evaluated. The experimental animals were divided into one control and three experimental groups of ten rats in each group. Feed given to animals in group A contained addition of pollen in concentration of 0.2%, in group B the addition of pollen was 0.5% and in group C 0.75%. Animals of the control group were fed without the pollen addition. Using the quantitative morphometrical methods, we have found statistically significant increase in the relative composition of epithelial tissue ($P < 0.0001$) and decrease in the connective tissue volume ($P < 0.0001$) of the small intestine in experimental groups B and C as compared to the control. The results of our work show that the addition of pollen in diet had concentration-dependent effects on the mucosa of small intestine and thus it could be used as a proof of another beneficial use of pollen as a feed supplement.

Key-Words: pollen, rat, small intestine

Introduction

Pollen grains are carriers of higher plants gender (sperm cells) containing genetic information for future sporophyte [1]. Pollen load represents a large number of pollen grains of different plant species. Pollen grains of each plant species have specific characteristics and properties [2].

Bee pollen has a complex chemical composition; it provides valuable nutrients such as carbohydrates, essential amino acids, proteins, fatty acids, lipids [3, 4, 5]. The significant components of bee pollen are pro-vitamins and vitamins in particular: thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, folic acid, beta-carotene (pro-vitamin of vitamin A), vitamin C, tocopherol and ergocalciferol (vitamin precursor of vitamin D2) [6, 7]. The most particular minerals contained in pollen grains are: phosphorus, potassium, magnesium, copper, iron, manganese and zinc [8]. Pollen is also rich in polyphenolic substances, flavonoids, phytosterols and other health-promoting substances [9, 10, 11]. Another biologically active and beneficial pollen load components are organic acids, terpentines, nucleic acids, purine compounds, essential oils, plant growth regulators and more than 100 enzymes [12].

From pharmacological and health effects which were tested on animals, bee pollen is best known for: antioxidant, anticancerogenic, antibacterial, anti-allergic, immune-stimulating and antianabolic effects, as well as the effect of specific enzyme inhibitors [13, 14, 15, 16, 17, 18]. As to its use against various diseases, pollen is used to treat: prostatitis, some serious skin diseases, chronic liver damage and acute chronic hepatitis, stomach ache and various intestinal disorders, it regulates bowel function, chronic constipation and chronic diarrhea [19, 20, 21]. Bee pollen also supports detoxification functions of the liver [22]. It has been shown to inhibit histamine reactions that cause inflammation and allergy symptoms. This feature is probably

duesss to the presence of quercetin in the bee pollen [23].

These features allowed using bee pollen as a widely known food supplement [24, 25].

Our scientific aim was to describe the changes in the structure of the small intestine using morphometric methods.

Material and Methods

Experiment was carried out in experimental facility of the Department of Veterinary Sciences of Slovak University of Agriculture in Nitra (SK 50004 PC). Wistar rats were used as experimental animals. Animals were fed with water and complete feed mixture for laboratory mice and rats M3 (Machal, Czech Republic) *ad libitum*. They were housed individually in plastic containers (Tecniplast, Italy) on bedding of wood shavings under basic requirements for living conditions (temperature 20 - 22° C, humidity 55 ± 10%, 12 h light regime).

At the age of 4 weeks, young rats were divided into 4 groups (control, A, B, C) for 10 animals in each group. The control group was fed with feed mixture without pollen additive from *Brassica napus var. Napus*. Experimental group A was fed with the pollen addition in concentration of 0.2 %, group B was fed with addition of pollen in concentration of 0.5% and group C with pollen addition in 0.75% concentration. The duration of the experiment was 90 days.

After 90 days of pollen intake, the animals were humanely killed in accordance to Government regulation no. 23/2009 coll. The samples of small intestine (*jejunum*) were taken immediately after sacrifice, they were treated by the special method, to ensure the elimination of autolytic processes in cells, so they could captured the actual state of the tissue. The samples were fixed in 10% formaldehyde solution and paraffin - watered. For the microscopic detection of any changes in the tissue of the *jejunum* the sections were stained with hematoxylin and eosin. Samples of experimental and control animals were treated in the same manner.

Histological preparation of the small intestine (*jejunum*) was assessed by light microscope (Olympus AX 70 Provis, Japan). The structure of the small intestine wall, especially the mucosa and the different types of epithelial cells were observed. The structure and any visible changes in the tissue of the small intestine were described. The changes in the small intestine were also evaluated using the quantitative morphometrical methods. The pictures were taken using the digital camera (Olympus C5050-Z) and light microscope (Nikon Eclipse E600). Ten different visual fields (approximately from 2 or 3 samples) from each experimental rat, totally 400 microphotographs of the small intestine were recorded and analyzed. The quantitative analysis was realized using the test grid containing 494 test points and the relative composition of the epithelium and connective tissue (*lamina propria mucosae*) in small intestine were evaluated. Morphometric measurements were based on computerized techniques with PC morphometric software M.I.S. Quick Photo and using light microscope Olympus AX 70 Provis (Japan).

The basic statistical indicators, the simple arithmetic mean, standard deviation, minimum and maximum were calculated and by one-way analysis of variance we determined the statistical significance of differences between groups using Scheffe test. All statistical analysis of the results was performed by the statistical program - SAS Enterprise Guide 9.1 (USA).

Results and Discussion

Assessing the small intestine mucosa under the light microscope we observed in all experimental histological samples: normal mucosa configuration, covered with single-layer columnar epithelium, enterocytes and goblet cells. Among villi were well observable Lieberkühn crypts and there were no signs of inflammatory process in the mucosa.

Using the quantitative morphometric methods we found that the percentage of epithelial volume in *jejunum* in the group A compared to the control was slightly increased by 1.02% and also a slight decline in connective tissue volume, identically by 1.02% was counted. These differences were not statistically insignificant.

In group B, we found statistically significant ($P < 0.0001$) increase in the epithelium volume by 5.20% and statistically significant ($P < 0.0001$) decrease in the connective tissue just the same by 5.20% when compared to the control. In group C we found also statistically significant ($P < 0.0001$) increase in the epithelium volume by 3.74% and statistically significant ($P < 0.0001$) decrease in the connective tissue 3.74% when compared to the control group. All differences between the groups are shown in Table 1 and Table 2.

The results show that the pollen in the diet given orally to experimental animals, during 90 days, at a concentration of 0.2% caused a slight increase of epithelium of the small intestine and the pollen concentration of 0.5% and 0.75% significantly ($P < 0.0001$) increased the percentage of epithelium volume and decreased the percentage of connective tissue volume (*lamina propria mucosae*).

Table 1 Morphometric analysis of epithelium relative volume in small intestine of rats

	X [%]	Significance	SD	Minimum	Maximum
Control	**64.18**		8.54	31.52	83.47
Group A	**65.20**		6.85	34.12	82.36
Group B	**69.38**	****	7.86	30.39	87.85
Group C	**67.92**	****	7.09	47.18	79.94

**** *P < 0,0001, X - arithmetic mean, SD - standard deviation*

Table 2 Morphometric analysis of soft tissue relative volume in small intestine of rats

	X [%]	Significance	SD	Minimum	Maximum
Control	**35.82**		8,54	16.53	68.48
Group A	**34.80**		6.85	17.64	65.88
Group B	**30.62**	****	7.86	12.15	69.61
Group C	**32.08**	****	7.09	20.06	52.82

**** *P < 0,0001, X - arithmetic mean, SD - standard deviation*

In weanling piglets the adition of dietary fibre differing in lignin content from *Pinus massoniana* pollen reduced apparent (faecal) digestibility of dry matter and crude protein [26]. Zhang, Diao and Tu (2010) had also concluded that supplement of bee pollen and polysaccharides in calves diet could improve the growth performance of calves. Bee pollen additive of 25 $g.d^{-1}$ and polysaccharides in dose of 5 $g.d^{-1}$ in milk replacer could get better performance and higher apparent digestibility in calves.

Supplementation of bee pollen-based product Dynamic Trio 50/50 increased the feed intake and thus nutrient retention of Arabian horses and that may have a positive effect on their performance [28]. Other results indicate that bee pollen possess a noticeable source of compounds with health protective potential and antioxidant activity. *Schisandra chinensis* pollen extract has strong antioxidant activities and significant protective effect against acute hepatotoxicity induced by carbon tetrachloride CCl_4, and it has been supported by the evaluation of liver histopathology in mice. The hepatoprotective effect may be related to its free radical scavenging effect, increasing antioxidant activity and inhibition of the lipid peroxidation [29].

In another study the effect of bee pollen on growing rabbit's performance was studied on 40 New Zealand White rabbits from 4 to 12 week of age. Bee pollen at 200 mg significantly ($P < 0.01$) increased body weight, conception rate, milk yield, litter size; improved biochemical profiles of blood and helps outstanding during both seasons. The same dose of bee pollen significantly increased growth and their survival rate until weaning [30]. Oxidant and antioxidant status, estrogenic and antiestrogenic activity and gene expression profile were studied in mice fed with *Cystus incanus* L. (*Cistaceae*) reach bee pollen. Bee pollen as a food supplement (100 $mg.kg^{-1}$ b.w. mixed with commercial food pellets) compared to control (commercial food pellets) modulated antioxidant enzymes in the mice liver, brain and lysate of erythrocytes and reduced hepatic lipid peroxidation [31].

In another similar study, the effects of bee pollen on the development of digestive organs were evaluated in broiler chickens. The control group was fed with a basic diet, while the pollen group was fed with a basic diet supplemented with 1.5 % bee pollen over a period of 6 weeks. The results demonstrated that compared to the control group, the small intestine villi from the *duodenum, jejunum*, and *ileum* were longer and thicker in the pollen group. These findings suggest that bee pollen could promote the early development of the digestive system and therefore is potentially beneficial food supplement for certain conditions, such as short bowel syndrome [32].

Conclusion

Using the morphometric techniques, we found that oral doses of pollen in the diet during 90 days at a concentration of 0.2% caused a slight increase in epithelial layer of the small intestine and in a concentration of 0.5% and 0.75% significantly increased the epithelium volume and decreased the connective tissue volume. The addition of pollen in the diet has proven effects on the mucosa of the small intestine in a concentration-dependent manner and could be used as a proof of another beneficial use of pollen as a feed additive.

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The effect of additives on the silage leaches quality of red clover preserved at low dry matter

LUCIA HODULIKOVA, JIRI SKLADANKA, ADAM NAWRATH, MICHAL KVASNOVSKY, IVA KLUSONOVA, MARIE BALABANOVA, DANIELA KNOTOVA

Department of Animal Nutrition and Forage Production
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

lucia.hodulikova@mendelu.cz

Abstract: The work deals with the evaluation of the quality of silage made from two red clover varietes (*Trifolium pratense* L.) - diploid varieties (Spurt) and tetraploid varieties (Amos). Experimental stands were established in locations Troubsko and Vatín. There were evaluated silages from the first cuts. The harvest was performed at the beginning of flowering (in late May and early June). The experimental silages were treated with a mixture of organic acids (formic acid and propionic acid). There were evaluated silage liquors (pH, lactic acid, acetic acid, butyric acid, ethanol and NH_3). The results showed that the content of fermentation acids, pH, ammonium and ethanol was not affected by variety ($P < 0.05$). There was observed the influence of a treatment on the content of acetic acid and ethanol ($P < 0.05$). The used preservative had a positive impact on the final quality of the silage.

Key-Words: red clover, silage, low dry matter, organic acids, ethanol

Introduction

To make high quality of silage is necessary to use forage of high quality. The better quality of roughage can means reduce of the cost of feeding day. By using of silage additives we can improve quality and there is also a favorable effect on the palatability of the resulting silage. In contrast, silages with lower quality may have a negative impact not only on palatability, but mainly on the health and productivity of animals [2, 11].

The principle is that the higher moisture in the silage means more intensive and spontaneously fermentation. It also causes the production of higher amounts of fermentation products. Fermentation products are a result of microbial activity [14].

The fermentation process is evaluated by the degree of proteolysis, which is great importance in terms of health, performance and reproduction. In our agricultural practice over the last 7 years has become customary to begin harvesting alfalfa and clover in the early formation of flower buds. The result is a silage which has a high content of nitrogen compounds. In this phenological growth has the vegetation low viscosity and wilting is very slow. In this silages with low viscosity can occur the buffering of silage leading to increase of pH above 4.6. This causes a high content of nitrogen compounds and potassium and at low osmotic pressure is started rapid proteolysis process supported by the activaties of clostridia [12].

Red clover is in agricultural practice, also known as red clover. Red clover is one of the basic fodder rich in proteins and vitamins. This perennial herb with a deep taproot in contrast to alfalfa is characterized by slower intensity of lignification. Despite the relatively short persistence in the stand (2-4 years) is red clover one of the most important forage. Red clover ensures the production of quality forage and improves soil quality [10]. The requires of red clover are soil with surface compaction and sufficient supply to the moisture [5].

It is an ideal crop for green feed. Red clover also thrives on the less fertile soils where alfalfa grows no longer, well tolerates and acidic soils. Red clover can be used in multiple ways - in the mix, for purposes grazing, for hay or silage. When drying there may be a problem with mashing at the higher layers during stock. In the Czech Republic is registered 40 varieties - 19 diploid and 21 tetraploid [10].

Tetreploid varieties characterized in compare with diploid varieties with higher production of fresh forage, increased stamina and increased competition in mixtures with grass species. The

forege of tetraploid varietes aging more slowly, is about 7 to 10 days later, which allows sequential harvesting for direct feeding of fresh forage [6].

Most of our diploid varieties is adapted to the environment, is less demanding and early. Tetraploid varieties have green forage yield about 12-20% higher and hay yield is higher of 2-3%. Crude protein content is higher of 4-5%. They are persistent and have a higher carbohydrate content. Most of them are later, slower aging and contain more water [17].

The aim of this study was to assess the quality of silage leachate of red clover varieties, diploid varieties (Spurt) and tetraploid varieties (Amos) and their suitability for silage.

Material and Methods

Small trial plots of red clover varieties SPURT (2n) and AMOS (4n) were founded in the Foraging Research Station of Vatín on the Bohemian-Moravian Highlands (Czech Republic), altitude 560 m above sea and at the Research Institute for Fodder in Troubsko by Brno (Czech Republic, altitude 270 m above sea level. Stands were used as three cuts. There were evaluated silages from the first cuts. The harvest was performed at the beginning of flowering (in late May and early June).

The experimental silages were prepared in containers with a diameter of 150 mm. Preparation of experimental micro silages describes in his work [20]. Silage samples were taken 60 days after ensiling.

Evaluated was the quality of the extracts (pH, lactic acid, acetic acid content, the content of the butyric acid, ammonia and ethanol). Analytical procedures including preparation of aqueous extract describing in work of [1]. The results were recalculated to 100% of dry matter. Results were evaluated by analysis of variance (ANOVA) followed by Tukey's test. The evaluation was carried at a significance level of $P<0.05$.

Results and discussion

The content of lactic acid was in the variety Amos 101.53 $g.kg^{-1}$ and in the variety Spurt 111.03 $g.kg^{-1}$ DM $g.kg^{-1}$ of dry matter. The content of acetic acid was 27.60 $g.kg^{-1}$ of DM, respectively 33.40 $g.kg^{-1}$ of dry matter (Table. 1). When comparing the impact of different varieties there were no significant differences between diploid and tetraploid red clover variety. Tetraploid varieties of red clover should produce more water [17]. The average values of dry matter suggest that but difference was not statistically significant. The silage of Amos varieties showed lower pH and lactic acid but these differences were not statistically significant (Table. 1). Butyric acid in the majority of the samples ranged zero values. Butyric acid was only detected in two samples of the untreated variants of diploid red clover (SPURT).

After treatment of silages by chemical preservative there occurred acidification and their pH reduction from 4.38 to 4.12 (Table. 2). According to [19] the pH in the ideal silage should by fall within the values 4 – 4.2. Doležal [3] discloses a pH of 3.7 – 5. The decrease in pH to 4 - 4.5 is also suitable measures against listeria in silage [5]. The optimum of pH values also achieved Silage samples that were not treated with a chemical additive. After addition of chemical occurred also decreasing of a lactic acid content. Lactic acid is an indicator of the quality and stability of the silage so is not so desirable decrease of lactic acid. Kotal [9] states that the lactic acid content in the silage should be 2/3 of the total amount of acids. This also indicates Zeman [21] in tables for evaluating the quality of silage, which specifies the minimum content of lactic acid 70% of the total acids.

Table 1 The influence of the type of dry weight, pH, lactic acid, acetic acid and ethanol [g.kg-1]

Varieties	Dry matter	pH	Lactic acid	Acetic acid	Ethanol
Amos 4n	159.59[a]	4.19[a]	101.51[a]	27.60[a]	8.39[a]
Spurt 2n	173.44[a]	4.31[a]	111.03[a]	33.40[a]	10.78[a]

Different letters in the columns indicate statistically significant differences at a level of P<0.05

Table 2 Effect of treatment on dry weight. pH. lactic acid. acetic acid and ethanol [g.kg-1]

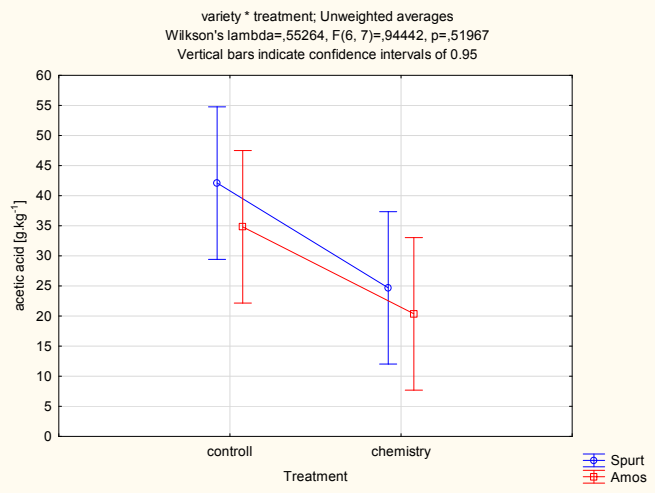
Treatment	Dry matter	pH	Lactic acid	Acetic acid	Ethanol
Control	164.94[a]	4.12[a]	85.53[a]	22.53[a]	5.43[a]
Kemisile	168.09[a]	4.38[a]	127.00[a]	38.47[b]	13.73[b]

Different letters in the columns indicate statistically significant differences at a level of P<0.05

These values were both variants of experimental silages. After addition of organic acids were reduced (P <0.05) the acetic acid in both monitored varieties. Chemically treated silages also contained significantly less (P <0.05) ethanol. The average value of ethanol decreased from 13.73 g.kg^{-1} to 5. 43 g.kg^{-1} (Tab. 2).

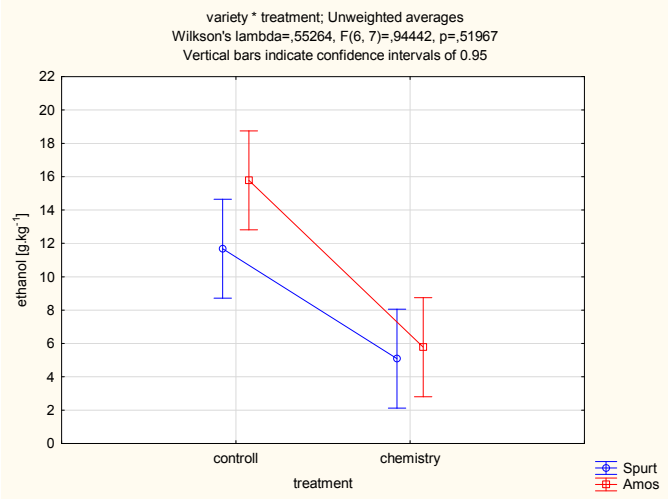
After the addition of organic acids reacted both varieties of clover by reducing the concentration of acetic acid. The interaction between variety and treatment was significant (P<0.05). The optimal acetic acid content of the total content of acids in silages should be 20 to 30% of dry matter [19]. Drevjany [4] indicates the proportion of acetic acid 4-9 g.kg^{-1} in the dry state from 35 to 35%. The acetic acid content is dependent on the number of cuts. On the second and each further mowing forms less acetic acid than in the first cuts [16]. Kocián [8] indicates that the acetic acid content should be within 20 g.kg^{-1} of DM. At this concentration is desirable in silage because it keeps the aerobic stability. The concentration of acetic acid over 30 g.kg-1 of DM is associated with high energy losses and reduced feed intake. Also Doležal [3] states the acetic acid content of 30 g.kg^{-1}. Formation of acetic acid in silages can prevent wilting and less time adding of silage additives [8]. In both variants of silages there were desired values of acetic acid (Tab. 2). More favorable acetic acid content contained silage from Amos varieties.

Fig. 1 The acetic acid content in red clover silages and interaction between variety and treatment



By using of chemical additives were reduced (P<0.05) ethanol content in both varieties. Spurt and Amos. The interaction between variety and treatment was significant (P<0.05). Wilkinson [19] reported the ethanol content <10 g.kg^{-1} of dry matter. Mitrik [10] presents the content of ethanol 4.06 g.kg^{-1}. The corresponding valuesof ethanol reached only variants treated by organic acids (Tab. 2). Higher content of ethanol may be a manifestation of the presence of yeast which fermented residual sugars. or lactic acid. Optimum ethanol content in silages should be according to Doležal [2] 8-10 g.kg^{-1}. Such content has a positive effect on palatability and cows preferred such silage. The high content of ethanol may negatively affect aerobic stability of silage and the rumen microflora [14].

Fig. 2 The ethanol content in red clover silages and interaction between variety and treatment



Conclusion

The evalueted experimental micro silages of red clover had comparable pH. fermentation acids. ammonia and ethanol. These results were achieved in both studied varieties (diploid varieties Spurt and tetraploid varieties Amos). Based on the results it can be assumed that for a production of silage can be used both diploid and tatraploid varieties of red clover. A silage quality can be influenced by using a chemical preservative. There was a significant effect on the concentration of acetic acid and ethanol content in monitored silages after a chemical preservative. This effect was observed for both varieties of red clover.

Acknowledgement

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The influence of the addition of chamomile extract to the diet of chickens

ZUZANA JAKUBCOVA, LADISLAV ZEMAN, PAVEL HORKY, EVA MRKVICOVA, PETR MARES, EVA MRAZKOVA, ONDREJ STASTNIK
Department of animal nutrition and forage production
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

zuzanajakubcova@seznam.cz

Abstract: The use of some herbs showing antimicrobial effects can be one of such alternatives to antibiotic growth promoters, which were banned in 2006.Our experiment involved 28 male chicks of the hybrid combination Ross 308. In this experiment, effects of three different concentrations of chamomile *(Matricaria chamomilla)* extract, (0.3%; 0.6% and 1.2%) in feeding doses on increments, feed consumption and carcass yield of broiler chickens were studied. Chickens were weighted every week at the age of 17, 24, 31 and 38 days. Feed consumption was measured every day. The experiment lasted 39 days that were slaughtered. The differences from in weight, feed consumption and carcass yield of broiler chickens were not significant.

Key-Words: chamomile extract, broiler chicken, weight, feed consumption, carcass yield

Introduction

Use of antibiotics concerning animal nutrition and as antimicrobial growth promoters is undoubtedly beneficial for the improvement of zootechnical performance parameters and prevention of disease. Nevertheless, because of the security threats for human and animal health which come from escalating resistance of pathogens to antibiotics and the accumulation of antibiotic residues in animal products and the environment, there is a global need to remove antimicrobial growth promoters from animal diets. The complete ban of all antimicrobial growth promoters has been in European Union since 2006. As a result, the demand for alternative products to antibiotics that can be used as prophylactic and growth promoting agents is very high [1].

Chamomile *(Matricaria chamomilla)* has been used in folk medicine throughout history [2]. *M. chamomilla* belongs to a major group of cultivated medicinal plants. It contains a large group of therapeutically interesting and active compound classes. Sesquiterpenes, flavonoids, polyacetylenes and coumarins are considered the most important constituents of the chamomile drug. The coumarins are represented in chamomile by herniarin, umbelliferone, and other minor ones (Z)- and (E)-2-β-d-glucopyranosyloxy-4-methoxycinnamic acid (GMCA), the glucoside precursor of herniarin, were described as native compounds in chamomile. Eleven bioactive phenolic compounds, such as herniarin and umbelliferone (coumarin), caffeic acid (phenylpropanoids) and chlorogenic acid, apigenin, apigenin-7-O-glucoside, luteolin and luteolin-7-O-glucoside (flavones), quercetin and rutin (flavonols), and naringenin (flavanone) are found in chamomile extract. More than 120 chemical constituents have been identified in chamomile flower as secondary metabolites, including 28 terpenoids, 36 flavonoids, and 52 additional compounds with potential pharmacological aktivity. Components, such as α-bisabolol and cyclic ethers are antimicrobial, umbelliferone is fungistatic, while chamazulene and α-bisabolol are antiseptic [3]. The *M. chamomilla* could be used as a natural antimicrobial agent for human and infectious diseases and in food preservation. Furthermore, the development of natural antimicrobial agents will help to ecrease negative effects (pollution of environment, resistance) of synthetic chemicals and drugs. The interesting antimicrobial effects observed in some cases support the traditional use of this plant, particularly by the local population, which needs cheap medicine [4].

Material and Methods

Experimental design

The experiment involved 48 male chicks of the hybrid combination Ross 308. All chicks were

seven days old. There were altogether 4 groups of these birds, viz. control and three experimental groups receiving chamomile extract supplements in concentrations of 0.3%, 0.6% and 1.2%.

Birds and experimental conditions

Prior to the beginning of the experimental period, chicks were weighed, identified with wing tags, assorted into four groups and placed into metabolic cages. All birds received water and feed mixture *ad libitum*. Composition of the feed mixture is shown in the table 1.Chamomile extract was added into the feed mixture in concentrations of 0.3%; 0.6% and 1.2%.

The light regime was 6 hours of darkness and 18 hours of light. On the 7th day of age chicks were kept at the ambient temperature of 29.9°C and relative humidity of 50%. The temperature was decreased daily by 1°C to 23°C.

Statistical processing

Obtained results were analysed using the programme Microsoft Excel 2010 and the software Statistica 10 CZ.).

Table 1 Composition of feed mixture (%)

Ingredient	%
Wheat	25
Maize	37
Soybean meal	28
Sunflower oil	6
Mineral-vitamin mixture without anticoccidial drugs	3
Monocalcium phosphate	0.8
Finely ground limestone	0.2

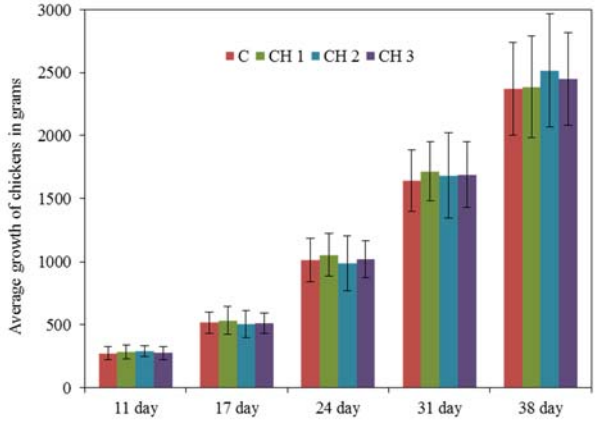
Results and Discussion

The experimental animals showed no health problems during the experiment.

Body weight

Body weight of chickens was measured in week intervals. There were no differences between groups with or without addition of chamomile extract.
Body weight of chickens is shown in Figure 1.

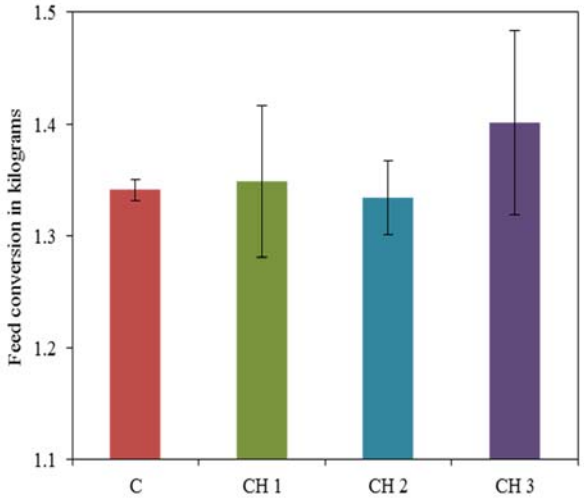
Fig. 1 Average body weight of chickens in grams.



C – concentration 0% of chamomile extract
CH 1 – concentration 0.3% of chamomile extract,
CH 2 – concentration 0.6% of chamomile extract,
CH 3 – concentration 1.2% of chamomile extract

Feed conversion is shown in Figure 2. There were no differences between groups

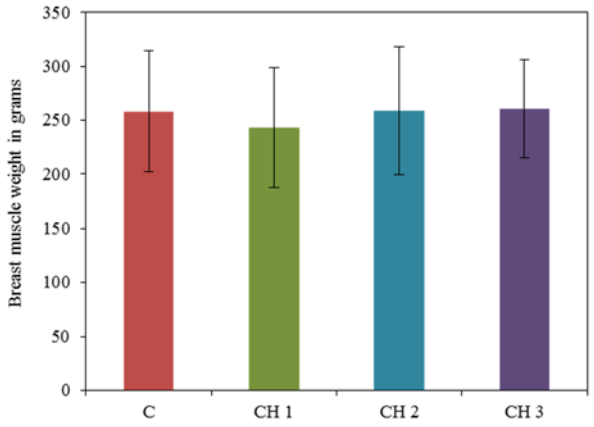
Fig. 2 Feed conversion in kilograms.



C – concentration 0% of chamomile extract
CH 1 – concentration 0.3% of chamomile extract,
CH 2 – concentration 0.6% of chamomile extract,
CH 3 – concentration 1.2% of chamomile extract,

Breast muscle weight is shown in Figure 3. There were no differences between groups

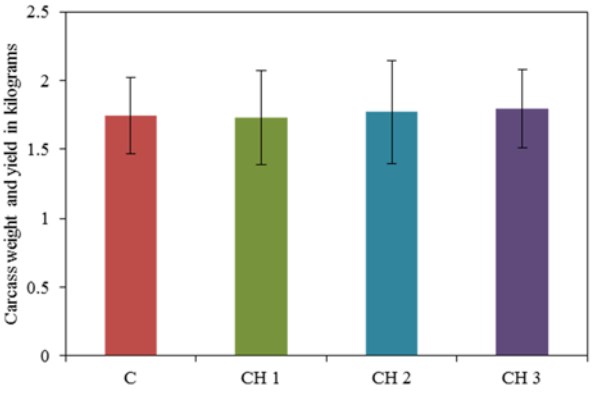
Fig. 3 Breast muscle weight in grams.



C – concentration 0% of chamomile extract
CH 1 – concentration 0.3% of chamomile extract,
CH 2 – concentration 0.6% of chamomile extract,
CH 3 – concentration 1.2% of chamomile extract,

Carcass weight and yield is shown in Figure 4. There were no differences between groups

Fig. 4 Carcass weight and yield in kilograms.



C – concentration 0% of chamomile extract
CH 1 – concentration 0.3% of chamomile extract,
CH 2 – concentration 0.6% of chamomile extract,
CH 3 – concentration 1.2% of chamomile extract,

According to (Cabuk et al., 2006) the herbal essential oil mixture may be considered a potential growth promoter. In their study demonstrated that supplementation of a mixture of herbal essential oils to the diet reduced feed intake and improved feed conversion ratio. These effects were different for broilers originating from young compared to old breeder flocks at 21 days of age. Broilers originating from young breeders had better feed conversion ratio than old breeders [5].
Use of herbal extracts especially garlic improved feed conversion ratio than old breeders comparable to virginiamycin in broilers. This effect could be attributed to improvement of digestive enzymes secretion. The most effective in immune function enhancement of the test herbal extracts were extracts from coneflower and garlic. These extracts were able to reduce the serum lipids. Remarkable was improvement of selected intestinal bacterial populations through these extracts [6].
Skomorucha and Sosnówka-Czajka (2013) demonstrated positive effect of the addition of 2 $ml \cdot l^{-1}$ chamomile in drinking water on broiler chickens organisms. Herb extract contributed to a decrease in cholesterol level and an increase in the immunoglobulin complex in the blood of experimental birds compared to the control group. The chamomile increased the body weight of the birds [7]. Supplementation of diets with thymol and cinnamaldehyde has positive impacts on gut microbiota, growth performance and welfare in monogastric animals [8]. Inclusion of 1 % anise and 1 % in broiler diets significantly improves the daily live weight gain and feed conversion ratio during a growing period of 6 week. These additives may be considered as a potential growth promoters for poultry due to stimulating digestive effect, antimicrobial effect and positive effect on performance [9].

Conclusion

In our experiment, which was based on supplementation of chamomile extract, we monitored its effect on increments, feed consumption and carcass yield of broiler chickens.
Our experimental hypothesis that the supplementation of chamomile extract, will affect the higher chickens weight, improve feed conversion and better carcass yield was not confirmed.

Acknowledgement

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Evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk

JANA JAVOROVA, MILENA VELECKA, DANIEL FALTA, JIRI ANDRYSEK, MILAN VECERA, STANISLAV STUDENY, GUSTAV CHLADEK
Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jana.javorova@mendelu.cz

Abstract: Objective of this research was evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk. During the period of 24th January 2013 to 11th March 2013 (winter period - WP; n=47) and 30th June 2013 to 30th September 2013 (summer period - SP; n=47) were analysed bulk milk samples obtained in herd of Czech Fleckvieh cows from farm GenAGRO Říčany, a.s. (GPS 49°12'32.319"N, 16°23'42.666"E). Monitored parameters were: average diurnal temperature (ADT), content of fat, protein, lactose, Ca, Mg, Cl, somatic cell count, chloride-lactose ratio, titratable acidity, rennet coagulation time (RCT). Between WP and SP were found in ADT, RCT and content of Ca statistically very highly significant differences ($P<0.001$) and in content of Cl statistically significant difference ($P<0.05$). In the evaluation of effect of ADT and milk composition on RCT was in WP found statistically highly significant ($P<0.01$; $r = -0.38$) correlation relationship between RCT and titratable acidity. In SP was found statistically highly significant ($P<0.01$; $r = 0.43$) correlation relationship between RCT and ADT.

Key-Words: Milk composition, rennet coagulation time, season, average diurnal temperature

Introduction

There is a great interest in changes in milk composition. The composition of milk varies with stage of lactation, feeding, health status or genetic factors [1]. No less important factors are climatic conditions [2]. Cows' thermoneutral zone is defined as air temperature range from 3 to 12°C. Heat stress starts already from temperature above 25°C [3]. Chládek [4] considers thermoneutral zone from 10 to 16°C. Heat stress has a negative effect on feed intake, leading to a reduction in milk yield [5]. On the contrary longer day in the summer feed intake increases [6]. Low temperatures in winter can also cause problems. Dairy cows tolerate low temperatures better than high, but extreme cold also affects them negatively [7]. Heck et al. [8] reported that the percentage of fat or protein was influenced by the seasonal variations. In winter is usually achieved the highest percentages of fat and protein, in the summer, these values are lower. Changes in lactose content during the year are usually small [9]. In the case of normally fed and reared cows salt content has certain regularity. When diseases of the mammary gland are detected content of calcium, potassium, magnesium, phosphorus and lactose decrease and content of sodium, chloride and somatic cells count increase [10]. To compensate osmotic pressure in the mammary gland, because of low lactose content, there is an increased transfer of sodium chloride from blood to milk [11]. Koestler (1920) used the ratio of chloride ions and lactose to indicate normal or mastitis milk [12].

Due to changes in milk composition are affected its technological properties [13]. Milk coagulation properties are an important aspect in assessing cheese-making ability and are influenced by many factors. The most important factors are the content of casein, calcium and phosphorus, acidity of milk or health. An important factor is also temperature [14, 15].

The aim of this study was evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk. A part of the research was also evaluation of changes in titratable acidity and its effect on rennet coagulation time.

Material and Methods

Characterization of the farm where the research was carried out and experimental design

During the period of 24th January 2013 to 11th March 2013 (winter period - WP; n=47) and 30th June 2013 to 30th September 2013 (summer period - SP; n=47) were analysed bulk milk samples obtained in herd of Czech Fleckvieh cows. Animals were kept on farm GenAGRO Říčany, a.s. with free stall barn (GPS 49°12'32.319"N, 16°23'42.666"E). Samples from WP were sampled daily, from SP were sampled every other day. Cows were fed total mixed ration *ad libitum* and were in various stage of lactation.

Description of the method of measurement and laboratory methods

Analysis of milk samples were performed in the laboratory of Department of Animal Breeding at Mendel University in Brno. **Lactose (L), fat (F), protein (P)** content were measured on instrument Julie C5 Automatic (Scope Electric) working on the principle of thermo analysis. **Chloride (Cl)** content in milk was determined after the addition of nitric acid by titration argentometric. Chlorides were precipitated by excess silver nitrate solution and for reverse titration was used a solution of ammonium thiocyanate. For the determination of **calcium (Ca)** and **magnesium (Mg)** content was used complexometric titration with EDTA, 2Na. **Titratable acidity (TA)** was detected by the methodology Soxhlet-Henkel titration of the sample with sodium hydroxide. **Rennet coagulation time (RCT)** was measured using nefelo-turbidic sensor of milk coagulation according to the methodology [16]. **Average diurnal temperature (ADT)** represents the arithmetic mean of the temperatures (detected all day before the date of sampling), measured every 15 minutes using 3 sensors (located in stable) with HOBO data logger (Onset Computer). **Somatic cell count (SCC)** was determined by apparatus NucleoCounter SCC-100 (Chemometec), which allows objective determination of somatic cells based on their automatic counting using a fluorescence microscope. **Chloride-lactose ratio (Cl/L)** number (x) was determined according to the formula:

a…chloride ions content ($g.l^{-1}$)
b…lactose content in milk ($g.100g^{-1}$)

$$x = \frac{a.100}{b.10}$$

For statistical analysis were used programs MS Office Excel 2010 and STATISTICA 10.

Results and Discussion

Evaluation of milk composition in winter and in summer period

Table 1 provides relationship between both monitored periods within measured properties of milk. The average diurnal temperature (ADT) in winter period (WP) was 0.58 °C in summer period (SP) was measured (ADT) 18.61 °C. As expected, between ADT in the monitored periods was found statistically very highly significant difference ($P<0.001$). Average calcium (Ca) content in WP was 1.04 $g.l^{-1}$ and in SP this value was 1.14 $g.l^{-1}$. There was found statistically highly significant difference ($P<0.001$) between observed periods. Ozrenk and Inci [9] found higher content of salts in summer period too. On the contrary Lukášová and Smrčková [17] found higher content of Ca in WP than in SP, Moreno-Rojas et al. [18] found in their research highest contain of Ca in November an lowest in May. Gaucheron [19] states that Ca content stay during the season almost unchanged, great impact on its content have phase of lactation, illness or diet. Average rennet coagulation time (RCT) in WP was 207 sec. and in SP was 190 sec. Between the monitored periods was found statistically highly significant difference ($P<0.001$). This result corresponds with data published by Chládek et al. [20]. On the other hand Polák et al. [14] in their study published opposite results that lower value of RCT were found in the SP. Regarding the chloride content (Cl) in WP was detected 0.88 $g.l^{-1}$ and 0.91 $g.l^{-1}$ in SP. With regard to these parameters was detected statistically significant difference ($P<0.05$). Castillo et al. [21] observed lowest Cl content in January and February. Gaucheron [19] states that Cl content is during season constant.

Between WP and SP in content of fat (F), protein (P), lactose (L), magnesium (Mg), somatic cell count (SCC), chloride-lactose ratio (Cl/L) and titratable acidity (TA) was not found statistically significant differences ($P>0.05$). Many authors observed statistically significant differences between content of F or P and season due to differences between temperature [20, 14, 22, 23, 24]. With statistically not significant differences between both monitored periods in TA agree Chládek et al. [20]. In case of SCC Rajčevič et al. [25] found higher SCC in winter, on the contrary Heck et al. [8] came to the opposite result. To L content same authors state that during year is its content relatively constant. In normal bulk milk samples is Cl/L 1.7–2.2. Values greater than upper limit points to secretory disorders in the mammary gland [26]. McSweeney and Fox [12] present upper limit to 3.

Table 1 Average values of the measured parameters within the monitored periods

Parameter	Unit	Average values			Significancy
		Total	WP	SP	
n	-	94	47	47	-
ADT	°C	9.60	0.58	18.61	***
TA	°SH	6.50	6.52	6.47	N.S.
F	$g.100g^{-1}$	3.84	3.87	3.81	N.S.
P	$g.100g^{-1}$	3.20	3.18	3.21	N.S.
L	$g.100g^{-1}$	4.78	4.75	4.8	N.S.
SCC	$ths.ml^{-1}$	147	156	137	N.S.
Ca	$g.l^{-1}$	1.09	1.04	1.14	***
Mg	$g.l^{-1}$	0.27	0.16	0.37	N.S.
Cl	$g.l^{-1}$	0.90	0.88	0.91	*
Cl/L	-	1.88	1.86	1.9	N.S.
RCT	sec.	199	207	190	***

*N.S.= non-significant (P>0.05), *= P<0.05, ***= P<0.001*

Effect of ADT and milk composition on RCT

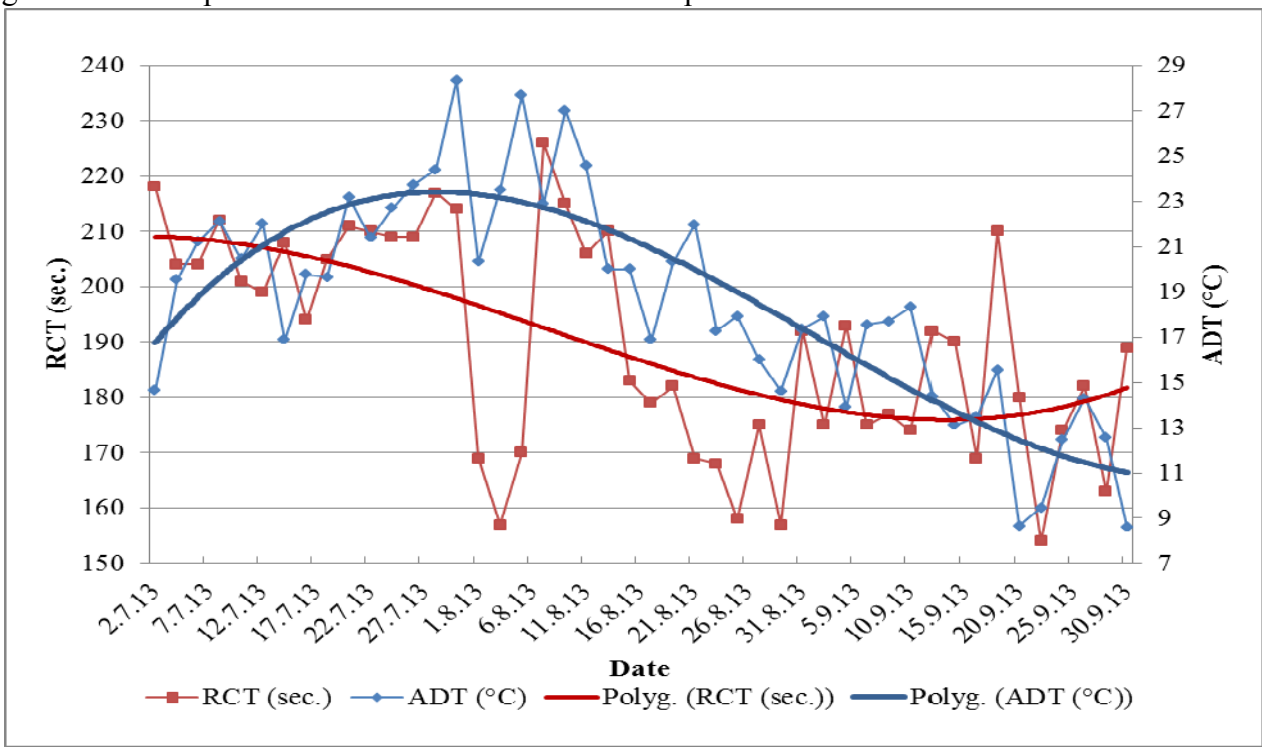
Table 2 provides correlation relationships of measured parameters and RCT in the monitored periods. In WP was not found statistically significant relationship between ADT and RCT (P>0.05; r = -0.15).

Table 2 Correlation relationship of RCT and measured parameters within the monitored periods

period/parameter	ADT	TA	T	P	L	SCC	Ca	Mg	Cl	Cl/L
WP	-0.15	-0.38**	0.04	-0.21	-0.23	-0.30	-0.09	0.08	-0.05	0.10
SP	0.43**	-0.21	0.03	0.14	0.14	-0.06	-0.08	0.10	0.27	0.24

*N.S.= non-significant (P>0.05), **= P<0.01*

Fig. 1 Relationship between ADT and RCT in summer period



In WP was not found statistically significant relationship between ADT and RCT (P>0.05; r = -0.15). On the other hand, in SP (see Fig. 1) was found statistically highly significant relationship between this two measured parameters (P<0.01; r = 0.43). Polák et al. [14] as well as Daviau et al. [27] found that the higher value of ADT means the shorter RCT. Our results in SP correspond with data published by Velecká et al. [28] who found similar relationship between ADT and RCT (P<0.01; r = 0.73). In WP was found statistically significant relationship between TA and RCT (P<0.01; r = -0.38) In SP was found similar trend but not statistically significant (P>0.05; r = -0.21). Chládek and Čejna [29] found very similar correlation relationship to ours findings (r = -0.50). Between RCT in both periods (WP and SP) within other measured parameters (F, P, L, SCC, Ca, Mg, Cl and Cl/L) was not found statistical significant relationship. In case of P (P>0.05; r = -0.21) and L (P>0.05; r = -0.23) in WP was found not statistically significant trend, that with increasing P and L in this period RCT decreased. A similar result reached Jõudu et al. [30]. On the other hand Daviau et al. [27] argue that the shorter RCT was in their research associated with a decrease in P content. Statistical not significant trend in WP was also found between SCC (P>0.05; r = -0.30) and RCT which means that with increasing SCC RCT decreased. But Chládek and Čejna [29] found opposite result (r = 0.36). In case of research carried out by Jõudu et al. [30]. was correlation relationship very weak (r = 0.02). In SP was in relationship of RCT and Cl content also found statistically not significant trend (P>0.05; r = 0.27).

Conclusion

Aim of this paper was evaluation of changes in milk composition in winter and summer period and its influence on rennet coagulation time of milk. Regarding the comparison of the composition of milk in the monitored periods were determined statistically very highly significant differences (P<0.001) in calcium content and rennet coagulation time of milk. Logically, was also found statistically very highly significant difference (P<0.001) between temperature in winter and summer period. Between summer and winter period was also found statistically significant difference (P<0.05) in chloride content.

In the case of determining the effect of the observed period (represented by average diurnal temperature in stable) and composition of milk on its rennet coagulation time was in summer period found statistically significant effect (P<0.01) in case of temperature. In winter period was found statistically significant (P<0.01) relationship between rennet coagulation time and titratable acidity. We can assume about statistically not significant (P>0.05) trend that in the winter period there is a relationship between rennet coagulation time and milk protein, lactose, somatic cell count.

Our research proved fact that during the year there are significant and not significant changes in milk composition. These changes affect the technological properties of milk. An awareness of these changes is important for primary producers as well as processors.

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Effect of ambient temperature on number of embryos obtained during embryo transfer process

ELISKA KABOURKOVA[1], HANS CORTEN[2]
[1]Department of Morphology, Physiology and Animal Genetics
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]CAH Dronten University of Applied Science, Dronten
De Drieslag 1, 8251 Dronten
THE NETHERLANDS

eliska.kabourkova@mendelu.cz

Abstract: The objective of the project was to evaluate the effect of the ambient temperature on the uterus flushing out during embryo transfer process. It was generally speaking about a comparison of the donors flushed out during the hot weather with the donors flushed out during the cold weather. It had regard to the number of the embryos obtained and to the ambient temperature. All numbers of the embryos obtained by the flushing out from the 33 milking cows were registered during the embryo transfer process. The three groups of the milking cows were measured during the *high ambient temperature* (24°C and higher), the *medium ambient temperature* (from 4.1°C to 23.9°C) and the *low ambient temperature* (4°C and lower) in the heat time. This research has been done on the dairy cow breed, specifically on the Holstein breed. However the results are easily extrapolated to the other dairy breeds. The experiment was focused on the embryos presented in the flushed liquid. All flushing out results were written down to the Excel MS and analyzed by the SPSS Statistics 17.0; exactly by the One-way ANOVA. The embryos obtained were found to fluctuate with the ambient temperature, being the lowest just during the *high ambient temperature*, high during the *medium ambient temperature*, high again at the time of the *low ambient temperature* during the heat phase of the cycle. *The low ambient temperature* had no significant effect to the embryos obtained. As the *high ambient temperature* had significant negative effect to the embryos obtained, it seems to be necessary to protect milking cows against the *high ambient temperature* during the heat time.

Key-Words: - embryo, ambient temperature, milking cow, flushing out

Introduction

Donor cows of particularly fine pedigree are treated with hormones to increase the number of eggs released at the ovulation [1]. The principle of the superovulation is basically simple: to induce more ovulations than the normal rate by giving a gonadotrophin stimulus (at critical moments of follicular development), followed by control of luteolysis, synchronous ovulation, high fertilisation and early embryonic development rates. The preparations to induce superovulation include: equine chorionic gonadotrophin, the follicle stimulating hormone and luteinizing hormone [2]. However the number of embryos obtained by flushing out is different every single time. There is a question mark regarding the ambient temperature and its effect on the number of embryos flushed out from the donor. The number has the huge influence to the farm economy issue. Holstein cows give 5.26 embryos in average per a flushing out [3] and a low number of embryos obtained causes worst farm economy. As need to have a sufficient economy farmers try to improve the conditions of the flushing out. The target is to obtain as much embryos as possible. Therefore the aim of the project was to evaluate the effect of the ambient temperature on the uterus flushing out during embryo transfer process. Reproduction suffers as a result of heat stress on folliculogenesis and oocyte maturation as well as embryonic development and survival [4].

Under the influence of heat stress, the duration and the intensity of the estrus are reduced. There is a clear decrease in the mobility and other manifestations of estrus [5]. Follicle-stimulating hormone secretion from the pituitary gland does not appear to be impaired in animals exposed to the high ambient temperatures [6]. In contrast a clear reduction of production in the pulse and amplitude of luteinizing hormone release has been observed in cows exposed to heat stress [5]. The decrease in productivity and the negative reproductive effects render periods of heat stress, and those periods following, less profitable for dairy operations, making heat stress abatement practices cost effective and advantageous [4].

Material and Methods

The study was carried out in 33 dairy farms in The Netherlands. In total, 33 cows were included. The different ambient temperatures were regarded because the research was focused on the ambient temperature effect on the embryos obtained during the embryo transfer process. The main research question was: *What is the effect of the ambient temperature on the uterus flushing out during the embryo transfer process?*

Established hypothesis were: *The number of the embryos obtained is not different during the high/medium/low ambient temperature (H0)* and *the number of the embryos obtained is different during high/medium/low ambient temperature (H1).*

The milking cows for the uterus flushing out process were selected. They were divided according the ambient temperature into the 3 groups with 11 members per a group. The groups were marked by the letters A – C. The level of the high ambient temperature meant ≥24°C [7]. The medium ambient temperature level was > 4°C to < 24°C. The low level of ambient temperature was established on ≤ 4°C [8]. The number of embryos obtained was high for > 8 embryos; the medium level was > 4 to < 8 and the low number presented < 4 embryos [3].

The cows in the group A were measured during *the high* ambient temperature. Group B was measured within *the medium* ambient temperature. The data about the group C were collected during *the low* ambient temperature. The group B formed a control group for the groups A and C.

The two veterinarians flushed out the uterus of the donor cows during the embryo transfer process. The experiment was focused on the embryos present in the flushed liquid. The flushed liquid was strained through a sterile sifter. After that it was observed under a microscope by trained assistants. The process of the uterus flushing out was done by the two different veterinarians. However the technical processes were absolutely uniform. Both of them had the similar workflow for the whole process of the uterus flushing out including the same tools used.

All flushing out results were written down to the Excel MS. Data were collected in MS Excel and analyzed by SPSS Statistics 17.0.

Results and Discussion

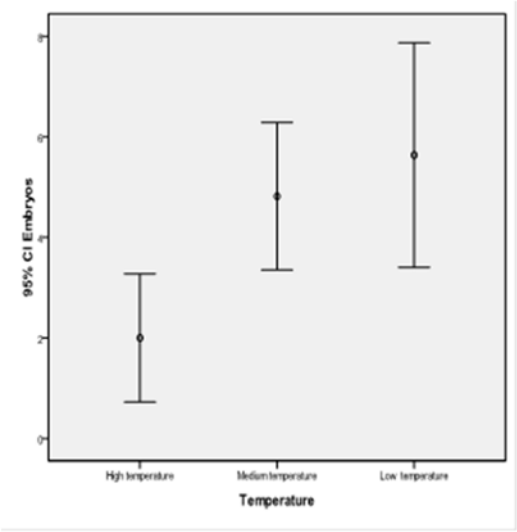
In regards to the project results the milking cows produced more embryos ($P<0.05$) during the low ambient temperature than the cows during the high ambient temperature. Further the cows produced more embryos ($P<0.05$) during the medium ambient temperature than the cows during the high ambient temperature. The high temperature had the significant ($P<0.05$) negative effect to the embryos obtained.

Due to many projects which has been done in this area the negative influence of high ambient temperature was expected. This result is consistent with the data reported by [9] who reported that the production of embryos by the superovulation is often reduced in periods of heat stress (climatic factors that may influence the degree of heat stress include: temperature, humidity, radiation, and wind [10] and the associated reduction in the number of transferable embryos is due to the reduced superovulatory response, lower fertilization rate, and reduced embryo quality. Further is reported that the success of in vitro fertilization procedures is reduced during warm periods of the year and the heat stress can compromise the reproductive events required for the embryo production by decreasing expression of the estrus behavior, altering follicular development, compromising oocyte competence, and inhibiting embryonic development [9]. High ambient temperature reduced embryo quality [11]. The superovulation result is highly dependent on the kind of the gonadotropin used and on the frequency of the application [12]. On the other hand no significant influence on the embryo

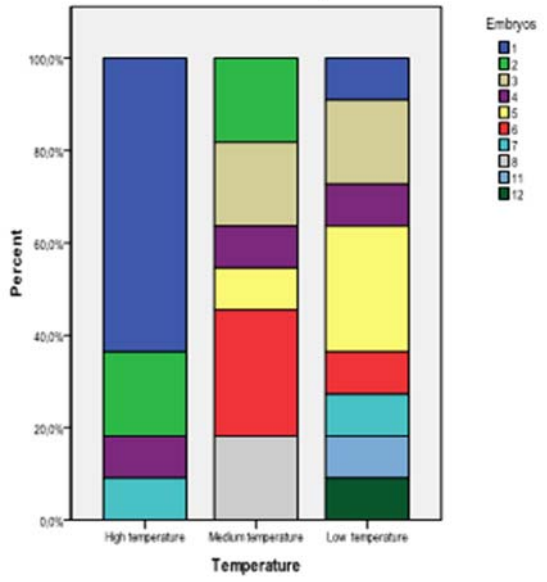
yield could be noted, regarding the day of the cycle on which the treatment is started [11]. Concerning the yield of embryos, the age of the donor has also no significant impact on the results [12].

The low ambient temperature had no significant (P>0.05) effect to the embryos obtained. Based on the outcome the zero hypothesis had to be rejected and at the same time the hypothesis number one was accepted. The number of the embryos obtained is different during *the high/medium/low* ambient temperature (H1).

The following (figure 1) shows the distribution of the population in every ambient temperature group (*the high, medium and low*). The every single segment illustrates the range of the distribution.



The other (Figure 2) shows frequency of the distribution. Every single color represents the certain number of the embryos obtained.



Conclusion

The negative influence of the high ambient temperature on the uterus flushing out during the embryo transfer process was expected. The embryos obtained were found to fluctuate with the ambient temperature, being the lowest (P<0.05) just during the high ambient temperature (24°C and higher), high during the medium ambient temperature (4.1°- 23.9°C), high again at the time of the low ambient temperature (4°C and lower) during the heat phase of the cycle. As the high ambient temperature had the significant (P<0.05) negative effect to the embryos obtained, it seems to be necessary to protect the milking cows against high ambient temperatures during their heat time. The low air temperature had no significant effect to the embryos obtained. There is no need to protect them during low ambient temperatures. A possible solution could be selecting the most suitable ambient temperatures for the estrus evocation and the following embryo transfer process by cow´s keepers. Even though the research has been done on the dairy breed specifically on Holsteins breed the results are easily extrapolated to other dairy breeds.

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The influence of colored wheat Konini feeding on antioxidant activity parameters in rats

FILIP KARASEK[1], EVA MRKVICOVA[1], ONDREJ STASTNIK[1], VACLAV TROJAN[2], TOMAS VYHNANEK[2], LUDEK HRIVNA[3], EVA MRAZKOVA[1]
[1]Department of Animal nutrition and Forage production, Faculty of Agronomy
[2]Department of Plant Biology
[3]Department of Food Technology
Mendel University in Brno
Faculty of Agronomy
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xkarase2@mendelu.cz

Abstract: The influence of feeding of purple wheat KONINI (14.01 mg/g of anthocyanins) on antioxidant activity measured in the liver tissue of rats was determined. 64 male rats of Wistar Albino strain were used in the experiment. Experimental group was fed by ration which content 100% of purple wheat KONINI. Control group was fed by ration with 100% of common wheat. Antioxidant activity measured by different methods DPPH, FR, FRAP and ABTS were higher in experimental group (10.06 ± 0.26; 543.88 ± 23.61; 39.56 ± 1.01; 458.76 ± 3.58, respectively) than in control group (9.20 ± 0.31; 482.46 ± 15.56; 36.73 ± 0.72; 445.38 ± 3.13, respectively. Differences were significant ($P<0.05$).

*Key-Words:*purple wheat Konini, rats, antioxidant activity

Introduction

Wheat is one of the most widely grown grain crops in the world, and durum and bread wheat represent staple foods for human nutrition, especially in the Mediterranean area [1]. The anthocyanins also have therapeutic roles for humans, against tissue inflammation, capillary fragility, cardiovascular disease, cancer, hyperglycaemia, and oxidative liver damage [2]. Unlike the carotenoids, for which plant breeding is mainly in response to the needs of the pasta producers, the anthocyanins represent a new target for genetic improvement due to consumer demand for foods with greater health benefits [1]. Several investigations of the anthocyanin content in coloured grain of spring wheat genotypes under spring-sown conditions were performed [3, 4, 5, 6, 7]. The purple colour is caused by anthocyanins accumulated in the pericarp [8]. It is well known that herbal anthocyanins are functioning as antioxidants and, in addition, they have anti-bacterial and anti-carcinogenic effects as well [9].
It is necessary to monitor the influence of anthocyanins in the food to human and animal organism.

Material and Methods

64 selected male laboratory rats of Wistar Albino strain at the age of 6 weeks were divided into 2 groups. Rats were marked by shaving of specific areas and keep in plastic bags with 8 rats per one. Average live weight of rats was 243 g. Room temperature (20 - 23°C) and humidity (50 - 60%) were controlled. Lighting system was 16 hours light and 8 hours dark. The experimental group (N = 32) was fed with dried granules of 100% wheat meal from purple wheat KONINI with content of Crude protein (CP) 16.8%. The control group (N = 32) was fed with dried granules of 100% wheat meal from common wheat in which content of CP was increased using wheat gluten to the identical in KONINI. Body weight gain was followed in two-day intervals and feed consumption was followed daily. At the age of 69 days they were killed using diethylether and liver tissue was taken for determination of antioxidant activity measured by different methods: DPPH (free radical 2,2-diphenyl-1-picrylhydrazyl), FR (Free Radicals*)*, FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

Determination of antioxidant capacity using the DPPH• test

The DPPH• test is based on the ability of the stable 2,2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors. The DPPH• radical displays an intense UV-VIS absorption spectrum. In this test, a solution of radical is decolourized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the following scheme: DPPH• + AH → DPPH•-H + A•, DPPH• + R• → DPPH•-R [10].

Determination of antioxidant activity by Free Radicals method

This method is based on ability of chlorophyllin (the sodium-copper salt of chlorophyll) to accept and donate electrons with a stable change of maximum absorption. This effect is conditioned by an alkaline environment and the addition of catalyst [11].

Determination of antioxidant activity by FRAP method

By the [12]: "The FRAP method (Ferric Reducing Antioxidant Power) is based on the reduction of complexes of 2,4,6-tripyridyl-s-triazine (TPTZ) with ferric chloride hexahydrate (FeCl3·6H2O), which are almost colourless, and eventually slightly brownish. This chemical forms blue ferrous complexes after its reduction. The method has its limitations, especially in measurements under non-physiological pH values (3.6). In addition, this method is not able to detect slowly reactive polyphenolic compounds and thiols."

Determination of antioxidant activity by ABTS test

The ABTS radical method is one of the most used assays for the determination of the concentration of free radicals. It is based on the neutralization of a radical-cation arising from the one-electron oxidation of the synthetic chromophore 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•): ABTS• – e- ABTS•+. This reaction is monitored spectrophotometrically by the change of the absorption spectrum. Results obtained using this method are usually recalculated to Trolox® concentration and are described as "Trolox® Equivalent Antioxidant Capacity" (TEAC). For chemically pure compounds, TEAC is defined as the micromolar concentration of Trolox® equivalents demonstrating the same antioxidant activity as a tested compound (at 1 mmol·L-1concentration) [13].

The data were processed through the use of MICROSOFT EXCEL (USA) and STATISTICA.CZ Version 10.0 (Czech Republic).

Results and Discussion

The results of antioxidant activity measured by DPPH, FR, FRAP and ABTS methods are in Table 1. Significant differences *(P<0.05)* between experimental group Konini and Control group was found using all method. DPPH Inhibition of 10.06 ± 0.26% on experimental group and inhibition of 9.20 ± 0.31% was measured on control group. In experiment [14] aimed at effect on inclusion of yellow Citrus wheat on antioxidant activity higher values were measured however these differences were insignificant.

Table 1 Antioxidant activity measured by different methods in the liver tissue of experimental rats

Methods	*KONINI*	*CONTROL*
DPPH	10.06 ± 0.26 [a]	9.20 ± 0.31 [b]
FR	543.88 ± 23.61 [a]	482.46 ± 15.56 [b]
FRAP	39.56 ± 1.01 [a]	36.73 ± 0.72 [b]
ABTS	458.76 ± 3.58 [a]	445.38 ± 3.13 [b]

DPPH is expressed in % of inhibition; FR, FRAP and ABTS values are expressed in gallic acid equivalent (GAE mg/l).

When using method Free Radicals (FR) values were higher in experimental rats fed Konini wheat (543.88 ± 23.61 GAE mg/l) than that Control wheat (482.46 ± 15.56 GAE mg/l). When using method FRAP, between experimental Konini group (39.56 ± 1.01 GAE mg/l) and control group (36.73 ± 0.72 GAE mg/l) significant differences were measured, which means that experimental Konini wheat has provable higher effect on antioxidant activity compared to control wheat. In experiment with yellow Citrus wheat [14] slightly higher values were measured in experimental group than in control.

Statistically significant higher value measured by method ABTS in experimental group Konini (458.76 ± 3.58 GAE mg/l) than that in control group (which achieved 445.38 ± 3.13 GAE mg/l) was discovered. In the experiment of Bendová [14] lower values were discovered in favour of experimental group but these values were not statistically provable.

Conclusion

In this experiment antioxidant activity on wheat Konini with higher anthocyanin representation was observed. Experiment took place on model animals of Wistar albino laboratory rat. When consuming this variety, effect of this wheat was discovered on

antioxidant activity when using methods DPPH, FR, FRAP and ABTS. Thus, it is possible to use this variety for feeding of farm animals as rats are model animals for pigs. It is possible to use the new variety Konini wheat in food processing industry also since consumer demand for functional food is getting higher. Because Konini wheat contains higher volume of anthocyanin which has positive impact on consumers' health condition, thus it is usable for production of functional foods.

Acknowledgement

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Relations of the leptin gene polymorphism and some internal environment parameters

LENKA KOVARIKOVA[1], ALES PAVLIK[1], ALES KNOLL[1], RADEK FILIPCIK[2]
[1]Department of Morphology, Physiology and Animal Genetics
[2]Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xkovari7@mendelu.cz

Abstract: The aim of this study was to evaluate effects of the single nucleotide polymorphism for leptin gene on selected metabolic parameters of Czech Pied cattle bulls. Missense mutation (cytosine-thymine switch) results in meat quality increase considering not only cattle but also another livestock. Animals with genotype TT distinguishes with higher leptin concentration in blood. These animals has higher amount of intramuscular fat (marbling score) in comparison to the animals with genotype CC. The leptin does influence the metabolism of lipids and energy, therefore the metabolic parameters of blood were observed and compared to genotypes. The relation between concentration of creatinine and genotype TT was found to be statistically significant ($P<0.05$), though other relations of parameters (glucose, urea, albumin) and genotypes were not.

Key-Words: SNP leptin, energy metabolism, urea, albumin, creatinine

Introduction

Leptin is the proteinaceous hormone produced by obese gene and synthetized by adipocytes. Leptin does circulate in blood in both free form and bound form [16] and it also shows retroactive effects [1]. There is influence of leptin on the regulation of the energetic metabolism [14]. It does signalize the amount of energetic provisions in periphery to the central nervous system [13]. The increase of leptin concentration in blood plasma stimulates leptin receptors located in hypothalamus, which does subsequently inhibit food intake and stimulates energy expenditure [7, 1]. Leptin does interfere with energy metabolism in peripheral tissues and probably influence insulin in adipocytes and muscles [3].

Leptin comprises 167 amino acids as a product of obese gene. The leptin gene was mapped at 4th chromosome in the case of cattle. It consists of three exons. The second exon bears point mutation (C/T), therefore the coding amino acid is changed from arginine to cysteine [4]. The genotype TT of animal does result in qualitative advantage of meatpacking parameters. These individuals indicates larger amount of intramuscular fat (marbling score) to the individuals distinguished by CC or CT, there is usually also higher evaluation in the means of body score condition. Single nucleotide polymorphism (SNP) is associated with the leptin concentration in serum [12] and amount of the stored fat in organism [3]. The level of leptin in blood or the amount of mRNA in fat tissue is in relation with body mass, food intake and body fat [5]. The similar research was performed on another livestock species, the leptin gene mutation correlates with the height of dorsal fat observed in pigs [15]. Leptin is engaged in processes of energy expenditure, food intake regulation, regulation of hormones secretion of endocrine system, reproduction, immune response and renal function [8].

Material and Methods

Animals

The experiment was performed on the Czech Pied bulls in age of 240 days. The animals were sorted out into three groups according to the leptin genotype. The genotype TT was represented by 18 animals, TC was represented by 133 animals and CC was represented by 102 animals. Animals were breed under identical conditions and fed with the same feeding ration based on the maize silage.

Genetic analysis

The blood samples were taken from animals into the test tube with anticoagulant agent EDTA. The samples were stored in the environment of -20° Celsius until the examination. The genome DNA of

animals was isolated by the means of QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA). The quality of isolated DNA was verified by the electrophoresis on 1% agarozoidal gel with ethidium bromide. The genotypes were differentiated on the basis of single nucleotide polymorphism in second exon of the bovine leptin gene. For testing, we used our own methodology. PCR primers were designed based on the nucleotide sequence of bovine leptin gene (GenBank U50365) (FW:5'TCGTTGTTATCCGCATCTGA3', REV: 5'TACCGTGTGTGAGATGTCATTG 3'). PCR were carried out in 12.5 µl volume containing 25 ng of cattle genome DNA, 1x HotStarTaq Master Mix (Qiagen) and 0.2 µM of straight and reverse primer. There is a multi-step process of PCR. A PCR thermal profile consisted of pre-denaturation at 95 °C for 2 min; followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 30 s; and final extension at 72°C for 7 min. The PCR products of 278 bp in size were separated on 3% agarose gel and sequenced using the ABI PRISM 3100-Avant Genetic Analyzer. The polymorphic locus (C/T) is located at position 204 base of the fragment.

Blood samples

The blood samples for biochemical analysis were taken from *vena jugularis externa*. All the bulls were sampled from 8:00 am to 9:30 am. Test tubes equipped with silicone separating gel and coagulation accelerator (Dispolab, Czech Republic) were used. The serum was separated by centrifugal machine set to 2,000 g for 10 minutes at 4° Celsius and further stored at temperature -20° Celsius.

Biochemical analysis

Urea, albumin, creatinin and glucose were analyzed by the Konelab T20xt (Thermo Fisher Scientific, Finland) using commercial kits (Biovendor-Laboratorní medicína, Czech Republic).

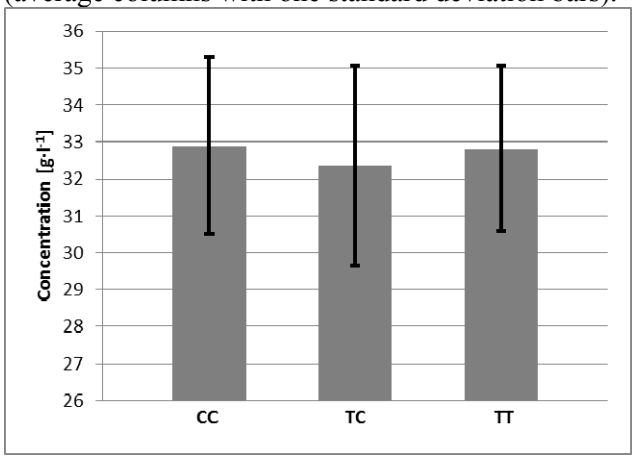
Statistical analysis

Measured data were sorted out and the univariate statistical analysis based on normal distribution was used in order to describe expected values and its dispersion characteristics within Czech Pied cattle population. The levels of significance of resulting differences were considered by the means of bivariate statistical analysis (Student's tests).

Results and discussion

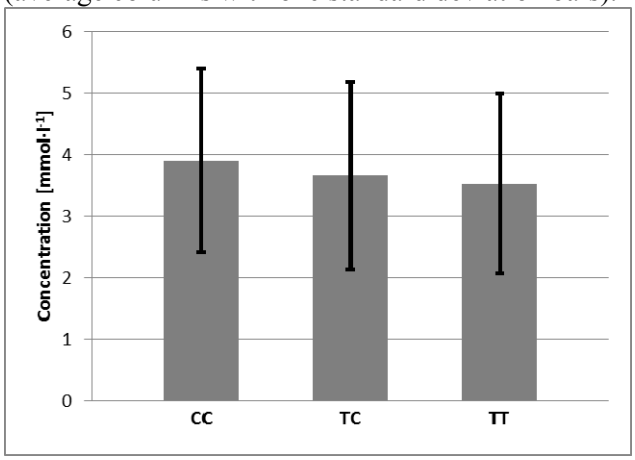
Leptin was reported to be a potent regulator of food intake. There was evidence indicating that at least some of the effects of leptin occured through receptor-mediated regulation of the hypothalamic protein neuropeptide Y (NPY), which was a potent stimulator of food intake [5].

Fig. 1 Relation of genotype of Czech Pied bulls for leptin gene and level of albumin in blood serum (average columns with one standard deviation bars).



Nutritional status, corresponding with food intake in animals could be evaluated by measurements of some biochemical markers such as plasmatic concentration of albumin. Several studies [6] were addressed to the relationship between plasma leptin concentration and nutritional status. But they did not find a significant correlation between leptinemia and plasma concentrations of albumin. These ascertainments corresponded with the results of our study. The albumin concentration in blood plasma in relation to leptin genotype was not found to be statistically significant.

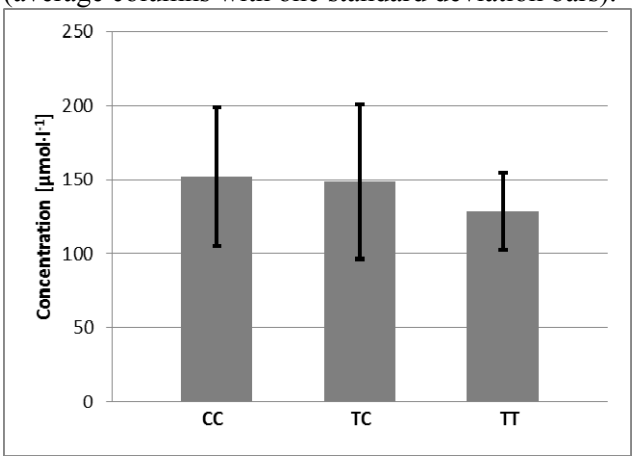
Fig. 2 Relation of genotype of Czech Pied bulls for leptin gene and level of urea in blood serum (average columns with one standard deviation bars).



Animals with the genotype CC for SNP leptin gene showed signs of higher value of urea concentration in comparison with the other genotype groups,

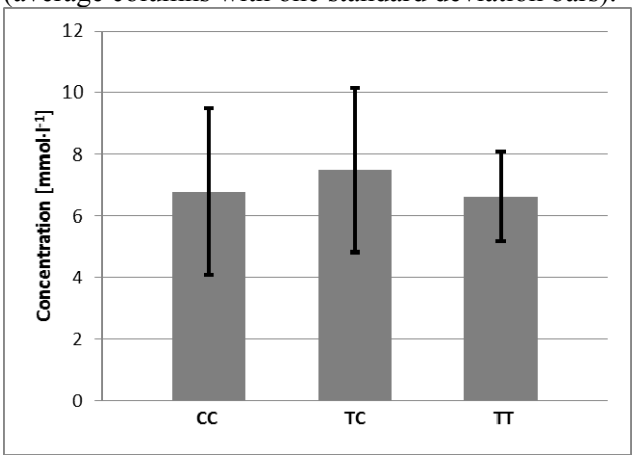
although the relation of urea to the leptin genotype was not found statistically significant. If genotype CC showed the lowest level of leptin in blood plasma, the results were in agreement with results of [6].

Fig. 3 Relation of genotype of Czech Pied bulls for leptin gene and level of creatinin in blood serum (average columns with one standard deviation bars).



Relation between creatinine and leptin genotype of Czech Pied bulls was significant. Animals with genotype TT did have the level of creatinin lower comparing to genotypes CC and TC. The study [2] focused on comparison of level of leptin and creatinin on Holstein and Charolais determined negative correlation between leptin and plasmatic creatinin.

Fig. 4 Relation of genotype of Czech Pied bulls for leptin gene and level of glucose in blood serum (average columns with one standard deviation bars).



Genotype TC had higher concentration of glucose but the influence was not significant. [9] stated that glucose did not influence leptin concentration in blood plasma of small ruminants in short horizon, even not the hormone with influence e.g. insulin. [9] On the contrary in the case of mice insulin influenced directly leptin production by adipocytes [2]. Compared the leptin and glucose of the both the dairy and beef cattle and observed the positive correlation.

Conclusion

The effects of SNP of leptin gene were determined. In this study, we found significant effect of leptin SNP on serum creatinine concentration, but any effect of leptin SNP on other investigated indicators was noted.

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The effect of the type of litter on the occurrence of footpad dermatitis in broiler chickens

ANNA MUSILOVA, MARTINA LICHOVNIKOVA, ALENA PRZYWAROVA
Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

anna.musilova@mendelu.cz

Abstract: The aim of the study was to evaluate the effect of the type of litter on incidence of footpad dermatitis in broiler chicken. Ross 308 and Cobb 500 hybrids were used in this study. Broilers were fattened in aviaries. This monitoring was performed in two experiments. Each experiment lasted 35 days. In the first experiment, straw, wood shavings and lignocel were used as litter material. Wood shavings, lignocel and peat were used in second experiment. Litter temperature and litter humidity were measured as indicator of the quality of bedding. Temperatures of litter were observed weekly at 5 locations in aviary. Litter humidity was measured in 35 day age of broiler. Samples of litter were collected from 2 locations in each aviary. Scoring of the paws was done in slaughterhouse according to six-point scale (0-5) [2]. To facilitate the evaluation of the paws damage the numbers in scoring groups were summarized as follows: negligible damage (0+1), intermediate damage (2+3) and severe damage (4+5). There was no statistically significant effect ($P<0.05$) between type of litter and litter temperature and litter moisture. The highest damage of paws was in boxes with the highest litter moisture (straw in first experiment and wood shavings in second experiment). These results show a tendency towards higher incidence of FPD depending on higher litter moisture.

Key-Words: broiler, footpad dermatitis, paws, litter

Introduction

Footpad dermatitis (FPD) is known by multiple names, such as pododermatitis and contact dermatitis, all of which refer to a condition that is characterized by inflammation and necrotic lesions, ranging from superficial to deep on the plantar surface of the footpads and toes. Deep ulcers may lead to abscesses and thickening of underlying tissues and structures [9]. The ulcers can cause swelling, redness, and heat under the skin and cause the surface area to thicken [16] and the ulcerations are often covered by crusts formed by exudate, litter and faecal material [9]. It is likely that FPD causes pain and therefore has a negative effect on bird welfare [13]. Animal welfare audits in Europe often use foot, hock, and breast burn-lesions as an indicator of housing conditions and the general welfare of the birds [12]. Concerns about the welfare of broilers have led to a new European Broiler Welfare Directive to be implemented by June 2010 [2]. Litter quality is of great importance for the welfare of broiler chicken, as they generally spend their entire life in contact with litter [18]. Litter serves several functions that include thermal insulation, moisture absorption, protective barrier from the ground, and it is allows for natural scratching behavior. Bedding material must not only be a good absorber of moisture but also have a reasonable drying time [10, 5]. A number of risk factors for wet litter have been suggested. High-moisture litter (i.e., >30%), type or quality of bedding material in broiler production systems has been clearly associated with an increasing incidence and severity of FPD [6]. Litter materials with a high water-holding capacity, such as wood shavings from coniferous trees, are believed to result in better litter quality than litter materials with poorer absorption capacity, such as straw [19]. Stocking density has been reported to influence litter quality, with poor litter quality when sticking density is increased leading to an increase incidence of footpad dermatitis [7]. Climatic conditions influence litter quality, with high relative humidity both outdoors and inside the house being associated with poor litter quality [17].

The aim of the study was to evaluate the effect of the type of litter on the occurrence of footpad dermatitis.

Material and Methods

Birds and management

Broilers were housed in twelve boxes. Boxes were designed for animals. Six smaller boxes provided 3.04 m^2 of floor area and six bigger boxes provided 3.96 m^2. In boxes were feeders and nipple water dispensers. All broilers were fed of standard commercial diets for broiler. Broilers were allowed *ad libitum* access to the feed and water. First week of age broilers was used 24-h photoperiod of light. From second week of age broilers until the end of experiment was used 18-h photoperiod from 5.00 am to 23.00. Broilers were fattened 35 days in both experiments. Weight of broilers was measured at the beginning and the end of the experiments.

In the first experiment eight hundred and nine broilers were housed. Hybrid Ross 308 was used in first experiment. Broilers were one day old. Average stocking density was 36.4 kg live weight/m^2 at the end of experiment. Average live weight of broiler at the end of the fattening was 1.95 kg.

In the second experiment five hundred seventy-five broilers of hybrid Cobb 500 were used. Broilers were one day old. Average stocking density was 25.9 kg live weight/m^2 at the end of experiment. Average live weight of broiler at the end of the fattening was 1.85 kg.

Broilers chickens were slaughtered in the slaughterhouse Modřice Vodňanská Drůbež a.s. company.

Litter management

Four type of litter were used in both experiments. It was straw, wood shavings, lignocel and peat. Quantity of litter used in boxes is shown in Table 1. Straw, wood shavings and peat were used in first experiment. Thus, broilers were divided into three groups according to type of litter and each type of litter was repeated in four boxes. In second experiment wood shavings, lignocel and peat were used.

Analysis and statistics

As parameters of litter quality were measured litter moisture and litter temperature. Both parameters were assessed 35 days of age of broilers. The temperature of the litter was measured at five locations in each box. Contact thermometer was used for measurement of litter temperature. Two samples were collected from each box for assessment litter moisture. First sample was collected between feeders and second sample was collected under the drinker system. Samples of litter were dried at 65 °C and moisture was calculated. Scoring of the paws was done in slaughterhouse according to six-points scale (0-5) Ask (2010). To facilitate the evaluation of the paws damage the numbers in scoring groups were summarized as follows: negligible damage (0+1), intermediate damage (2+3) and severe damage (4+5).

Data obtained from this experiment were analyzed using the single factor analysis of variation. Data were followed by LSD test using the software package Unistat 5.1 (UNISTAT Ltd, ENGLAND).

Table 1 Type of litter, quantity of litter and stocking density in experiment

Type of litter	1. experiment		2. experiment	
	Quantity of litter kg/m^2	Stocking density kg/m^2	Quantity of litter kg/m^2	Stocking density kg/m^2
Straw	0.72	36.5	-	-
Wood shavings	0.64	35.5	0.64	25.7
Lignocel	0.80	37.1	1.00	25.8
Peat	-	-	1.05	26.2

Results and Discussion

Litter temperature and humidity

Four type of litter were compared to ascertain their effect on litter temperature, litter moisture and occurrence of footpad dermatitis in broiler chickens. In Table 2 and Table 3 is expressed effect of the type of litter on litter temperature and litter moisture in both experiments. In first experiment average temperature of the litter in the 5th week age of broilers was 33.3°C. The highest temperature of the litter was in boxes with lignocel and the lowest temperature of the litter was in boxes with straw (Table 2). There was not significantly difference ($P<0.05$) in litter temperature between different types of litter. In second experiment average temperature of litter was 30.8°C. The highest

Table 2 Effect of the type of litter on litter temperature and moisture in first experiment

Parameters	Type of litter		
	Straw	Wood shavings	Lignocel
Litter temperature (°C)	33.2[a]	33.3[a]	33.4[a]
Litter moisture (%)	53.8[a]	52.5[a]	51.6[a]

Table 3 Effect of the type of litter on litter temperature and moisture in second experiment

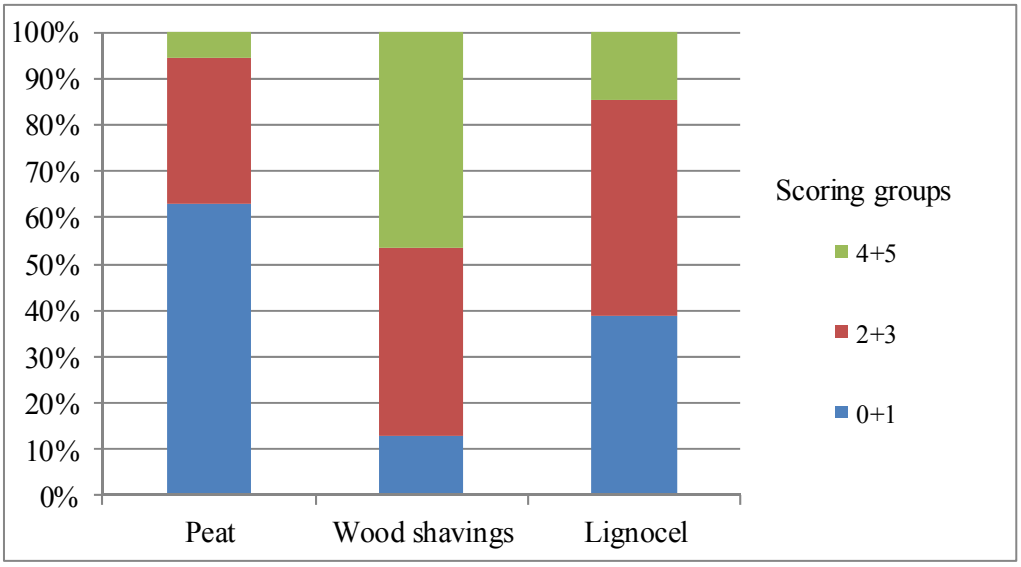
Parameters	Type of litter		
	Peat	Wood shavings	Lignocel
Litter temperature (°C)	31.0[a]	30.8[a]	30.7[a]
Litter moisture (%)	45.6[a]	50.2[a]	46.6[a]

temperature was in boxes with peat and the lowest temperature was in boxes with lignocel (Table 3). Statistically significant difference (P<0.05) between litter moisture and type of litter was not found. Type of litter had not influence on litter temperature. The lower average temperature of litter in the second experiment could be due to lower stocking density in the second experiment than in the first experiment. The average moisture of litter in the 5th week age of broilers was 52.6% in first experiment. The highest moisture of litter was in boxes with straw and the lowest moisture was in boxes with lignocel. There was no statistically significant difference (P<0.05) between litter moisture and type of litter. In second experiment the average litter moisture was 47.5%. The highest moisture was in boxes with wood shavings and the lowest moisture was in boxes with peat. There was no statistically significant difference (P<0.05) between litter moisture and type of litter. Type of litter had no effect on litter moisture. The lower average litter moisture in second experiment could be due lower stocking density in second experiment. [1] assumed that the "critical moisture content" for the development of FPD lesions as about 35% litter moisture content. Furthermore, doubling exposure time (4-8h) led to only slightly increased severity of FPD for the low litter moisture contents (35% and 50% moisture) and a higher rise for the wettest litter treatment (65% moisture) at the end of trial.

Fig. 1 Classification of paws in groups in first experiment

Fig. 1 shows the percentages of different degrees of damage of paws in the first experiment with different type of litter. Better results were obtained in boxes with wood shavings and lignocel. The lowest percentage of representation paws with severe damage (group 4+5) was observed in these both types of litter. Paws classified in group 4+5 are considered as paws with ulcer, which occurs over

Fig. 2 Classification of paws in groups in second experiment



almost the entire plantar surface (25-80%) [2]. Moreover, lesions on the paws may be a gateway for bacteria which moght affect carcass quality [14]. The highest percentage of paws with negligible damage (group 1+2) was found with lignocel. The worst damage of paws was observed at broilers fattened in boxes with straw. Also, the highest litter moisture was found in this type of litter. On the other hand, the best results of classification of paws were achieved in boxes with lignocel, which had the lowest litter moisture. Fig. 2 shows the percentages of different degrees of damage of paws in the second experiment with different type of litter. Markedly better results were obtained in boxes with peat. The lowest percentage of representation paws with severe damage was observed in this type of litter. The worst damage of paws was observed in boxes with wood shavings. Even in this case, the highest damage of paws was in boxes with the highest litter moisture. These results show a tendency towards higher incidence of FPD depending on higher litter moisture. Footpad dermatitis lesions have been found to be more severe as litter moisture increases, especially when the litter contains high moisture with sticky fecal droppings [9]. In general, a high incidence of FPD can be produced in broilers by increasing the moisture level of the litter, as suggested earlier by [11]. According to research conducted with broilers and turkeys, litter conditions (i.e., type, particle size, and moisture level) are significant factors in the development of FPD [6]. This study shows a tendency towards higher incidence of FPD in broiler chickens depending on higher litter moisture. [15] also reported that FPD lesion scores increased rapidly following wetting litter after 1 wk. More recently, [14] showed a similar effect in turkey poults and concluded that water alone was sufficient to cause FPD in a very short time. However, the FPD lesions appeared to regress (i.e., improve) in birds with time, especially with improvements in litter conditions. As the conditions under which broilers are raised vary between different parts of the world, it is extremely difficult to give efficient general advice on how to prevent contact dermatitis [3]. One thing that is common among most previous research is that litter moisture is a significant factor in the onset of FPD.

Wood shavings and lignocel were used in the first experiment and in the second experiment. Better results in the classification of damage of paws in boxes with these both type of litter was achieved in the second experiment. Better results in the second experiment could be due to lower stocking density in the second experiment than in the first experiment. Some studies have reported that higher stocking densities are associated with a greater incidence of FPD than lower stocking densities [12, 16]. The sudden onset of poor litter conditions associated with higher stocking densities is considered to be the biggest influence on the development of FPD. Litter conditions deteriorate rapidly and litter moisture increases as stocking density increases [4]. [8] found that as stocking density increased, water consumption increased per bird. As birds drink more water, their feces may become more watery and thus contributes to overall litter moisture.

Conclusion

There was no statistically significant effect ($P<0.05$) between type of litter and litter temperature and

litter moisture. In the first experiment the highest temperature of the litter was in boxes with lignocel and the lowest temperature of the litter was in boxes with straw. The highest moisture of litter was in boxes with straw and the lowest moisture was in boxes with lignocel. The lowest percentage of representation paws with severe damage (group 4+5) was observed in boxes with wood shavings and lignocel. The worst damage of paws was observed at broilers fattened in boxes with straw. In second experiment the highest temperature was in boxes with peat and the lowest temperature was in boxes with lignocel. The highest moisture was in boxes with wood shavings and the lowest moisture was in boxes with peat. The lowest percentage of representation paws with severe damage was observed in boxes with peat. The worst damage of paws was observed in boxes with wood shavings. The highest damage of paws was in boxes with the highest litter moisture. These results show a tendency towards higher incidence of FPD depending on higher litter moisture.

Acknowledgement

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The comparison of selected breedings of Hucul horses bred in the Slovak republic and Hungary

IVANA ORAVCOVA, EVA SOBOTKOVA, IVA JISKROVA
Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xoravco1@node.mendelu.cz

Abstract: The aim of this work was to compare basic body dimensions and hippo-metrical indices of Hucul horses in the various breedings in Slovak republic and Hungary and found out some differences between these countries in the terms of age, gender, line and environment. First of all in each herd we measured basic body dimensions, then we calculated from measured body dimensions hippo-metrical indices and finally these resultings were analysed statistically and compared with each other. Most of the statistically significant differences were related to the effects of the gender and environment. Out of the 9 body dimensions and indices, 6 were highly signifianted. The line had a statistically highly significant effect on 2 body dimensions and the age was not statistically significant.

Key-Words: Hucul horse, measurement of the horses, basic body dimensions, hippo-metrical indices

Introduction

The Hucul horse is a primitive breed of horses which were very popular with our ancestors for their much appreciated qualities: the horses have a strong constitution; they are surefooted on the rough terrain; easy to feed; of undemanding nature and endurance. These qualities have been successfully preserved to the present day in spite of the fact that for many years the breeders strived to “improve” the Hucul horse by crossing with draught and Fjord horses (efforts to make it stronger) or with Arabian horses (efforts to upgrade the breed). Due to these tendencies at one point in time the Hucul horse came close to extinction. Fortunately due to intensive breeding efforts, in 1979 this small horse was entered in the protected FAO gene pool. Later this breed was ranked among genetic resources not only in Slovakia and in the Czech Republic, but also in Hungary, Poland, Germany, Austria and Ukraine [1]. The gene pool of the Hucul horse is classed among rare resources of genetic diversity. To preserve the high quality of the breeding material permanent monitoring, evaluation and comparisons deem necessary in all countries where this genetic resource exists in order to prevent any greater deviations from the breeding standard.

The Hucul horse is a small mountain breed of well-defined type and typical pace when walking and when overcoming mountain obstacles. Their conformation is correct; the body is long with short and bony legs and tough hoof horn [1]. The head of the Hucul horse is dry and robust, of medium length, wide brain base and strong jowl. The neck is muscular, strong, of medium set, sometimes arched. Not very high-withered, the chest is large, deep and wide. The back is firm, flat, the hips are well bound and short; the croup is short, wide and slightly sloping, the tail is set low. The formation of the front legs is regular, hind legs sometimes bow-legged with converging thighs (allowing better movement in mountain terrains) [2]. Thanks to its body conformation the horse can be used for work in the mountains and also as a draught horse, as a pack horse and for riding [1]. In colour the Hucul horse is usually bay, fawn, black, or chestnut and piebald [3]. Atavistic features appear very frequently: dorsal (eel) stripe (distinct darker stripe on the back), zebra stripes on the knee and forearm and sometimes shoulder stripes (cross). Some individuals show what is called a pale rosette in the area of the dock which is a marking of wild horses. Albinotic markings are undesirable [3].

In the present study we focused on 2 important herds bred in Slovakia, i.e. the National Stud Farm Topoľčianky and Slovakian Hucul Club in Lom nad Rimavicou and 1 big herd in Hungary. In each herd we analysed 5 basic body dimensions, calculated the hippo-metrical indices and statistically processed the results. The objective of our study was to carry out comparisons of selected herds of Hucul horses

in terms of their age, gender, line and environment; to detect potential deviations and to analyse the possible causes.

Material and Methods

The own results of field measuring of the herds of Hucul horses in Slovakia and in Hungary served as groundwork material for our research.

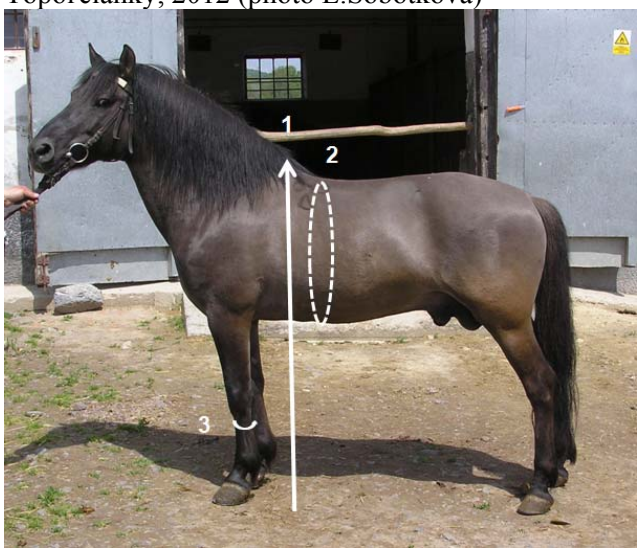
In total we measured 132 Hucul horses. In Hungary we measured 87 horses (65 breeding Hucul mares, 11 breeding Hucul stallions and 11 Hucul geldings) from Aggtelek national park and in Slovakia we measured 45 Hucul horses. In the National Stud Farm Topoľčianky we measured 22 breeding Hucul mares and 5 Hucul stallions. In private sphere horses of the M. Gonda breeder from Slovak Hucul club in Lom nad Rimavicou were measured in the number of 17 Hucul mares and 1 Hucul stallion.

As aids for measurement we used the stick measure (a three-part square folding metal stick with an engraved scale in cm, with two perpendicular arms of which one arm was adjustable) and the tape measure for horses (wax tape with a scale). All the horses were measured in October – December by two assistants using the same aids (therefore eliminating errors in the results). Each dimension was measured three times and in the study we included the resulting average value from these 3 dimensions. During measuring the horses were standing on a flat firm surface and all the 4 limbs were equally weighted. When viewed from the side the chest and pelvis limbs overlapped. In all the measurements we took into account the height of the horseshoe which was then subtracted from the measured values. To obtain correct and as accurate as possible data we used only dimensions which were not in any way disturbed during the measuring process.

Dušek et al. [4] defined the body dimensions as follows:

1. *Stick measure (SM)* – perpendicular distance of the highest point of withers from the ground
2. *Tape measure (TM)* – distance from the outside heel of the left front limb to the highest point of the withers
3. *Heart girth (HG)* – measured behind the shoulder blade and withers at the point of the smallest girth
4. *Circumference of the shank (CS)* – measured at the weakest point of the cannon bone on the left front limb, i.e. at the point where the upper third of the cannon bone passes into the second third

Fig. 1 Basic body dimensions of horses, Topoľčianky, 2012 (photo E.Sobotková)



Legend: 1 – Stick/tape measure, 2 – Heart girth, 3- Circumfence of the shank

The following indices of the body conformation were calculated for all the analysed horses based on field measurements of body dimensions [4]:

1. *Index of the body conformation (IBC)* – (diagonal length of body / stick measure) x 100
2. *Index of robustness (IROB)* – (heart girth / tape measure) x 100
3. *Index of compactness (ICOM)* – (heart girth / diagonal length of body) x 100
4. *Index of boniness (IBON)* – (circumference of the shank / stick measure) x 100

The data were processed using the programme MICROSOFT EXCEL 2000. The database was divided according to:

A) Age
- horses of 3 - 6 years (n = 53)
- horses of 7 - 12 years (n = 49)
- horses of 13 – 18 years (n = 24)
- horses of 19 years and older (n = 6)

B) Gender
- stallions (n = 17)
- mares (n = 104)
- geldings (n = 11)

C) Line
- Goral (n = 36)
- Gurgul (n = 7)
- Hroby (n = 43)
- Oušor (n = 20)
- Prislop and Pietrosu (n = 12)
- Polan (n = 14)

D) Environment
- Slovakia (n = 45)
- Hungary (n = 87)

We used the database to monitor correlations among the body dimensions and indices and age, gender, line and environment. Data on the conformation and indices were statistically processed using the statistical programme UNISTAT version 5.1. :
- by overall numerical characteristics of mean values and rates of variations (the following mean values and rates of variation were evaluated: dispersion and variation coefficient)
- by the GLM (linear model with consistent effects) method.

Model equation of linear model with consistent effects (GLM):

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

Where:
y_{ijklm} = observation of the body rate or index
μ = total mean
a_i = consistent effect of the age of the horse (i = 1,2,3,4)
b_j = consistent effect of the gender of the horse (j = 1, 2, 3)
c_k = consistent effect of the line (k = 1, 2, 3, 4, 5, 6)
d_l = consistent effect of the environment (l = 1, 2)
e_{ijklm} = random residual error

If the impact of the monitored effect was statistically significant we specified the differences among the respective factors of age, gender, line and environment by means of the method of multiple comparisons (Scheffe method).

Result and Discussion
Statistical processing of the data by means of the programme Unistat 5.1 revealed statistically significant and highly significant differences. Table 1 gives a basic outline illustrating the measures and indices of the given effects which came out as statistically significant.

Comparisons of the stick measure
Analysis showed that the effects of the age and environment were not statistically significant on stick measure. Multiple comparisons showed statistically highly significant differences, particularly among the gender and line. Mares (139.02 cm) exhibited the lowest values of the SM and stallions (142.15 cm) and geldings (140.55 cm) the highest. This fact is in agreement with breeding standard of Hucul horses, where Hucul stallions have acceptable higher SM as Hucul mares.

Table 1 Overall results of statistical analysis

Dimens/Effects	Gender	Line	Enviro.
SM	**	**	
TM	**	**	**
HG		*	**
CS	**		**
IBC	**	*	
IROB	**		**
ICOM			**
IBON	**		**

Legend: SM-stick measure, TM-tape measure, HG-heart girth, CS-circumference of the shank, IBC-index of the body conformation, IROB-index of robustness, ICOM-index of compactness, IBON-index of boniness
** indicates a statistically significant effect $p \leq 0.05$*
*** indicates a statistically highly significant effect $p \leq 0.01$*

Jakubec et al. [5] who measured the Old Kladrub horses discovered small differences in the SM between stallions and mares also. Multiple comparisons showed statistically highly significant differences among lines. Lines Pietrosu and Prislop (142.58 cm) exhibited the highest values and line Polan (136.68 cm) the lowest. In her dissertation thesis Matoušová-Malbohanová [6] reached the same conclusions when comparing Hucul horses bred in the Czech Republic and in Poland. Some of the body dimensions of the lines Pietrosu and Prislop showed higher average values than other lines. If we study the average values of body dimensions in greater detail we see that the line Pietrosu has preserved the large body dimensions of its founder. It is of common knowledge that the body dimensions of both the line Prislop and the line Pietrosu are larger than of the other Hucul lines. Krzemień [7] declare that foundation sire of Pietrosu line in Poland, stallion Pietrosu VI-111, exhibited 141 cm SM, 168 cm HG and 19 cm CS. Also the body dimensions of the sire Prislop IX-90 bred in Topoľčianky were above-standard (SM 149 cm, TM 161 cm, HG 190 cm, CS 20,0 cm) Matoušová-Malbohanová et al. [8].

Comparisons of the tape measure
Analysis showed that the effects of the gender, line and environment were statistically significant on tape measure. Multiple comparisons showed statistically highly significant differences among the

gender. Mares (146.57 cm) exhibited the lowest values of the TM and stallions (150.24 cm) and geldings (149.32 cm) the highest. Simčič et al. [9] who measured the primitive breed of Posavje horse in Slovenia and Croatia discovered statistically highly significant differences in the TM between the gender. The TM of stallions was statistically significantly larger by 3.22 cm than the TM of mares. Multiple comparisons showed statistically highly significant differences among lines. Again lines Pietrosu and Prislop (150.17 cm) exhibited the highest values and line Polan (144.14 cm) the lowest. Multiple comparisons showed that Hucul horses bred in Slovakia (148.63 cm) exhibited the higher values of the TM as Hucul horses bred in Hungary (146.56 cm). Between the highest and lowest average value the difference is more than 2 cm. This fact is not in agreement with the highest and lowest average value of the SM (difference is about 0.9 cm). TM is largely influenced by the HG, which, as we can see in the chapter below, is significantly higher in Hucul horses bred in the Slovakia.

Comparisons of the heart girth

Analysis showed that the effect of the age and gender were not statistically significant on HG. Effect of line was statistically significant and environment was statistically highly significant on HG. With the help of multiple comparisons we found out that Hucul horses bred in Slovakia (181.79 cm) exhibited markedly higher values of the HG as Hucul horses bred in Hungary (166.49 cm). Even though the GLM method showed that the line had a statistically significant effect on the heart girth, multiple comparison did not show anything. In the present study the authors gives at least the basic average values of the lines, because the difference between them is relatively big (between the highest and lowest average value the difference is 11.95 cm). Here again the lines Pietrosu and Prislop reached the highest average value of HG and the line Polan the lowest (176.88 and 164.93 cm, respectively).

Comparisons of the circumference of the shank

Analysis showed that the effects of the age and line were not statistically significant on the circumference of the shank. On the other hand, multiple comparisons showed statistically highly significant differences among the gender and environment. Hucul horses bred in Slovakia (18.51 cm) exhibited lower values of the CS as Hucul horses bred in Hungary (19.32 cm). With the help of multiple comparisons we found out that Hucul mares (18.85 cm) exhibited the lowest values of the CS and stallions (19.71 cm) and geldings (19.86 cm) the highest. A similar conclusion was also present by Matoušová-Malbohanová et al. [8], when comparing the body conformation between Hucul horses bred in the Czech Republic and in Poland and Purzyc et al. [10], when comparing the body conformation of 243 Hucul horses bred in Poland. They discovered differences in the CS based on the effect of the gender. Here again we see differences between stallions and mares. The mares have a lower CS than the stallions. Sobotková et al. [11] discovered statistically highly significant differences in the CS between the genders of the Old Kladrub horses (the CS of stallions was statistically significantly larger by 0.6 cm than the CS of mares) as well as Simčič et al. [9] who measured the primitive breed of Posavje horse in Slovenia and Croatia (the CS of stallions was statistically significantly larger by 2.1 cm than the CS of mares).

Hippo-metrical indices

Comparison analysis revealed that the gender and line have a significant effect on the IBC of Hucul horses. In warm-blood breeds this index ranges roughly around 100, in heavy horses around 109 [12]. In Hucul horses this index ranges within these two values. Multiple comparisons showed statistically highly significant differences, particularly among the gender. The stallions (100.37) have a lower IBC than the mares (106.04). A similar conclusion was also present by Matoušová-Malbohanová et al. [8], when comparing the body conformation between Hucul horses bred in the Czech Republic and in Poland – Hucul mares had statistically significant higher of IBC than stallions. Even though the GLM method showed that the line had a statistically significant effect on IBC, multiple comparison did not show anything. IBC is in relation with body dimension *diagonal length of body*. The higher the diagonal length of body is the higher is also IBC. Analysis showed that the effect of the environment was not statistically significant on diagonal length of body and IBC so we can suppose that Hucul horses bred in Slovakia and Hungary are proportional well-balanced.

Analysis showed that the effect of the gender and environment were statistically significant on IROB. The IROB decreased from heavy horses to the lighter breeds [13]. Multiple comparisons showed statistically highly significant differences among the environment, where Hucul horses bred in Slovakia (129.7) are robuster as Hucul horses bred in

Hungary (119.62). IROB is in relation with body dimension heart girth, which was statistically significant in our work also. HG of Hucul horses bred in Slovakia was statistically significantly larger by 15.3 cm than the HG of Hucul horses bred in Hungary so higher average value of IROB in Slovakia´s Hucul horses is not surprising. Multiple comparisons also showed statistically highly significant differences among the gender, where mares (124.12 cm) exhibited the highest values of the IROB and stallions (119.556 cm) and geldings (118.47 cm) the lowest. A similar conclusion was also present by Matoušová-Malbohanová et al. [8], when comparing the body conformation between Hucul horses bred in the Czech Republic and in Poland; i.e. that Hucul mares were robuster as stallions and Purzyc et al. [10] reached the same conclusions; i.e. that adult Hucul mares bred in Poland had a larger heart girth and smaller dimension of the bone than stallions. Furthermore, they consider the hypothesis that subsequent pregnancies in mares additionally transfer some of the function of the thoracic diaphragm, through indirect pressure, to inhaling muscles of the chest; this could result in greater arching of the ribs, and by extension – greater circumference of the chest, in mares. It is therefore likely that Hucul mares are more robust and deeper than stallions which are bonier and which become considerably stronger after castration.

By means of multiple comparisons we discovered that the environment has a significant effect on the index of compactness. The most compact horses are bred in Slovakia. ICOM is in relation with body dimension heart girth, which was higher in Slovakia´s Hucul horses.

Comparative analysis established that the gender and environment had a significant effect on the index of boniness of Hucul horses. Multiple comparisons detected statistically highly significant differences between the genders. Mares exhibited the lowest values of the IBON and stallions and geldings the highest. When comparing the body conformation of Hucul horses bred in the Czech Republic and in Poland Matoušová-Malbohanová et al. [8] reached the same conclusions; i.e. they found differences between stallions and mares where stallions had a higher IBON than mares. Multiple comparisons also revealed statistically highly significant differences among the environment. The index of boniness was higher in country which also exhibited the highest values in the circumference of the shank so Hucul horses bred in Hungary (13.88) exhibited higher values as Slovakia´s Hucul horses (13.2).

Conclusion

The objective of the present study was to analyse the effect of the age, line, gender and the environment on the body conformation of Hucul horses bred in some herds in the Slovak Republic and in Hungary. Most of the statistically significant differences appeared when analysing the effect of the environment and gender. Out of the 8 measured body dimensions and indices 6 were statistically highly significant in both effects. Hucul horses bred in Slovakia exhibited higher average values in almost every body dimensions and indices. From the results it can be assumed that Slovakia´s Hucul horses are higher, wider and they are robuster and more compact as Hucul horses bred in Hungary which are bonier. The effect of the environment embraces not only the environment of the horse, but also the technique and technology of rearing. The effect of the gender showed important statistical differences; i.e. mares are deeper and more robust than stallions. Stallions are bonier and after castration they become considerably stronger but do not increase their height any more. The line has a significant effect on the HG and IBC and highly significant effect on the SM and TM. Horses of the Prislop and Pietrosu line achieved the highest average values and Polan line the lowest. The effect of the age was not significant.

The results indicate that there are certain differences among the populations of Hucul horses from measured herds in Slovakia and Hungary. To raise the standard and improve the herd the authors recommend, in particular, a higher level of selection and breeding to be implemented as well as to change the conditions of rearing the Hucul horses to accommodate the demands of the horses and to avoid changing their precious conformation due to unfavourable conditions. Further recommendation is to harmonise the standard of breeding the Hucul horses within the HIF (Hucul International Federation) (to be identical for all countries where this horse is bred) and to agree on where the breeding should be headed. The Hucul is a horse resistant to harsh conditions, undemanding, easy to feed, healthy, calm with good disposition and we should do our utmost to preserve it in this condition for the future generations.

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The effect of the stable environment and age on the semen production in the Czech Fleckvieh bulls

MICHAELA PALDUSOVA[1], TOMAS KOPEC[2], GUSTAV CHLADEK[1], MARTIN HOSEK[1], LADISLAV MACHAL[1], DANIEL FALTA[1]
[1]Department of animal breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Czech Fleckvieh Breeders Association
U Topíren 2, 170 41 Praha 7
CZECH REPUBLIC

michaela.paldusova@mendelu.cz

Abstract: The objective of this study is to evaluate the effect of stable environment and age on semen production in the Czech Fleckvieh bulls. During the period from March 2014 to August 2014, semen samples (n = 232) were collected using an artificial vagina. Immediately after collection, laboratory examinations were made for all samples, which included finding out ejaculate volume, sperm concentration and total sperm count. Ejaculate volume was measured using the graduated tube and sperm concentration was determined using a spectrophotometer. Total sperm count was calculated by multiplying ejaculate volume by sperm concentration. For statistic evaluation of the effect of stable environment, the average values of THI_1 (temperature-humidity index from the one day before semen collection) were used and the bulls were divided into the groups according to the age. Monitored characteristics were expressed in weighted average and standard error. The effect of the stable environment and age on bovine semen production was tested by the general linear model (PROC GLM) in SAS 9.1. Based on the results we can conclude that age had the significant influence ($P < 0.05$) on all monitored parameters. In case of THI_1, no statistically significant differences among determined classes were found ($P > 0.05$).

Key-Words: age, bovine semen, Czech Fleckvieh bulls, semen production, stable environment

Introduction

Artificial insemination (AI) is one of the most powerful and the most valuable biotechnology methods that allows to dairy cattle breeders to use of quality proven AI sires and thus to improve genetic potential and increasing profitability of their herds.

For the last sixty years, AI has become one of the most important tools in Czech cattle breeding programs. At present in the Czech Republic, more than 98% [1] of breeding stock of Czech Fleckvieh dairy cows is artificially inseminated. It is clear that the need of knowledge of factors affecting the sperm production and quality of bovine semen is of considerable with regard to reproductive efficiency.

Photoperiod, temperature, relative humidity, nutrition, diseases and some other factors can affect welfare of farmed animals. Cattle production can be affected by heat stress, when under high ambient temperature and relative humidity of stable air, internal thermoregulatory mechanisms are unable to increase body heat loss and body temperature increases above physiological limits [2]. In case of AI sires, heat stress can cause a decrease of quantitative and qualitative parameters of their ejaculates [3]. The size of heat stress, can be defined as a sum of external factors on the animal that act to displace body temperature from set point, is caused by the combined effects of temperature, humidity, solar radiation and wind speed [4]. A variety of indices were used to estimate the degree of heat stress affecting cattle. The temperature-humidity index (THI), uses temperature and relative humidity of stable air to estimate the size of heat stress [5].

The present study was conducted to determine the effect of stable environment and age on the semen production of a selected population of the Czech Fleckvieh bulls kept in AI center.

Material and Methods

Characterization of location

The project was realized in AI center of Chovatelské družstvo Impuls, družstvo at Vysočina region (GPS:

49°28'25.137"N, 16°4'3.303"E and 558 m above sea level). This region is characterized by mild climate throughout the all year. Average daily temperature in stable was 14.9ºC with a minimum of 3.8ºC and a maximum of 23.6ºC and average relative humidity was 65.9 % (43.1 – 94.5%), during the experimental period.

Animals and treatment

The study was carried out on a biological matherial consisting of semen samples from the 18 Czech Fleckvieh AI bulls, aged between 1.5 and 8.5 years, as Table 1 shown. Bulls were kept intensively and were fed ad libitum of hay and 3 kg of a 14% protein concentrate diet per bull per day. Water was available ad libitum too.

Tab. 1 Division of bulls according to their age

Class of age	*n*	*Age of bulls*
1	*8*	< 2 years
2	*4*	2 – 5 years
3	*6*	> 5 years
Total 18		

Semen samples and analyses

In total, the 232 semen samples were collected in period from the 1st March, 2014 till the 31st August, 2014. A standard bovine artificial vagina at a temperature of 42°C was used to collect the semen from all bulls. The bulls were paraded around a teaser bull to increase the libido prior to semen collection [6].

Immediately after collection, macroscopic and microscopic examinations were made for all samples, which included finding out ejaculate volume, concentration of sperm and total sperm count. The ejaculate volume was read directly from the tube after semen removal from the artificial vagina. Concentration was established through spectofotometric method, using a spectrofotometer calibrated for bull semen. Total sperm count was calculated by multiplying ejaculate volume by sperm concentration.

Data of stable environment

The effect of stable environment was analyzed by using THI_1 (temperature-humidity index one day before collection) which was counted based on data of temperature and relative humidity of stable air. Data of temperature and relative humidity were measured by HOBO data logger at 30 minutes intervals. THI_1 was determined using the following equation [7]:

$$THI_1 = \frac{(0.8 \times t_{db} + ((t_{db} - 14.4) \times RH))}{100} + 46.4$$

Where:

- *t_{db} is temperature of stable air (°C)*
- *and RH is relative humidity (%).*

For statistic evaluation of the effect of stable environment, the average values of THI_1 from the one day before semen collection were used and divided, as indicated in Table 2.

Tab. 2 Division of observation according to THI_1

Class of THI_1	***n***	***Range of THI_1***
1	*17*	< 50
2	*59*	50.1 – 55
3	*57*	55.1 – 60
4	*40*	60.1 – 65
5	*59*	> 65.1
Total 232		

Statistical analyses

Statistical analyses of the data were done using the general linear model (GLM) procedure of the Statistical Analysis System [8]. The least square means (LSM) option of general linear model (GLM) was used for analysing of effect of age and THI_1 for each trait separately, as follows:

$$y_{ij} = age_i + thi_j + e_{ij}$$

Where:

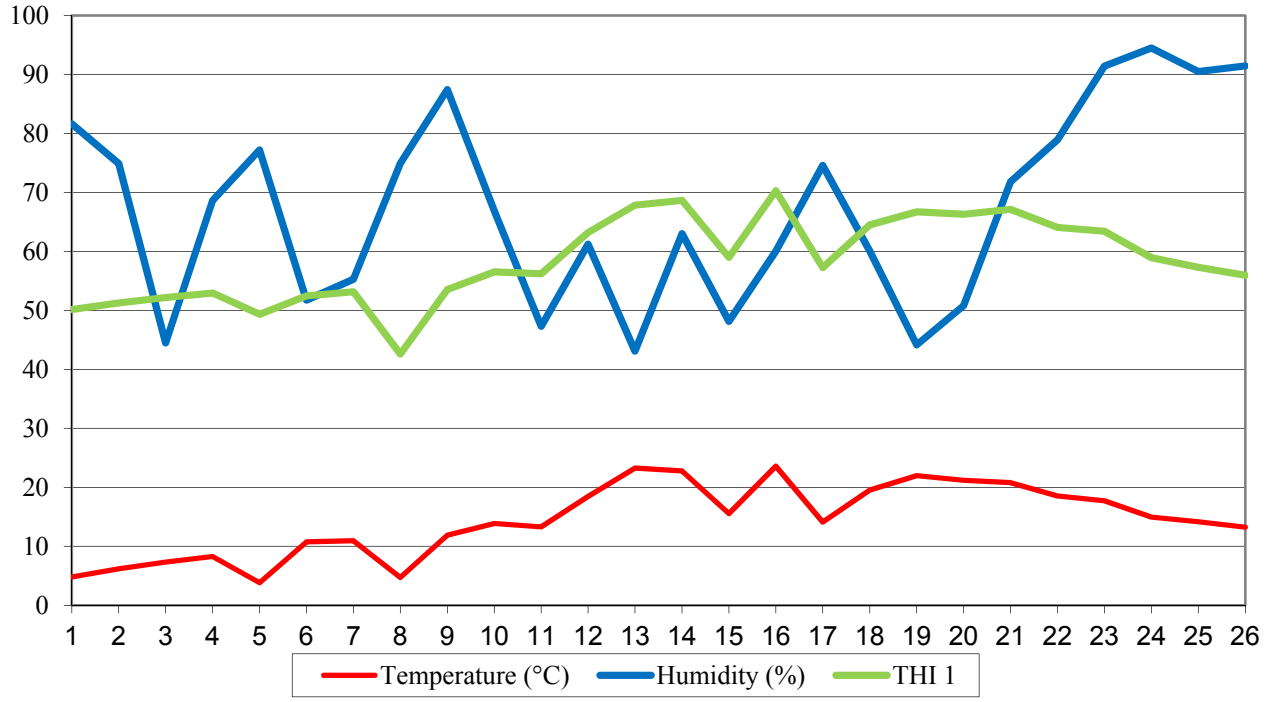
- *y is observations of ejaculate volume, sperm concentration and total sperm count;*
- *age is fixed effect of i^{th} group of age of bulls ($i = 1 – 3$);*
- *thi is fixed effect of j^{th} group of THI_1 index ($j = 1 – 5$);*
- *and e was residual error.*

Results and Discussion

Analysis of temperature, humidity and THI_1

In the course of observation period, the trend of temperature and relative humidity responded the typical climate of Vysočina region, when extreme values were not found, as indicated in Figure 1. The same trend was observed in the case of THI_1.

Fig. 1 Trend of temperature, relative humidity and THI_1 during the observation period



Effect of age on the bovine semen production

The results recorded indicated that age had a statistically significant influence ($P < 0.05$) on all monitored characteristics.

The values of ejaculate volume ranged from 7.26 ± 0.25 to 11.91 ± 0.24 ml with an overall average of around 9.67 ± 0.21 ml. Figure 2 illustrates that the highest ejaculate volume was obtained from the class of age 3 (> 5 years) and the smallest values was found in class of age 2 (2 – 5 years). Class of age 3 had a significantly higher ejaculate volume than class of age 1 (< 2 years) and class of age 2 ($P < 0.01$). Mathevon *et al.* [9] reported that the the volume of the ejaculate improves with an increase in the age of bulls. The same trend was recorded in our study (Figure 2).

In case of sperm concentration, values were moving in variation ranging from 1055.70 ± 31.22 × 10^6 to 1244.42 ± 56.64 × 10^6 with an overall average of around 1101.04 ± 22.21 × 10^6 spermatozoa/ml of ejaculate. Statistically significant difference ($P < 0.05$) was found between class of age 2 and the class of age 1, and highly statistically significant difference ($P < 0.01$) was proved between class of age 2 and the class of age 3, as indicated in Table 3.

In terms of total sperm count, values of monitored groups were very balanced (8649.53 ± 404.54 × 10^6 and 8699.79 ± 502.39 × 10^6) except the class of oldest bulls (12427.05 ± 427.65 × 10^6) with an overall average value of around 10464.02 ± 292.10 × 10^6 spermatozoa per ml of ejaculate. In case of this class, a highly statistically significant difference ($P < 0.01$), between it and the remaining classes, was found.

Tab. 3 Effect of age on semen production

Efekt		*n*	*Ejaculate volume (ml)*		*Sperm concentration (×10⁶ spermatozoa/ml)*		*Total sperm count (×10⁶ spermatozoa/ml)*	
			Mean	*Std.*	*Mean*	*Std.*	*Mean*	*Std.*
Age	**1**	*88*	7.76^{A}	2.31	$1104.45^{a,A}$	343.28	8649.53^{A}	3794.88
	2	*33*	7.26^{A}	2.24	$1244.42^{b,A}$	325.39	8699.79^{A}	2886.04
	3	*111*	$11.91^{A,B}$	2.57	1055.70^{B}	328.89	$12427.05^{A,B}$	4505.60

A, B – between values with different letters in a column were proved statistical highly significant differences ($P < 0.01$)
a, b – between values with different letters in a column were proved statistical evidential differences ($P < 0.05$)

Our results are confirmed by Fuerst - Waltl *et al.* [10] who analyzed effect of age and environmental factors on semen production too and state, that total number of sperm per ejaculate followed those for ejaculate volume. This trend was also observed in our study (Table 3) and was probably due to the size of the bull´s testicles, which continue to grow up to five years of age [11].

Fig. 2 Effect of age on ejaculate volume (ml)

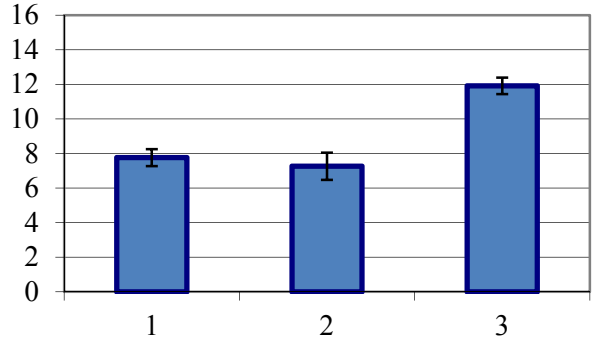


Fig. 3 Effect of age on concentration ($\times 10^6$)

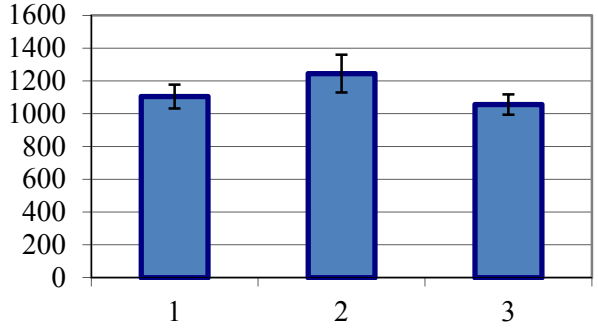
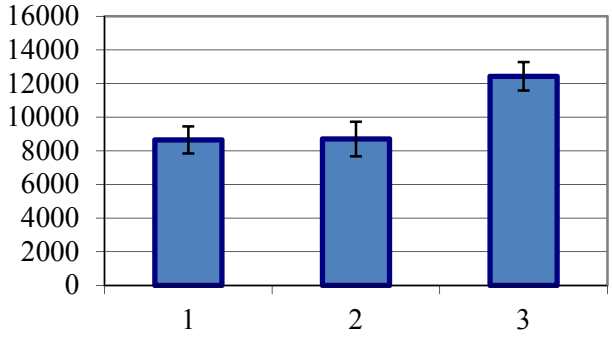


Fig. 4 Effect of age on total sperm count ($\times 10^6$)



Effect of THI_1 on the bovine semen production

Salah *et al.* [12] reported in their study, that association among the temperature-humidity index (THI) and sperm concentration and total sperm count, was negative and significant ($P < 0.01$) where it was non-significant with ejaculates volume. As our results indicate, in case of effect of THI_1 on ejaculate volume, sperm concentration and total sperm count, the measured values were very balanced (Figure 5, 6, 7) and the differences recorded among them statistically insignificant (Table 4). This fact could be cause by the small variation in range of monitored climate parameters.

Fig. 5 Effect of THI_1 on ejaculate volume (ml)

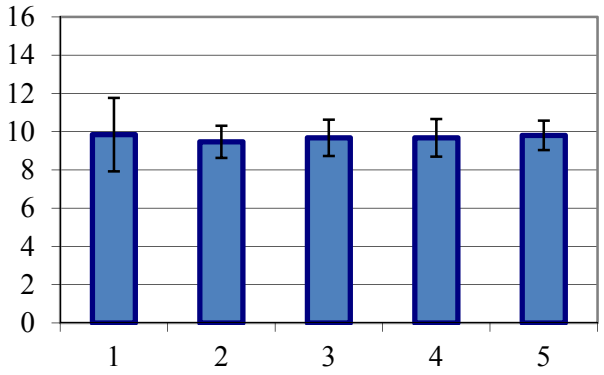


Fig. 6 Effect of THI_1 on concentration ($\times 10^6$)

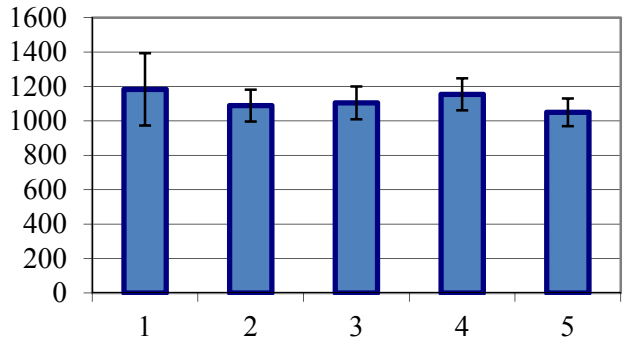
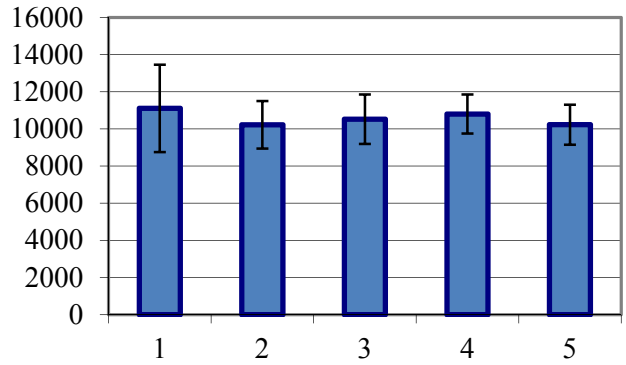


Fig. 7 Effect of THI_1 on total sperm count ($\times 10^6$)



Conclusion

According our results, the class of age has a significant influence on the observed parameters of the bovine semen. Oldest bulls have highest volume of ejaculate and total sperm count. In case of sperm concentration, the class of age 2 shows highest values. All these differences were statistically high significant ($P < 0.01$). In case of THI_1, no significant effect on the monitoring parameters was observed. This facts could be caused by mild climate without extreme values of daily temperature and humidity.

Tab. 4 Effect of THI_1 on the bovine semen production

Efekt		*n*	*Ejaculate volume (ml)*		*Sperm concentration (×10⁶spermatozoa/ml)*		*Total sperm count (×10⁶spermatozoa/ml)*	
			Mean	*Std.*	*Mean*	*Std.*	*Mean*	*Std.*
THI_1	**1**	*17*	9.85	3.74	1183.12	408.98	11106.50	4569.91
	2	*59*	9.47	3.24	1088.58	354.61	10224.38	4916.00
	3	*57*	9.68	3.54	1104.81	360.46	10526.61	5015.41
	4	*40*	9.68	3.06	1154.45	290.04	10802.93	3285.99
	5	*59*	9.81	2.98	1050.00	308.70	10228.31	4128.48

Acknowledgement
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Evaluation of light parameters in terms of breeding boars

HANA PECINOVA, ZDENEK HAVLICEK
Department of Morphology, Physiology and Animal Genetics
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xpecino3@node.mendelu.cz

Abstract: One of the key factors affecting achievement of good reproductive skills within the microclimatic conditions is lighting. The environment in which animals are kept can be defined as an overall influence of external physical, chemical and biological factors, all of them being among those substantially affecting the animal welfare. The lighting parameters play a significant role. Light intensity which is evaluated in this experiment was conducted at weekly intervals at ISK Velké Meziříčí from March 2012 to March 2013. The experiment included such breeds of pigs as Duroc, Danish Duroc and Landrace. Light intensity was evaluated in relation to parameters of sperm quality, namely: sperm motility (%), semen volume (ml), sperm concentration (thousand/mm^3) and the percentage of sperm with pathological changes (%). The analysis of collected data showed statistically a significant light effect on the ejaculate quality (P=0.01). With an increase of light intensity (65.7 lx), the volume and portion of pathological changes decreased (to 231.3 ml from 279.7 ml and 8.7 % from 9.6%), while the concentration has risen (426.8 thousand/mm^3 from 346.2 thousand/mm^3) in opposite to lower level of light intensity (52.3 lx).

Key-Words: Boar, light intensity, light meter, semen quality

Introduction

Stable climate is a key to successful healthy status of animals bred in closed space [1]. The surroundings is defined as an overall influence of external physical (temperature, relative humidity, airflow, light, noise), chemical (gasses) and biological (microorganisms, dust) factors which have impact on animals' health, satisfaction and efficiency [9].

Fluctuation of luminous portion of a day has an impact on breeding animals from middle and higher latitudes, through the influence of photoperiodism and its perception via the organ of sight [7].

Stall lighting significantly affects regulation of the reproduction cycle. If a minimal standard 40 lx for a period of 8 hours a day is not complied, particular changes in animals' behaviour as well as in structure of single tissues and organs occur as a result of physiological processes [3, 8].

The physiological importance of lighting lies in fulfilling vital processes thanks to so-called dual sight function. It involves visual function (medium for processing the sight stimulus) and non-visual function (medium for providing important biological processes). If the availability of lighting is insufficient, the balance of physiological, biological and mental processes is broken. A long-term lack causes a decrease of organism immunity [6].

In relation to venereal activity, lighting functions through hormones produced by germ cells of gonads during a cycle of oestrus, which means venereal sexual characteristics, temper and managing the oestrus [4].

The whole process of adolescence is led by gonadotrophic hormones which, among others, shape physical features and males' behaviour. Under the influence of such hormonal changes spermiogenesis happens. The venereal adolescence is affected by factors such as heredity, nutrition and external conditions of farming. Axis hypothalamus-hypophysis-gonads or hypothalamus-adenohyphophysis-testicle in males' case are extremely important for regulation of endocrine function [5]. Innervation of venereal centres is arranged in two ways – by stimuli of internal and external

backgrounds when such stimuli penetrate and reactions arisen from them are hinged on each other [2].

Material and Methods

Methodology of this experiment was constant for the whole year and measuring took place from 2.3.2012 to 1.3.2013. Intensity of lighting was measured at the same time and the same place (at the level of boar's eyes) with Luxmeter. The intensity was evaluated in relation to parameters of the ejaculate quality, including motility of sperms (%), volume of ejaculate (ml), concentration of sperms (thousand/mm^3) and rate of sperms' abnormalities (%). Data analysis of the experiment was statistically evaluated.

Results

The results supported by statistical argumentativeness comment, first of all, the effect of stable and light intensity on the value of ejaculate, followed by the effect of light intensity on boars, sorted by breeds (duroc, Danish duroc, landrace), in different seasons of a year. The effect of light intensity at higher average light intensity (65.7 lx against 52.3 lx) caused a decreased volume of ejaculate (from 279.7 ml to 231.3 ml), increased concentration (from 346.2 thousand/mm^3 to 426.8 thousand/mm^3) and a percentage decrease in pathological changes (from 9.6% to 8.7%).

Concerning the stable aspect, it was detected that it was necessary to unify lighting conditions of different hall units where the decrease was about 20 lx in comparison to the others (47 lx against 68 lx). While the volume of sperm in this particular unit was high (292 ml against 219 ml), there was a fall of sperm concentration (from 432 thousand/mm^3 to 354 thousand/mm^3), not mentioning nearly double deterioration of percentage abnormality of sperms (up to 12% against 7%).

Furthermore, the influence of breed was compared to the influence of light intensity. Landrace breed in particular was affected by low light intensity (52 lx against 66 lx). Although Landrace breed provided the highest volume (445 ml against 322 ml), it showed the worst percentage value of sperm abnormalities (12.3% against 5.6%) and reduced concentration of sperm (from 289 thousand/mm^3 to 220 thousand/mm^3). Duroc displayed the highest sperm concentration during high light intensity (from 390 to 473 thousand/mm^3) and abnormalities were reduced by 0.3%. Danish duroc proved also an increase in concentration during higher light intensity (from 377 to 439 thousand/mm^3) and abnormalities were lower as well (from 12.1% to 10.6%). It can be stated that in duroc case the concentration during higher light intensity increased nearly by 90 thousand/mm^3 and in Danish duroc case it went up by more than 60 thousand/mm^3. Having taken the evaluated breeds into account, it can be concluded that the influence of lower light intensity has a negative impact on quality of ejaculate, which varies at different breeds.

Fig. 1 The volume of semen at boars and intensity of lighting in different stables

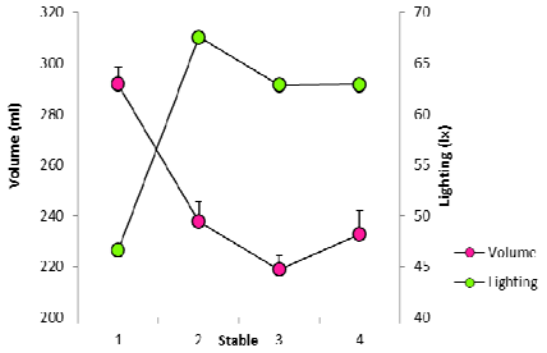


Fig. 2 Sperm concentration at boars and intensity of lighting in different stables

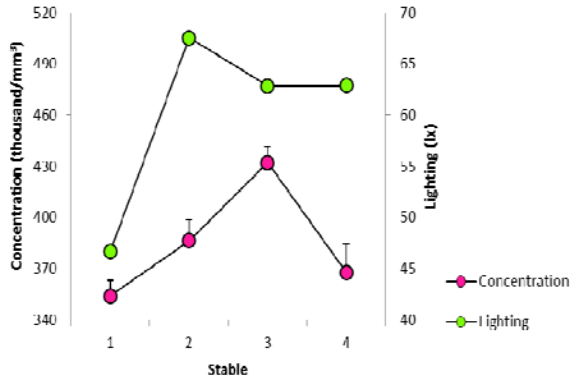
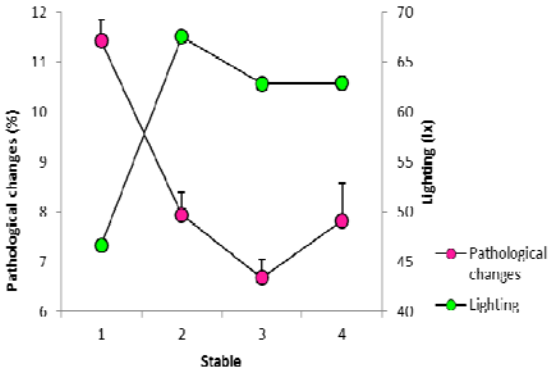


Fig. 3 Pathological changes of sperm at boars and intensity of lighting in different stables



Discussion

Light modes in pigsties are a subject of continuous discussions. The element of seasonality can be noticed in both male and female wild swine species. Domestic pig appears to stem from the wild swine and many experiments have proved that sows tend to acyclicity during summer months. Equally, boars' potency goes down during summer season as well as quality of their semen worsens, which has been affirmed by results of an experiment carried out at ISK Velké Meziříčí.

Fig. 4 Volume of ejaculate at boars of *Landrace* breed and the effect of light intensity in different seasons of a year

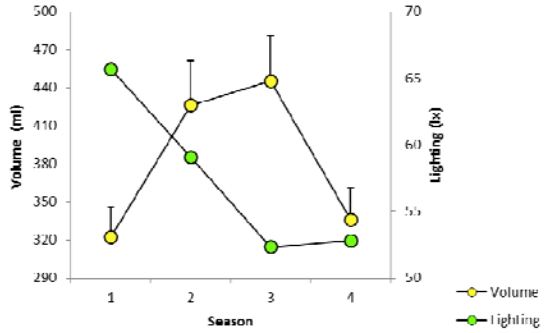


Fig. 5 Concentration of sperm at boars of *Landrace* breed and influence of light intensity in different seasons of a year

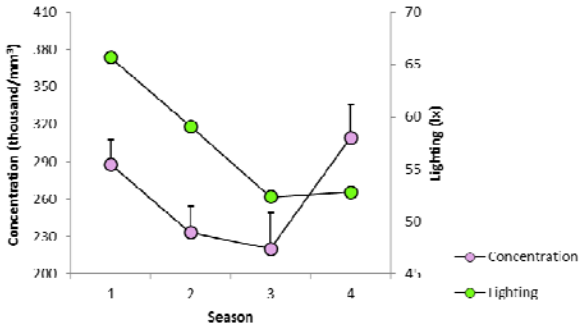
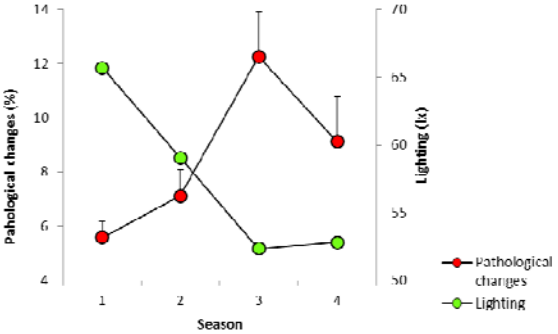


Fig. 6 Pathological changes of sperm at boars of *Landrace* breed and intensity of lighting in different seasons of a year



Some experiments dealt with an assessment of illumination quality as regards sows and boars, focusing on elimination of sexual function deviations controlled by light modes. When it comes to sows, phase light modes evidently appear much more successful than the constant ones. This particular thesis can be applied to boars, too, with the exception that light programs are based on development of young boars' sexual adolescence, which has faster progress during spring and summer, but in contrast to winter season, less sunlight and shorter daytime period, it gets much slower. One of the experiments has also proved that blackout of a pigsty during summer months can lead to a higher sperm production (when compared to a naturally long light day), which is in conformity with the results of my research. It states that lower illumination intensity causes increased volume. Another experiment related to improved indicators of sows' fertility showed that a 3% decrease in elimination of sterile pieces was remarked at 300 lx illumination intensity and litter quantity increased by half a piglet.

More recent studies from Australia, Germany, UK, Hungary and Czech Republic also prove that pigs' sexual function lowering in the course of summer is caused not only by high temperatures, but also by other reasons, such as photoperiod, constant daytime shortening in autumn being the main starter of sexual function activation [7].

Conclusion

Analysis of the experiment proved direct impact of illumination intensity upon quality of boar semen. The average intensity recorded at the beginning of spring and during summer was higher (63 1x) than in the course of autumn and winter season when there is less sunlight and therefore the average intensity reached lower level (53 1x). Increased illumination intensity caused smaller amount in terms of volume. On the contrary, decreased volume made sperm concentration goes up and consequently decreased percentage of pathological modifications was considered a positive feature.

The highest illumination during spring months (66 1x):
- the lowest volume – Danish duroc (170 ml),
- the highest concentration – duroc (473 thousand /mm^3),
- the lowest pathological modifications – landrace breed (5.6%).

The lowest illumination intensity during autumn months (52 1x):
- the most obvious impact upon landrace breed – the largest volume (445ml),
- the lowest sperm concentration (220 thousand/mm^3),
- the highest (i.e. the worst) percentage of sperm abnormalities (12.3%).

Illumination intensity significantly affects the resulting semen quality which is straightforwardly related to processes and financial aspects of the entire company. Its insufficient level may have fatal consequences as far as insemination dosage quality is concerned.

In conclusion, it can be stated that high illumination intensity causes decrease in the amount of semen volume; however, it positively affects quality of concentration indicators and percentage of pathological modifications of sperms. This thesis confirms a theory about importance of illumination in general to boar insemination stations as well as its impact upon semen quality parameters. Light modes of boars may still be a subject to further questions and research.

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Genealogic structure of Slovak Pinzgau cattle population

VERONIKA SIDLOVA, RADOVAN KASARDA, NINA MORAVCIKOVA
Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2, 949 76 Nitra
SLOVAKIA

veron.sidlova@gmail.com

Abstract: The aim of the study was improve knowledge about genealogical structure of Slovak Pinzgau cattle using genetic markers. Observed population structure was characterized by use of eight microsatellites. Each locus was tested for deviations from the Hardy-Weinberg Equilibrium (HWE). In general, breed was in genetic equilibrium, only locus BM1824 deviated from HWE. The overall fixation index values per locus ranged from -0.039 (CSRM60) to 0.0535 (BM1824) and average F_{IS} was close to zero (-0.0039 ± 0.0122). The highest average F_{ST} was observed in subpopulation divided based on paternal lines. The value 0.0669 from all the loci indicated that 93.31% of the genetic variation was caused by differences among individuals and 6.69% due to differentiation among the origin of animals. Division of the population into logical groups was confirmed and clarified on the basis of genetic analyses. The chosen set of microsatellites confirmed the suitability for genetic structure assessment and its usefulness in determination of the subpopulations for Pinzgau cattle in Slovakia.

Keywords: genetic structure, microsatellites, Pinzgau cattle, subpopulations

Introduction

Many industrial breeds currently suffer from inbreeding, and genetic resources in cattle, sheep, and goats are highly endangered, particularly in developed countries [1]. Genetic diversity within farm animal species refers to the extent of genetic variation within and among breeds, strains and lines in order to preserve the highest intraspecific variability [2]. Maintaining genetic variation is an important requirement for future animal breeding strategies, to match animals to a variety of husbandry systems and for adaptation to environmental changes. In addition, genetic diversity of livestock species is of considerable scientific interest for understanding phenotypic variation [3] and for reconstructing the history of livestock [4].

Slovak Pinzgau cattle are divided into two separate populations. The first is represented by dual-purpose type (dairy) and the second by beef suckler cows (beef). Pinzgau cattle are an original Alpine breed, which had been imported to Slovakia approximately 200 years ago. Thanks to its unique traits as longevity, fertility, health, grazing ability it had been bred in mountain regions of northern Slovakia, but there is significant decline of the population in recent years. Due to this, the population can be considered endangered and it is necessary to assess genetic variability. Taking in the account the situation alternatively breeding programs were optimised [5], development were monitored [6] and analyses of genetic diversity were performed [7].

Microsatellite markers have been widely used for population genetic analyses and structure of livestock species, as they are informative and can successfully elucidate the relationships between individuals and populations, including also cattle populations [8]. Microsatellites have been commonly used to assess within-breed genetic diversity and inbreeding levels, introgression from other species, genetic differentiation, admixture among breeds [9] and to define conservation priorities [2].

The most widely used measures of population structure are Wright's F statistics [10], which partition the genetic variation in a within-subpopulation component (average subpopulation inbreeding coefficient F_{IS}) and between-subpopulations component (fixation index F_{ST}), with the inbreeding in the total population described by the inbreeding coefficient F_{IT} [2]. In case of heterozygosity decreasing in population F_{IS} value will be positive and opposite, if there is a sufficient number of heterozygotes, this value will be negative [11]. F_{ST} measure provide important insight into the evolutionary processes that influence the structure of genetic variation within and among populations, and they are among the

most widely used descriptive statistics in population and evolutionary genetics [12]. To calculate these indices, one needs first to define groups of individuals and then to use their genotypes to compute variance in allele frequencies. Thus, a fundamental prerequisite of any inference on the genetic structure of populations is the definition of populations themselves. Population determination is usually based upon geographical origin of samples or phenotypes. However, the genetic structure of populations is not always reflected in the geographical proximity of individuals. Populations that are not discretely distributed can nevertheless be genetically structured, due to unidentified barriers to gene flow. In addition, groups of individuals with different geographical locations, behavioural patterns or phenotypes are not necessarily genetically differentiated [13].

The aim of this study was to assess genetic structure of Slovak Pinzgau cattle population based on polymorphism at microsatellite loci using statistical programs. This should allow improve our knowledge of population structure and genetic variability with using for preservation of the breed in the original phenotype supported by the current selection schemes and breeding programmes.

Material and methods

Random selected 302 cows of Pinzgau cattle from four Slovak farms were analysed. Beef and dual-purpose farming types, as well as purebred and crossbred animals were represented. DNA was isolated from hair roots and amplified in one multiplex PCR with 8 microsatellites (TGLA122, CSSM66, TGLA227, ILST006, CSRM60, ETH3, BM1824, SPS115). To determine the polymorphism of microsatellite DNA sequences was used fluorescent fragmentation analysis by ABI PRISM 310 Genetic Analyser and the allele sizes were evaluated. All loci were tested for deviations from the Hardy-Weinberg equilibrium (HWE) using a permutation version of the exact test given by Guo and Thompson [14] provided in PowerMarker V3.25 software [15]. Observed animals were divided into subpopulations based on farm, where are the animals living, breed type, respectively level of admixture of other breeds, year of the birth and line of father. To describe the properties of a subdivided population F-statistics, genetic identity and distance measures were estimated using above-mentioned software. F_{IS} and F_{ST} values per locus with standard deviation (SD) estimated on 1000 bootstrap replicates were computed. Genetic distance according to Nei [16] was calculated based on similarity matrix for all substructures of selected animals and then visualized to the form of phylogenetic tree using MEGA6 [17].

Results and discussion

Out of the 8 analysed loci only BM1824 showed highly significant ($P \leq 0.001$) HWE deviations across breed. Overall F_{IS} ranged from -0.039 for CSRM60 to 0.0535 for BM1824 (see Table 1). Fixation index (F_{IS}) measures the reduction of heterozygosity in an individual because of non-random mating within population and hence F_{IS} values significantly higher or lower than 0 reveal inbreeding or outbreeding, respectively. F_{IS} is usually estimated from the heterozygote deficit (1 minus the ratio of observed and expected heterozygosity) and hence F_{IS} values significantly higher or lower than 0 reveal inbreeding or outbreeding, respectively. A positive F_{IS} may also be the result of genetic subdivision (the Wahlund effect), and negative estimates can arise from crossbreeding. Inbreeding depression, or the reduction of fitness of populations by inbreeding, is caused mainly by homozygosity of deleterious mutations [18]. Whereas the average value of F_{IS} reached a negative number, generally we can notice there is no reduction of heterozygosity, but this value was close to zero (F_{IS} = -0.0039) and few microsatellites have a positive values, what means this situations can be easily changed in the next generations. Positive F_{IS} values could be derived from inbreeding or from the presence of a substructure within the population.

As is shown in Table 1 the highest average F_{ST} value reached subpopulation based on paternal lines. The value 0.0669 ± 0.0049 from all the loci indicated that 93.31% of the genetic variation was caused by differences among individuals and 6.69% only due to differentiation among the origin of animals. In subpopulation divisions by farm, breed type and year of the birth were observed following values: 0.0188 ± 0.0049, 0.003 ± 0.0013 and 0.0053 ± 0.0012, respectively. Generally, F_{ST} values between 0.05 and 0.3 are typical for differentiation of livestock breeds, with a value over 0.15 indicating significant differentiation [19], although much smaller values can be significant [2].

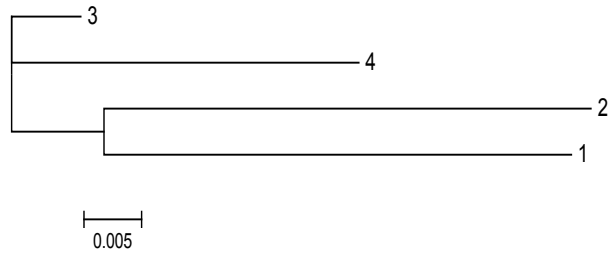
Table 1 Genetic structure of Slovak Pinzgau cattle subpopulations

markers	F_{IS}	F_{ST}			
		farm	breed type	year of the birth	paternal lines
TGLA227	-0.0318	0.0101	0.0027	0.0039	0.0547
SPS115	0.0139	0.0254	0.0014	0.0129	0.0908
ETH3	-0.0330	0.0232	0.0047	0.0049	0.0582
BM1824^{+++}	0.0535	0.0524	0.0090	0.0046	0.0720
CSRM60	-0.0390	0.0165	-0.0030	0.0067	0.0841
CSSM66	-0.0304	0.0074	0.0069	-0.0005	0.0484
ILST006	-0.0048	0.0178	-0.0016	0.0052	0.0632
TGLA122	0.0399	0.0014	0.0030	0.0059	0.0673
Mean	-0.0039	0.0188	0.0030	0.0053	0.0669
SD	0.0122	0.0049	0.0013	0.0012	0.0049

Legend: $^{+++}$Significance level of Hardy-Weinberg disequilibrium (P≤0.001)

Individuals are characterized by sufficient genetic diversity on separate farms. Slight decrease in heterozygosity is visibly at the farm 4 (PD LČV Čimhová – Vitanová), as indicated by a positive F_{IS} value (F_{IS} = 0.0207). Farm 3 (Agria a.s. Liptovský Ondrej – Liptovská Porúbka) is dual-purpose type only and 4 beef type, whereas farms 1 (PD Smrečany – Veterná Poruba) and 2 (PD Spišské Bystré – Kvetnica) are changing the type of the farming from beef to dual-purpose, confirming also Fig. 1. Animals from the farm 1 and 2 are genetically closer to each other than animals of the other two farms, because they are both in one cluster, whereas farm 3 and 4 constitute separate cluster.

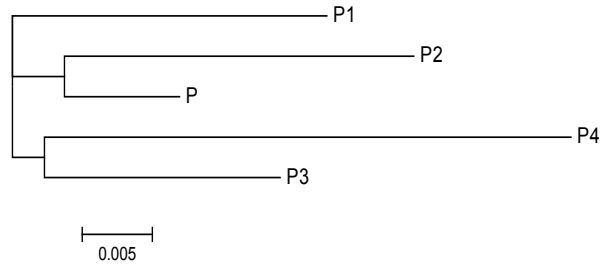
Fig. 1 Genetic structure of the population by farm



Legend: 1 - PD Smrečany – Veterná Poruba, 2 - PD Spišské Bystré – Kvetnica, 3 - Agria a.s. Liptovský Ondrej – Liptovská Porúbka, 4 - PD LČV Čimhová – Vitanová

Fig. 2 shows the distribution of the animals into five groups by breed type. It is clear that P (purebred animals) is genetically more similar to P2 (12.5% of other breeds) as to P1 (6.25% of other breeds), which may be caused by inaccurate data in the pedigree information. We can observe small reduction in heterozygosity based on F_{IS} values in P2 (F_{IS} = 0. 018) and P3 (25% of other breeds; F_{IS} = 0.0034), which may be the result of genetic subdivision or lower number of the animals in these groups.

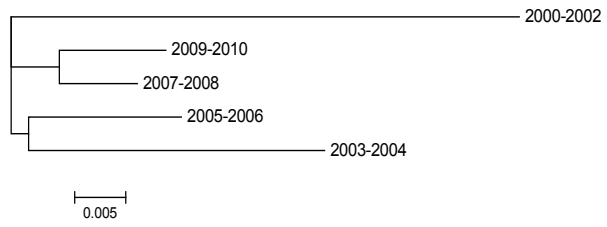
Fig. 2 Genetic structure of the population by breed type



Legend: P - purebred animals, P1 - 6.25%, P2 - 12.5%, P3 - 25%, P4 - 50% of other breeds

Logical division of animals based on year of the birth as shown in Fig. 3 has proved that the oldest animals are genetically the most distant from the younger grades. We can notice that with the passing years, the genetic information preserved in DNA is changing, which may be caused by considerable genetic contributions from migrants while constraining breeding values of the offspring, but also due to type of farming, unification of breed and hence reduction of genetic variability in younger animals. As well positive F_{IS} value (0.0207) in the animals born in years 2009-2010 has confirmed decrease of heterozygosity.

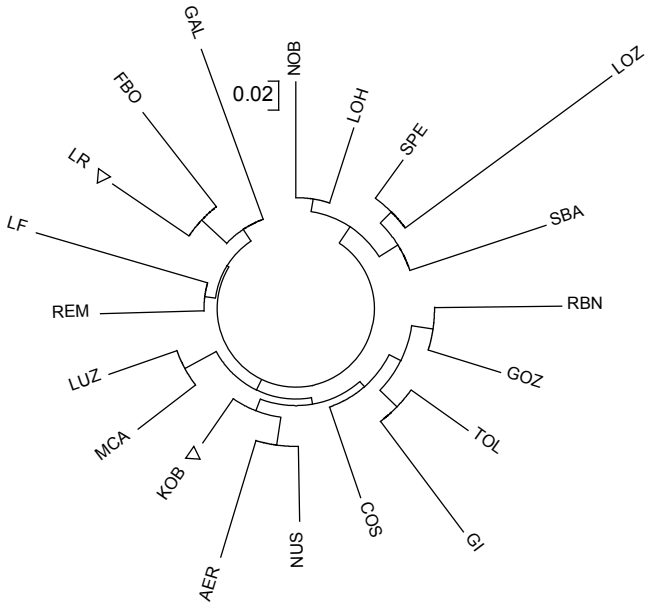
Fig. 3 Genetic structure of the population by year of the birth



A slight reduction of heterozygosity was

occurred in lines LR and KOB based on the positive F_{IS} value after division of the population into groups based on paternal lines. We can observe distribution of the lines into 3 main clusters. These tree groups are marked in Fig. 4. First cluster is created by lines GAL, FBO, LR, LF and REM. Second one consists from lines NOB, LOH, SPE, LOZ and SBA. The remaining animals belong to the third cluster. All clusters create 2 subclusters, while it is being understood that the each 2 smallest subclusters are genetically more similar to each other than to remaining animals.

Fig. 4 Genetic structure of the population using paternal lines



Conclusions

Genetic structure of Pinzgau cattle population has been analysed using set of 8 microsatellites. F_{IS} and F_{ST} values proved that the population is divided into substructures. In most cases, it was logical division confirmed by analysis of genetic structure. The overall average of fixation index was close to zero (F_{IS} = -0.0039) which means the reduction of heterozygosity in the whole population was not observed. The F_{ST} has reached following values according to the division method: 0.0188 by farm, 0.003 by breed type, 0.053 by year of the birth and 0.0669 by paternal lines. Detection of possible subpopulation structures provided us with detailed information of the genetic structure of Slovak Pinzgau cattle. Positive F_{ST} values indicate a deficiency in heterozygotes in the subpopulations, whereas in the whole population appears to be sufficient heterozygosity, what may imply the Wahlund effect. The used set of microsatellites can be applied in more detailed studies in the future by analysing more breeds, larger numbers of animals per breed. This should allow improve our knowledge of origin and phylogenetic relationships to other breeds and provide a basis for preservation of the breed in the original phenotype favoured by the current selection schemes and breeding programmes

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Effect of cattle stable environment in terms of welfare

ANNA SIMKOVA, KATERINA SVEJDOVA, MILOSLAV SOCH, KRISTYNA SIMAK LIBALOVA, LUBOS ZABRANSKY
Department of Animal husbandry sciences
University of South Bohemia in Ceske Budejovice
Studentska 13, 37005 Ceske Budejovice
CZECH REPUBLIC

anna-simkova@seznam.cz

Abstract: The high summer temperatures and high humidity can negatively affect stabled animals and cause thermal stress. The combination of high temperatures and humidity represents a burden. It is especially for dairy cows, which get rid of excess heat too difficult. The negative effect of heat stress for dairy cows is reflected, for example, worsening health condition, reproduction and reduced of milk production. Using the stable reconstruction is possible affect the microclimate of stable and meets the demands of housed dairy cows to environment, comply with welfare. Among the methods used for cooling of dairy cows include the installation of an active cooling system with fans. In the experiment was used of DeLaval fans DF 1300 in the stable for cows and heifers of the breed Holstein, which were added to the current cooling system. It was measured air flow using Testo 425 in the stable. It is the thermal anemometer with permanently attached thermal probe. The experiment was carried out during two years. It was collected a primary data in the first year. The additional fans were installed in the second year. The values of air flow were compared with the total milk production for the period 2013 and 2014. The air flow was slightly reduced and the direction of airflow was streamlined in the stable. The resulting values showed a positive effect on the milk production, which constantly increased in the second year of the experiment. In the warm months of the year it was created for dairy cows and heifers adequate climatic environment in the stable, which was reflected by an increase in milk production.

*Key-Words:*stable microclimate, air flow, ventilation, heat stress, dairy cows, milk production

Introduction

The stable microclimate is defined as a condition of stable environment, which is consisted by the file of physical, chemical and biological elements. One of the important physical factors influencing stabled animals is the speed of air flow [1].

Among the factors affecting the microclimate of stable are sorted for example: the number of animals in the stable, method of solving peripheral construction of stable in terms of its thermal insulation properties, the intensity of ventilation or stable position in the terrain due to the prevailing winds and climatic region [2]. In the temperate zone of Central Europe were recorded significant losses in milk production. The reason is a combination of high temperature and humidity. As a result of the combination of these factors is exerted a burden on the body of dairy cows, which stabled animals feel in the form of excess heat [3].

In the animal husbandry should therefore be providing conditions that allow them to not only express their natural behavior but also to cope with environmental conditions [4]. In ensuring of suitable welfare can stabled animals express positive and negative feelings at the same time [5], which is finally reflected in the total production. The production is directly proportional to the stress, because the animals spend a part of their energy to overcome of stressful situations. The production is decreases with increasing stress [6].

The loss of milk production can be prevented with the suitable stable environment, which can be achieved for example ventilation. Ventilation can be natural and artificial. Natural ventilation is achieved by the correct orientation of the stable using the prevailing wind direction.

Among the methods of artificial ventilation are sorted passive elements of ventilation such as heat insulated roof or ridge vent fissure. The active elements are the cooling fans systems [3].

The value of air flow should not exceed a value above 0.3m/s at lower temperatures [7]. At higher values of air flow is reduced temperature tolerance of housed animals. Heat loss is increased with convection and evaporation from the surface of the body, especially in places that are dirty and wet [8].

At high temperatures is suitable the value of air flow in the range from 0.5m/s to 1.5m/s, which has a favorable effect on the blood circulation and metabolism [7].

The aim of the study was to determine how changes the environment in terms of installation of active cooling system in the stable. If it is possible influence the welfare of dairy cows in relation to the total milk production.

Material and Methods

The experiment was carried out in the stable of Agricultural company Petrovice u Sedlčan a.s. for dairy cows and heifers of the breed Holstein with free boxing barns. Data was measured in the period from April to July 2013 and from April to July 2014. The interval of measurement was one to two weeks. The primary data collection was carried out in the first year of experiment. It was measured airflow in the second year. The values were already affected by installed fans of DeLaval type DF 1300 to improve the welfare of dairy cows and heifers.

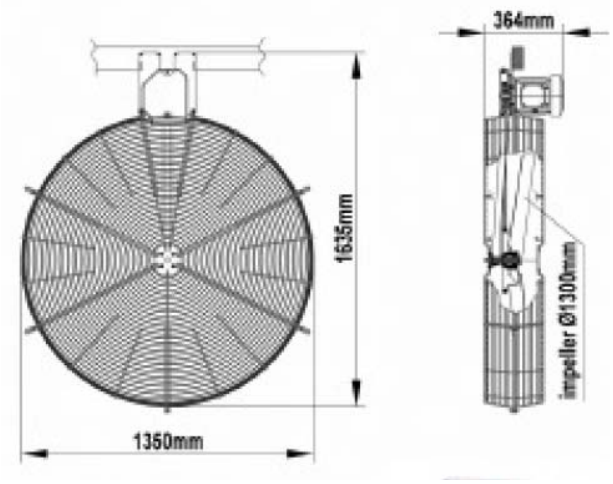
The stable is roughly situated north-south with a ratio of construction 3:1. Thermally isolated part of building is only the roof with ridge slot. In the side profile of the stable there are rolling the sails. Longitudinal profile of the stable is in the implementation phase of opening and installation of automatic sails with weather station. Inside the stable there is the feeding corridor split lengthwise into two halves, each half is further divided into three sections. In the sections there are boxing beds placed in three rows.

The air flow was always measured by thermal anemometer Testo 425 with permanently attached thermal flow probe. The measuring range of the probe is from 0m/s to 20m/s with an accuracy of ± 0.03m/s and a resolution of 0.01m/s [9].

The airflow was measured at several locations of environment in the living area of dairy cows and heifers in the stable. The measured values were averaged for clarity. Fans of DeLaval DF 1300 have a diameter of 1300mm with a maximum replacement of air up to 48,500m^3/h. The noise level of running fan is 67 dB. This value does not disturb the peace of stable environment. For the safety the fans are provided with protection cage from all sides. The fans are started automatically by the temperature sensor [10].

Data of total milk production was taken from farm evidence and together with the measured values of airflow recorded in tables and evaluated in a chart in Microsoft Excel.

Fig.1 Fan DF 1300 [10]



Results and Discussion

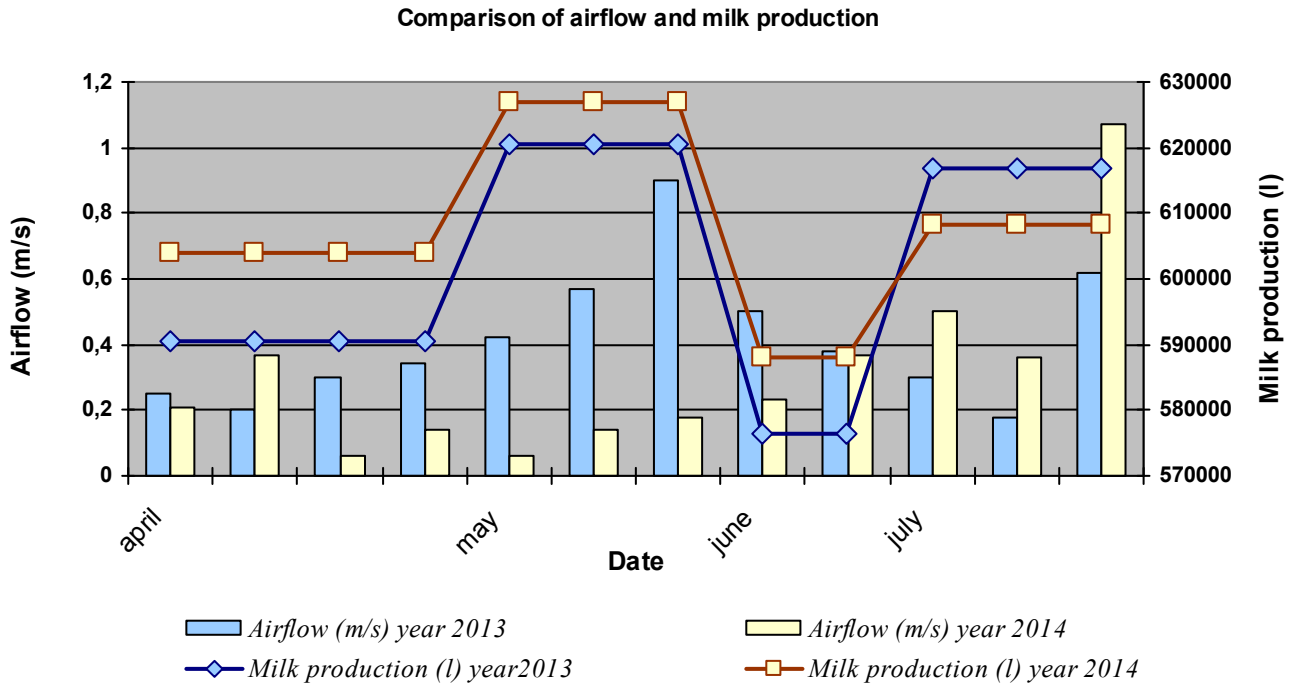
In the Figure 2 are shown the values of air flow measured for stretch period in 2013 and 2014, interspersed with values of milk production for the same period.

The average value of the speed of air flow for 2013 was 0.42m/s. Kursa et al. [11] reported a value above 0.3m/s as undesirable, which may negatively affect stabled animals, for example hypothermia or poor blood circulation in certain parts of the body. However, the measurement was carried out in the period of higher temperatures, where a higher airflow was used for cooling of dairy cows and used for avoiding to heat stress which could reduce milk production.

The stable was oriented in a north-south direction. The suitability of the stable orientation is confirmed by Smith [12] according to it is the best use of natural ventilation. In the side profile there was stable closed with the rolling sails. In the longitudinal profile there was closed by stable windows. Above the middle of lying down double-boxes was used existing cooling of ventilation system. Location of ventilation was justified even by Havlik [3], whose states that the best place to install a fan in the stable is above the center of lying down double-boxes. The main corridor of the airflow is in the middle line of double-boxes, which use dairy cows in days with high temperatures to reduce heat stress. Dairy cows lying a longer time and resting in the stable.

In the following year 2014, the average air velocity decreased by 0.11m/s to 0.31m/s. One of the reasons for the change of air flow velocity in the barn were

Fig. 2 Comparison of airflow and milk production



initiated modifications to improve the welfare of dairy cows. The previously used cross-ventilation of barn, comb slots and existing fans above the centre of lying down double-boxes added new elements of ventilation. Fans DF1300 were installed due to the feeding area. The airflow directed to the animals that were feding at the time. Havlik [3] in his publication says that the installation of the fans is above the feeder error. Dairy cows stand here longer time, in some cases they even lie down on the floor. In the experimental stable was not negative behavior of dairy cows recorded. Although there was directing air flow velocity an average of 0.11m/s, it was showed a positive effect on total milk production. Lactation has evolved in the opposite trend and overall it increased in the second year of experiment. The increase of total milk production was an average of 5,755 liters. With the results identifies Wells [13], who in his book says that the proper setup and use of ventilation in the barn for dairy cows is possible to achieve of better health and increase milk production. Also Frazzi [14] concluded that adequate optimization of microclimate in the barn during the summer months is possible to increase milk production.

Conclusion

The results of the experiment, which took place in the barn with dairy cows and heifers during two years shows that the location of the fans above the feeder was justified. Although vertical fans are most commonly placed over the center lying down double-boxes, adding additional the fans to the feeder caused a change in velocity of air in the barn. Positive values were obtained even in milk production. It is therefore possible to attribute positive results in an increase in total milk production ventilation system. In warmer periods of the year it provided the cows and heifers sufficient cooling of the body surface. Animals were less affected by temperature stress. Improved living conditions in the stable were resulted in the resulting evaluation in an increase in total milk production, which is especially useful for breeders.

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The influence of colored wheat feeding on broiler chickens performance parameters

ONDREJ STASTNIK[1], EVA MRKVICOVA[1], FILIP KARASEK[1], VACLAV TROJAN[2], TOMAS VYHNANEK[2], LUDEK HRIVNA[3], ZUZANA JAKUBCOVA[1]
[1]Department of Animal nutrition and Forage production
[2]Department of Plant Biology
[3]Department of Food Technology
Mendel University in Brno
Faculty of Agronomy
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xstastni@mendelu.cz

Abstract: The aim of this study was to determine effect of purple wheat Konini included in feed ratio for fattening male broilers of hybrid combinations Cobb 500. Konini wheat has a higher content of anthocyanins (14.01 mg / g) in grain. The content of crude protein in the common wheat was increased using wheat gluten to the identical in KONINI. In both feed mixtures was the same CP content. The effect on feed consumption, weight gain and carcass yield were evaluated. The content of purple wheat Konini in experimental diet was 78%. Control diet contains 78% of common wheat. We observed higher live weight (2530 ± 19.89 g SD) in Konini group. Average total feed consumption was 3.40 kg in Konini group. Effect on performance parameters was not significant (P > 0.05).

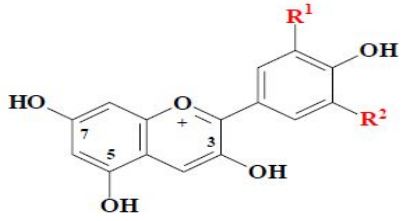
*Key-Words:*wheat Konini, anthocyanins, Cobb 500, nutrition of chickens

Introduction

Wheat grain is considered as a good source of fiber, phenols, tocopherols and carotenoids.

Anthocyanins, another group of bioactive agents, are in blue and purple wheat grains. It is well known that herbal anthocyanins are functioning as antioxidants and, in addition, they have anti-bacterial and anti-carcinogenic effects as well [1]. In addition to white and red grains, wheats with purple grain may occur. Purple grain colour is caused by anthocyanins in the pericarp. Purple grains occur in tetraploid wheats from Ethiopia, and in one bread wheat accession apparently native to China [2].

Fig. 1 Structure of anthocyanins, which are formed by two benzene rings and an oxygen-containing pyran ring [3]



The aim of our experiment was to determine if replacement of common wheat Konini wheat not worsen performance parameters of broiler chickens.

Material and Methods

202 chickens of hybrid combination COBB 500 were divided into 5 groups at the age of 19 days and were marked by the wing sign with number. They were breeding in balance cages. Room temperature and humidity were controlled. Lighting system was 16 hours light and 8 hours dark. Every group obtained ration with different content of purple wheat KONINI. Compositions of experimental rations are given in Table 1. Chickens were fed ad libitum. Approach to the water was permanent. Percentage of wheat was 78%. Content of Crude protein (CP) in the wheat KONINI was 16.8%. The content of CP in the common wheat was increased using wheat gluten to the identical in KONINI. In both feed mixtures was the same CP content. Ration was calculated using recommendations in [4].

Table 1 Composition of experimental rations

	KONINI	CONTROL
Wheat KONINI	78%	0%
Common wheat + gluten*	0%	78%
Soy-bean meal	13.1%	13.1%
Wheat starch	0.6%	0.6%
Monocalciumphosphate	0.7%	0.7%
Milled Limestone	0.3%	0.3%
Rapeseed oil	4%	4%
Premix - concentrate of microelements* *	3%	3%
Cr_2O_3	0.3%	0.3%

**The content of CP in the common wheat was increased using wheat gluten to the identical in KONINI*
*** lysine 60,0 g; methionine 75,0 g; methionine + cysteine 75,0 g; calcium 195,0 g; phosphorus 55,0 g per; sodium 46,0 g per kg; copper 4,0 mg; zinc 3,70 mg; tocopherol 1,50 mg; biotin 6,0 mg per kg and retinol 450 IU; calciferol 166,70 IU*

The feed consumption were measured every day, chickens were weight in the 3-day intervals.

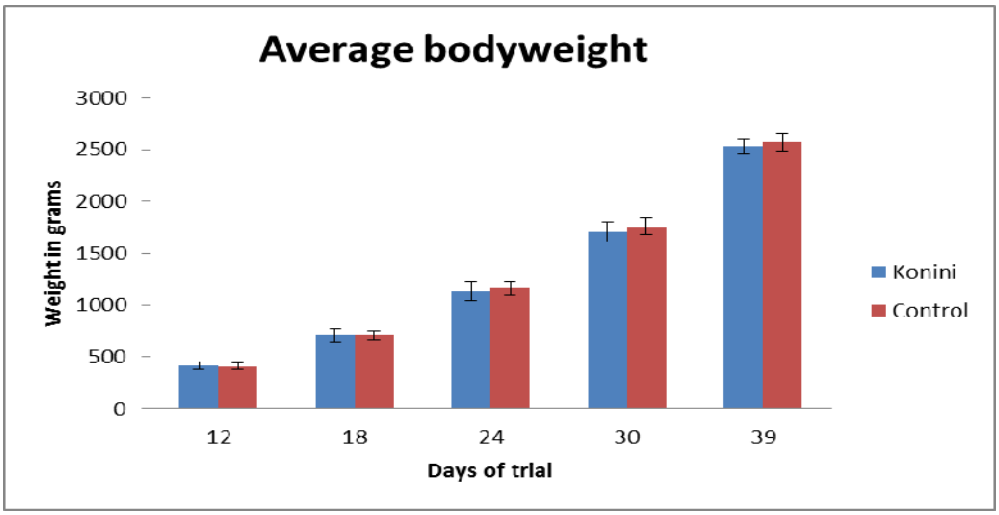
At the age of 39 days they were slaughtered and percentage of breast meat and leg meat (meat from thigh and drumstick without skin and bone) were calculated.

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ).

Results and Discussion

At the end of trial we observed non-significant ($P > 0.05$) higher weight (2530 ± 19.89 g SD in Konini group and 2577 ± 24.31 g SD in Control group). Average bodyweight of cockerels are shown in figure 2.

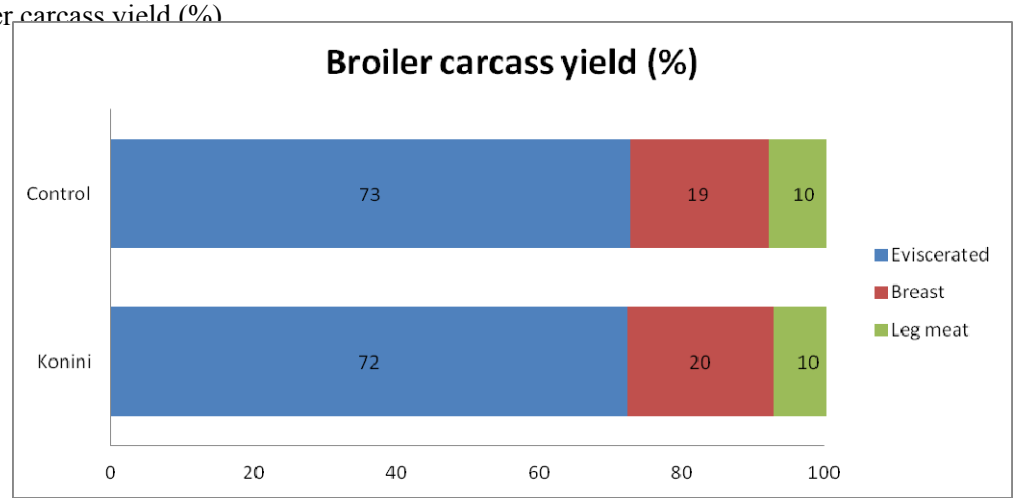
Fig. 2 Average bodyweight of chickens (g)



Average total feed consumption in the period from 19th to 39th day of life was in Konini group 3.40 kg and the control group 3.41 kg for chicken. The addition of Konini wheat had no significant influence on bodyweight gain even on carcass yield. Broiler carcass yield shows in Figure 3.

We observed that groups with wheat Konini and groups with common wheat to be balanced in our trial. These values are statistically non-significant ($P > 0.05$). The average weight gain in a day of slaughter were 2386 ± 47.89 g SD in a group of Konini and 2380 ± 38.00 g SD in the control group.

Fig. 3 Broiler carcass yield (%)



Eviscerated carcass is calculated with feet and shanks removed from the hock joint. Boneless breast is as a percentage of live weight.

The highest carcass yield was found in the control group, the average carcass was 72.71 ± 0.74% SD. The lowest values 72.49 ± 0.49% SD was found in the Konini group. The results are not statistically significant. In [5] was the carcass yield higher (75%) to compare with our results. In [6] which feeding wheat Citrus is lower percentage of carcass yield (71.49%) to compare with our experiment. But the yield of breast (23.15%) and thigh muscle (15.88%) was higher than in our experiment.

Percentages of breast muscle of body weight were highest for experimental group Konini (20.32 ± 0.69% SD), while the lowest was observed in the control group (19.40 ± 0.57% SD). In the manual of hybrid Cobb 500 [5] are higher percentages of breast muscle (23.73%) of body weight to compare with our experiment.

Percentages of thigh muscle of body weight was attempted highest for Control group (10.29 ± 0.65% SD), while the lowest value was observed in experimental Konini group (10.10 ± 0.58% SD). The manual for the hybrid Cobb 500 [5] indicates a higher proportion of thigh muscle 14.14%.

Conclusion

In the experiment has been found that feeding of purple wheat Konini does not significant effect ($P > 0.05$) on performance parameters, namely feed consumption, weight gain and carcass yield compared to feeding control wheat. In the experiment it was found that even with the inclusion of 78% of color wheat Konini in the diet, was achieved a good results during of fattening and good carcass yield.

Konini wheat not worsen parameters of fattening and health condition of chickens.

Acknowledgement

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The influence of technology housing with regard to thermal comfort

KATERINA SVEJDOVA, ANNA SIMKOVA, MILOSLAV SOCH,
KRISTYNA SIMAK-LIBALOVA, LUBOS ZABRANSKY
Department of Animal husbandry sciences
University of South Bohemia in Ceske Budejovice
Studentska 13, 370 05 Ceske Budejovice
CZECH REPUBLIC

Ramandu@seznam.cz

Abstract: The thermal state of environment is the most important- from the basic components affecting the welfare of animals housed. The thermal state of environment consists of air temperature, relative humidity, air velocity and effective temperature of the surrounding surfaces. The overall effect of these four components under normal conditions significantly influences feed consumption and their use in production. Factors affecting thermal comfort organism can significantly affect the thermoregulatory mechanisms, conversion of nutrients, the performance and health of the animals. Temperature is the main climatic factor that drives the organism of animals with a constant body temperature in order to adapt the production and distribution of heat environmental status, which in extreme cases can affect performance, or even animal health. Heat stress is a major cause of the decline in milk production and fertility, especially in high-yield dairy cows. The best indicator of physiological response to stress is body temperature, since under non-stressed conditions, is nearly constant. The aim of this study was to evaluate the influence of selected housing technology for thermal comfort of the animals housed. In selected groups of cows and heifers was measured rectal temperature and given in relation to the ambient temperature. It was examined how high the temperature effect on the body, what is their impact on heat stress and how it is influenced by the average yield of dairy cows and heifers. In the case of this experiment, it was found that the effect of high temperatures during the summer months, which ranged up to around 26.4 ° C, there was no significant decrease in the average yield of animals.

Key-Words: temperature, feed consumption, production, animal health, heat stress, thermal comfort, dairy cows

Introduction

In environments with high temperatures occur in fattening cattle and especially cows to reduce feed intake, resulting in a decrease in performance [1]. With increasing efficiency, increasing body frame, but also with the growing tendency of a tropical summer days, the phenomenon of heat stress in cattle is becoming an important factor for maintaining a stable yield, but also the health and reproduction of the herd [2]. According to Broucek [3] hot weather causes heat stress in dairy cows, which leads to a decrease in milk production. Higher temperatures are manifested in cattle adversely, especially a decrease in performance, changing the composition of milk (fat content decreases) and a decrease in feed consumption [4, 5, 6]. The consumption of drinking water rises [7]. High-yield dairy cows are the most affected category in cattle [8]. Milk yield of dairy cows is influenced by many internal and external factors. Stable microclimate is undoubtedly one of the important external factors, since it has a significant impact on well-being (welfare) housed animals. Only the cow, which has ideal conditions and welfare and is nothing further stressed can (within their physiological options) to bring maximum performance [9]. For animal products like milk and meat, the conditions in which animals are reared and slaughtered are of prime importance [10, 11]. Individual kinds of animals react to create a suitable microclimate conditions differently. in dairy cows is to increase milk production, fattening improves feed intake and therefore are even higher weight gain [12]. Beede a Collier [13] identified three management strategies to minimize the effects of heat stress: 1) physical modification of the effects of the environment (shading, cooling), 2) genetic development of heat-tolerant breeds, and 3) improved nutritional management practices. Adaptation and acclimatization to heat is more difficult than adaptation and acclimatization to cold. It is easier to increase the production of heat, especially if there is enough food, than to reduce the production of heat by metabolic processes necessary to sustain life [14,15]. In both the heat and cold, homeothermic animals utilize autonomic and

behavioral responses to regulate their body temperature (i.e. thermoregulation) [16]. The cattle generally belongs to animals with very good thermoregulation abilities. Yet ruminants are able to maintain strict homeothermic. In the case of very significant effect of heat stress in cattle the body temperature may fluctuate by up to 3 ° C [2]. The importance of optimal temperatures can be seen in the optimum conversion of nutrients. When temperatures are below the lower boundary optima, affects mainly by reducing the conversion of nutrients, or the next drop increases by reducing disruption to health. On increasing the temperature above the upper limit of the optimum occurs primarily to a reduction in feed intake, body to prevent overheating mainly by evaporation (mainly breathing), a further rise in temperature leads to overheating [17]. The best indicator of physiological response to stress is the body temperature, since under non-stressed conditions, is nearly constant. On the basis of the changes is the fastest way to deduce the thermal load on the body and on the involvement of adaptive mechanisms [18].

Material and Methods

This experiment was carried out in the period from July 2013 to August 2014th. Measurements were carried out in the agricultural cooperative Petrovice. There were selected two groups of cows and heifers. First group consisted of cows and heifers from day to two months after calving, at the beginning of lactation. The second group consisted of cows and heifers in seven to eight months after calving, just prior to hooking. This experiment was conducted in the stable, where the measured cows and heifers were fixed in boxing. First group of cows and heifers was in the barn with different microclimatic conditions than those in the stable, where they were placed cows and heifers from the second group. Rectal temperature was measured using a digital rectal thermometer in selected animals. The rectal temperature was also given in correlation with ambient temperature, which was sensed by a thermal TESTO 425 anemometer with permanently attached thermal probe. Operating temperature of this unit is in a range from -20 to +50 ° C and the probe measuring range is from -20 to +70 ° C. The probe is measured with an accuracy of ± 0.5 ° C and 0.1 ° C resolution. From an agricultural cooperative Petrovice was obtained data on the average performance of dairy cows and heifers for each month and monthly need for raw materials in tonnes (Table 1). The resulting values are summarized in graphs using Microsoft Excel.

Table 1 Monthly raw materials needed in tonnes

Hay	300	Alkalage	252
Alfalfa haylage	2520	Maize silage	5040
Grass silage	1680	Extruded corn grain	132
GPS (a mixed bag wheat with peas)	960	EKPO (waste in the production of confectionery)	72
Fresh dregs	960	A mixture of dairy cows (Preparation for childbirth)	66
CCM (wet corn grain)	300	A mixture of calves	120
A mixture of dairy cow (lactation)	1500	A mixture of dairy cows (rozdoj)	144
molasses	144	cut straw	96

Source: Agricultural cooperative Petrovice

Results and Discussion

The results of correlation rectal temperature of each group of dairy cows and heifers with environmental temperature and yields are shown in graphs 1 and 2. The average rectal temperature measured with animals ranged between 37-38.5°C. Literature gives a range of rectal temperature in cattle 37.5 to 39.5°C. [19] states based on the actual measurement of fluctuations in rectal temperature in dairy cows from 36.9 to 39.1°C. According to [8] temperatures above 39.5°C are already considered as a response to high temperature environments.

The highest values, the average rectal temperature was in the summer, when they were measured also in high temperature air. According to [14] as the threshold temperature, considered to be to heat stress, is usually considered to be 20°C. Regarding the effect of high temperatures on the welfare of

dairy cows, for example [20] argues that in the interval 16-21°C there is no significant changes in production performance, animal behavior and the quality of their products. Likewise [21] indicate that dairy cows thermoneutral zone is placed in the range of -5 to + 24°C, and for high-yield cows are moved to the upper limit of 21°C. Increased heat stress induces behavioral and physiological responses including increased body temperature and respiration reduction in the activity, food intake and milk production. Considerable differences in the measured values of rectal temperatures, especially in summer, are found in the second group of cows and heifers. In this group were in dairy cows and heifers with the highest degree of lactation compared to the previous group, therefore fluctuations were most pronounced in rectal temperature values.

Fig. 1 Effect of temperature on the average performance at first group of dairy cows and heifers

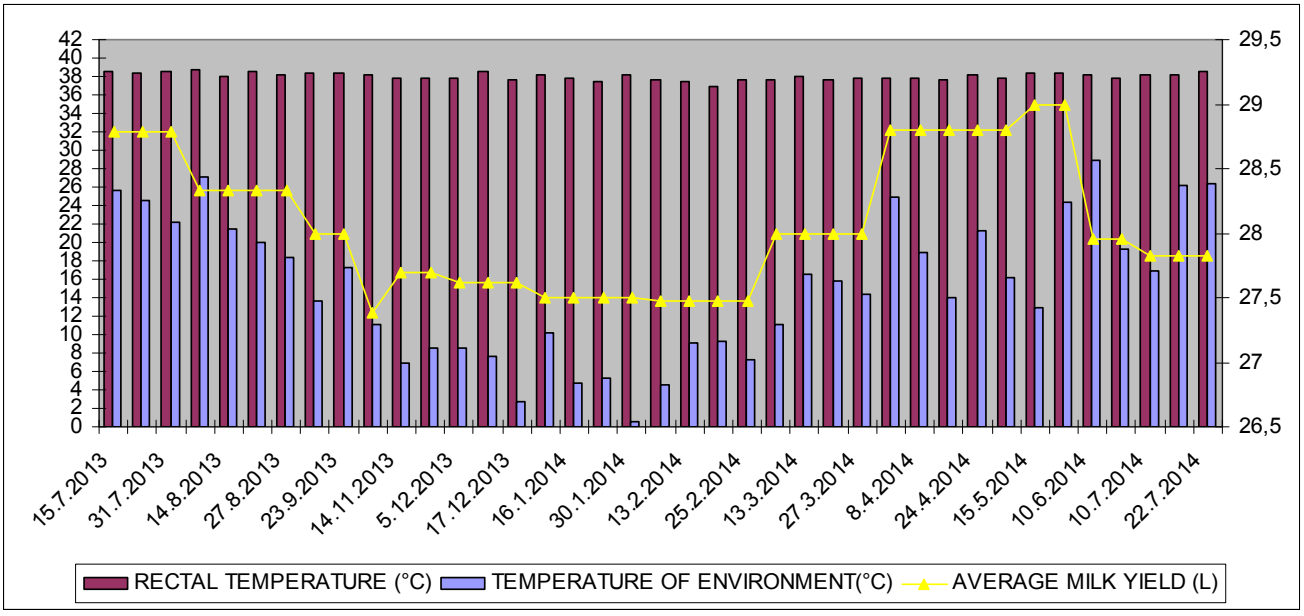
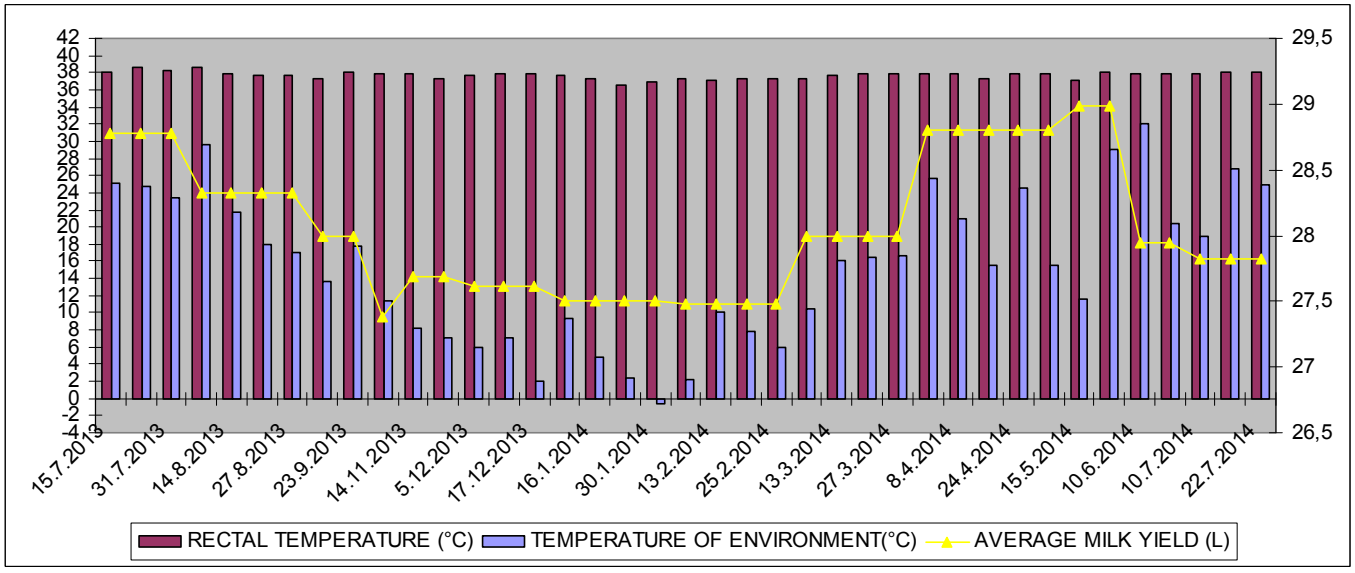


Fig. 2 Effect of temperature on the average performance at second group of dairy cows and heifers



This group of cows and heifers was in the barn, which took place only ventilation through open doors and windows. In contrast to the stables, where was the first group of dairy cows and heifers and where ventilation was used by fans and open doors. Significant fluctuations in ambient temperatures

were recorded during the spring and autumn. As claimed by [22] from the point of view of barn ventilation management, spring and autumn are difficult periods of the year because of frequent changes in air temperature, relative humidity and air velocity. A specific finding is that in an environment of high temperature fluctuates in cattle feed consumption and this decline is usually given in a context of declining milk production [2]. In the case of this experiment, it was found that the effect of high temperatures during the summer months, which ranged up to around 26.4 ° C, there was no significant decrease in the average yield of animals. In contrast, in this experiment were recorded the lowest values in the lowest ambient temperatures, i.e. in the winter months. In the case of low temperatures below thermoneutral zone increases in feed intake and water intake reduction, and usually will increase the consumption of dry matter per unit of production because part of metabolisable energy must be used to produce heat [23]. According to [24] negative effect of low temperature environment is not the result of direct cooling of animals, but is the result of disruption of feeding, watering, etc, i.e. disruption of the dynamic stereotype.

Conclusion

In selected groups of cows and heifers was measured rectal temperature and given in relation to the ambient temperature. It was examined how high the temperature effect on the body, what is their impact on heat stress and how it is influenced by the average yield of dairy cows and heifers. The average rectal temperature of the animals was between 37-38.5°C. The most significant fluctuations in rectal temperature were measured in a group of cows and heifers that are on the highest stage of lactation compared to the second group, it was in dairy cows and heifers immediately after calving. Group of cows and cows in 7 to 8 months after birth is also found in the stable with different microclimatic conditions, which could affect differences in measurement. These results indicate relatively large variability cows as regards their relationship to ambient temperature.

Regarding performance in this experiment, it was found that the effects of high temperatures during the summer months, which ranged up to around 26.4 ° C, there was no significant decrease in the average yield of animals. Contrary to expectations, the lowest performance will be recorded in the summer months, when the animals are most exposed to heat stress, were the lowest in the performance recorded in the period with the lowest ambient temperatures, i.e. in the winter months.

Acknowledgement

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Iodine Teat Dips: A Comparison of Three Iodine Concentrations

EVA VAVROVA[1], ZBYSEK SLADEK[1], VACLAV TROJAN[2], MIROSLAV MACHACEK[3]
[1]Department of Morphology, Physiology and Animal Genetics
[2]Department of Plant Biology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
University of Veterinary and Pharmaceutical Sciences
Palackeho trida 1-3, 612 42 Brno
CZECH REPUBLIC

evavrova24@gmail.com

Abstract: The aim of this study was to compare disinfection properties of 0.4%, 1.5%, and 3% iodine concentration in teat dip. The samples were obtained from teat skin of 9 clinically healthy dry cows of czech fleckvieh breed. After cleaning their teats with wet towels, we used sterile swabs to wipe the skin of the teats to get the control samples. Then the swabs were put into test tubes with BC7 broth. Three cows were treated with 0.4%, 1.5%, and 3% iodine dip respectively. After 30, 60, 90, and 120 minutes, we took samples the same way previously. Each time 4 samples of every concentration were gained. These samples were examined by modified suspension method according EN on Malthus AT machine in the Institute for State Control of Veterinary Biologicals and Medicines. Every swab was cultivated in 4ml of BC7 broth for 24 hours at the temperature of 37°C. Antibacterial properties of distinct disinfection concentrations are shown by the changes of electric conductivity of the broth. After that the suspension was inoculated to blood agar and cultivated for another 24 hours, so that the colony forming units (CFU) could be counted. The bacteriostatic properties are proven by decline of CFU in the control sample compared to exposure times for each concentration of iodine. The results have shown that bacteriostatic effect of the 0.4% iodine is not sufficient. In 30 minutes after dipping, the colony forming units (CFM) decreased in 2.31 logarithmic orders. To declare its disinfective properties, we need it to be 3 logarithmic orders or more. The higher concentration of iodine is more likely to be effective. The iodine concentration of 1.5% reached its nadir after 60 minutes from exposure, and stayed almost the same for the next half an hour. Until 120 minute the CFU increased but still there was certain bacteriostatic effect. The strongest bacteriostatic effect was proven at the concentration of 3% iodine solution. The onset of the effect appeared after 30 minutes after exposure and went on until the end of sampling.

Key-Words: iodine, disinfection, teat dip, bacteriostatic

Introduction

Aim of our research was to evaluate the effect of three different concentrations of iodine in post-milking teat dips. Teat dipping with a proven disinfecting teat dip has been demonstrated to be one of the most effective mastitis control practices [1]. Teat dips are commonly used before and after milking to reduce new infections induced by mastitis-causing bacteria in lactating dairy cows [2]. The dips are designed to effectively reduce infection caused by environmental bacteria as well as reducing the spread of infections caused by contagious bacteria.

After milking the teat canal remains opened for at least an additional 15 minutes allowing pathogenes to enter. Application of the teat dip immediately after milking kills the significant proportion of the pathogens on teats and reduces the possibility of the pathogenes entering the teat canal. Iodine teat disinfectants provide a broad spectrum efficacy with rapid kill while providing a persistent film on teat skin which offers extended protection through the formation of a physical and chemical barrier [1].

Iodine is the active ingredient in the majority of teat dips used today and is recognized as effective as documented in the National Mastitis Council´s teat dip bibliography.

Iodine teat dips are effective at reducing the spread of bovine mastitis, and free iodine is an

important factor in determining the germicidal efficacy of these teat dips [3].

Material and Methods

The trial was performed at ŠZP Nové Dvory of University of Veterinary and Pharmaceutical Sciences. 9 healthy dry cows of czech fleckvieh breed were placed in the parlour. Their teats were cleaned with wet towels and we used sterile swabs to wipe the skin of the teats to get the samples of bacterial colonization (control). The swabs were put into test tubes with 4ml BC7 broth. Afterwards three cows were treated with 0.4%, 1.5%, and 3% experimantal iodine dip respectively. The products were left to dry on teats.

Teat dips were provided ready to use. 30, 60, 90 and 120 minutes after treatment, we took the samples as we did in the beginning of the trial. Each time 4 samples of every concentration were gained. The analyses were made in the laboratory of Institute for State Control of Veterinary Biologicals and Medicines. The samples were examined by modified supsension method according EN on Malthus AT machine. The test tubes were put into this machine and their contents were cultivated for 24 hours at the temperature of 37°C. Gauging of electric conductivity is possible in these tubes thanks to their metalic parts so antibacterial properties of distinct disinfection concentrations are shown by the changes of electric conductivity of the broth. After cultivation the suspensions obtained from the tubes were inoculated to blood agar and cultivated for next 24 hours at the temperature of 37°C. The other day colony forming units (CFU) were counted. The bacteriostatic properties are proven by decline of CFU in the control sample compared to exposure times for each concentration of iodine. Appropriate broth is inoculated with suspension of a microorganism in order to achieve total repression of microorganism under trial conditions – GE (germicidal effect). GE is sufficient, if the CFU declines in more than 5 logarithmic orders (the disinfectant is killing microbs) in certain exposure time. When the decrease is in 3 logarithmic orders, the disinfectant has bacteriostatic properties (supression of bacterial gowth).

Results and Discussion

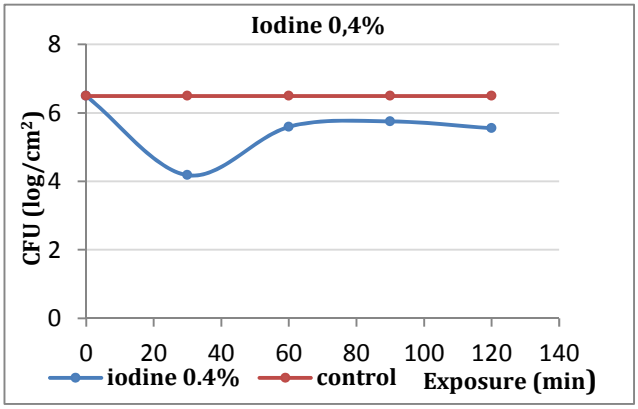
0.4% iodine teat dip

As for the 0.4% iodine teat dip, the results have shown that bacteriostatic effect is not sufficient. In 30 minutes after dipping, the colony forming units (CFU) decreased in 2.31 logarithmic orders. To declare its disinfective properties, we need it to be 3 logarithmic orders or more. The higher concentration of iodine is more likely to be effective.

Table 1 0.4% iodine teat dip - effectiveness

Exposure time	CFU (log/cm^2)	CFU (log/cm^2)
0 min	6.49	
30 min	4.18	
60 min	5.59	6.49 (control)
90 min	5.75	
120 min	5.55	

Fig.1 0.4% iodine teat dip - effectiveness



1.5% iodine teat dip

The onset of disinfection effect of this concentration was a bit slower. The iodine concentration of 1,5% reached its nadir after 60 minutes from exposure, and stayed almost the same for the next half an hour. Until 120 minute the CFU increased but still there was a certain bacteriostatic effect. The higher concentration of iodine is significantly more effective than the concentration of 0.4%.

Table 2 1.5% iodine teat dip – effectiveness

Exposure time	CFU (log/cm^2)	CFU (log/cm^2)
0 min	6.49	
30 min	5.59	
60 min	3.30	6.49 (control)
90 min	3.33	
120 min	4.00	

3% iodine teat dip

As expected the teat dip with 3% content of iodine happened to be the most effective disinfectant. Yet bacteriostatic, its antimicrobial properties are very strong. The onset of the effect appeared after 30 minutes after exposure. The difference between CFU pre-dipping and samples taken after 30

minutes was more than 4 logarithmic orders. This concentration is meeting the requirements to disinfective solutions for maintaining the udder health.

Fig. 2 1.5% iodine teat dip – effectiveness

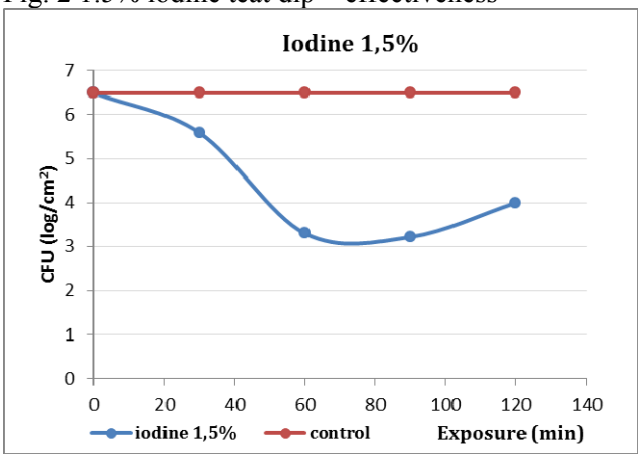
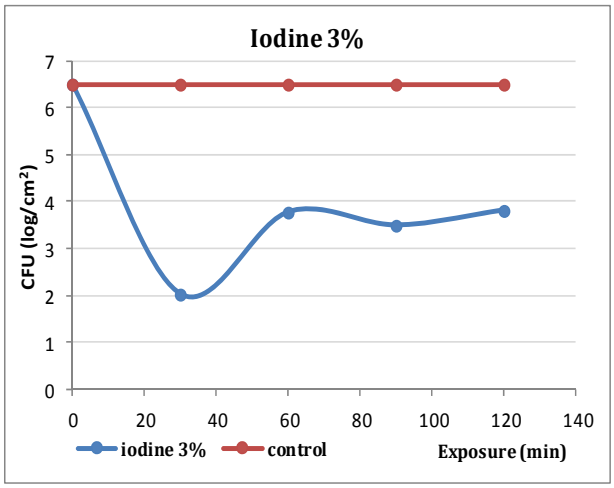


Table 3 3% iodine teat dip – effectiveness

Exposure time	CFU (log/cm^2)	CFU (log/cm^2)
0 min	6,49	
30 min	2,03	
60 min	3,78	6,49 (control)
90 min	3,50	
120 min	3,82	

Fig. 3 3% iodine teat dip – effectiveness



Conclusion

Proper milking hygiene is essential for the production of good quality raw milk and for the udder health of the cows [4]. One of the most important practices in the parlour is the proper after-milking teat dipping. This fact has been proven in many studies. The teat needs to be fully covered with disinfectant to avoid bacterial colonization of the teat skin and to stop replicating present bacteria. Based on experimental results, the 0.4% iodine solution is insufficient in its germicidal properties. The teat dip containing 1.5% iodine had a certain bactericidal effect. The concentration of 3% iodine in solution is the best choice for treating cows´ teats after milking. .

Acknowledgement

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The effect of stable microclimate on composition of bulk milk samples from Holstein cows

MILENA VELECKA, JANA JAVOROVA, JIRI ANDRYSEK, MILAN VECERA, DANIEL FALTA, GUSTAV CHLADEK
Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

milena.velecka@mendelu.cz

Abstract: The aim of this study was to determine the effect of stable microclimate on the composition of bulk milk samples from Holstein cows. The study lasted 25 weeks (milk samples were sampled daily and analysed weekly) from April 2013 to September 2013. Data and milk samples come from University farm in Žabčice (49° 0′ 43″ N, 16° 36′ 8″ W; 182 m.a.s.l.). Measured stable parameters were: cooling power, air velocity, light intensity, air temperature and relative humidity. In the samples of milk were monitored: content of fat, protein, lactose and minerals (calcium, magnesium, and chloride). Based on the correlation of milk composition and stable microclimate was found with increasing air temperature decreased cooling power ($r = -0.77$, $P < 0.001$). Furthermore, results indicated that with increasing air velocity decreased cooling power ($r = -0.52$, $P < 0.01$). With increasing lactose content increased fat content ($r = 0.64$, $P < 0.01$) and protein content ($r = 0.57$, $P < 0.05$). Had also been found negative correlation between calcium content and magnesium content ($r = -0.41$, $P < 0.05$). Air temperature had a statistically significant effect on magnesium content ($r = 0.42$, $P < 0.05$) and protein content. ($r = -0.49$, $P < 0.05$). Positive correlation was found between the cooling power and the protein content ($r = 0.48$, $P < 0.05$) and a negative correlation was found between the relative humidity and the magnesium content ($r = -0.45$, $P < 0.05$) in the milk samples.

Key-Words: stable microclimate, composition of milk, Holstein cows

Introduction

The main function of a livestock building is to protect farm animals against adverse and variable atmospheric conditions and to raise them in a way that ensures herd health and welfare as well as profitability of production. This is possible when the building is equipped with technological, functional and structural solutions that largely determine indoor microclimate conditions [1]. One of the most important challenges in modern stables is to maintain appropriate microclimate, i.e. sufficient air temperature, humidity, air flow velocity, low pollution and low content of gases [2].

Air temperature - cows are the homoioterm animals, which mean that the animals keep a constant body temperature. Physiological functions of cattle work relatively independently of environment temperature. This stability is relative, because the enormous decrease or increase of environmental temperature can induce undercooling or overheating of the organism [3]. Cattle tolerate low temperatures better than high ambient temperature [4]. High environmental temperature causes lower feed intake and lower milk yield [3].

Relative air humidity is another main indicator of the quality of stable microclimate immediately after air temperature [5]. Seasonal and daily changes of relative air humidity values are suppressed owing to production of heat and water vapor from animals and air ventilation in stable [6]. The amount of evaporation depends mainly on the air temperature, degree of water vapor saturation and air flow. High relative humidity has a negative impact on the welfare and milk production of dairy cows [5].

Cooling power (refrigeration) is physiologically significant factor that results from the simultaneous action of air temperature, relative air humidity and air velocity. Cooling power expresses the loss of heat from the surface of the organism and also the thermal comfort of the animal. This is the amount of heat that is released from the unit body surface for a certain time period [7].

Air velocity (air flow) – wind is the basic meteorological element, which describes the air flow in a particular location of the atmosphere at

any given time with regard to Earth. Air flow around animals can have either positive or negative effects. Air flow takes heat and water vapor and supports thermoregulation, or it causes unpleasant draught [8].

Light intensity – light is visible part of the spectrum of solar radiation in the wavelength range (about 260-760 nm). Cattle are sensitive to light intensity. In the stable should be for light (intensity of 150-200 lux) a period of 16-18 hours. Exceeding this time has a negative effect on physiological function of cattle [8].

Factors that influence milk composition include two aspects: external factors (e.g. season, feeding system and milking frequency) and internal factors (e.g. gene, parity, and stage of lactation) [9]. Some studies have shown that seasonal variation affects milk composition through several aspects, such as ingestible diets, photoperiod, and temperature [10]. Monitoring of milk components is evident in the whole world [11]. Minerals found in milk such as Se, Ca, K, Zn, Mg, and P, contribute to several vital physiological processes (e.g. Ca and P play an important role in bone metabolism; Ca, K, and Mg in the regulation of blood pressure [12]. Minerals represent a small fraction of solids, but play an important role in the structure and stability of casein micelles [13].

The aim of this work was to assess the effect of stable microclimate on the composition of bulk milk samples from Holstein cows.

Material and Methods

Characterization of animals, housing and feeding

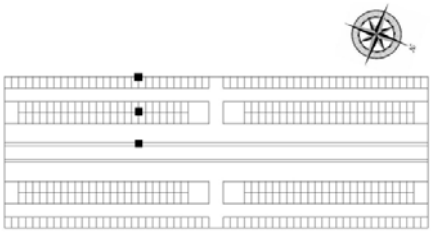
The observation was carried out on the University farm in Žabčice (49° 0′ 43″ N, 16° 36′ 8″ W; 182 m.a.s.l.) where are reared Holstein dairy cows. During the period (from April 2013 to September 2013) were collected data and milk samples (n = 25) after the morning milking. All cows were milked twice a day, but this study does not deal with afternoon milk yield. Cows were housed in free-stall stable bedded stalls and they were fed a TMR ("total mix ratio"). During the study were all cows healthy, untreated and milked (average 401) and were in various stages (average 191 days) and in a different number (average 2.34) of lactation.

Analysis of stable microclimate

Stable microclimate was measured one a day before the control day.

Air temperature represents the average of daily temperatures. It was measured every 15 minutes by 3 sensors with HOBO data logger (Onset Computer). The sensors were stationed in a stable (in height at the withers) (Figure 1).

Fig. 1 Location of sensors in the main stable of production



Relative humidity in stable was recorded by the same sensors and in the same intervals like air temperature.

Hill's kata thermometer was immersed in hot water to heat up to more than 38 °C. Refrigeration was measured at three locations in a stable (near the HOBO sensors) and then averaged into a single value. Refrigeration was detected by using kata thermometer (F = 24,751.82 W/m^2). Kata value was calculated according to the formula [7]: K = F/t

(F - kata thermometer factor (W/m^2) – a constant amount of heat loses 1 cm^2 flask of kata thermometer when cooled from 38 °C to 35 °C; t - time (s) decrease in alcohol column of kata thermometer from 38 °C to 35 °C).

Air velocity (air flow) was measured by an anemometer Testo 405-V1. Air velocity was measured near HOBO sensors (i.e. at three places in the stable). Data were averaged.

Light intensity was measured by lux meter Testo 540. Measurement was carried out at three locations in the stable (near the HOBO sensors - the height at the withers of cows). Data were averaged.

Analysis of milk samples

Milk sample were taken in control day. Analyses of samples were carried out the day after sampling in the laboratory at the Department of Animal Breeding at Mendel University in Brno.

Measured milk compositions were: fat content (g.100g^{-1}), lactose (lactose monohydrate; g.100g^{-1}) and proteins (g.100g^{-1}). Milk composition was measured on instrument Julie C5 Automatic (Scope Electric) working on the principle of thermo analysis. Chloride content in milk was determined after the addition of nitric acid by titration argentometric. Chlorides were precipitated by excess silver nitrate solution and for reverse titration was used a solution of ammonium thiocyanate. For the determination of calcium content was used complexometric titration with EDTA, 2Na.

Magnesium content was observed by titration of a mixture of milk with an ammoniacal buffer solution (pH 10) with 2Na.

Statistical analysis

Milk production was obtained from the computer database of university farm in Žabčice. MS Office Excel 2010 was used to evaluate of data. Statistical evaluation of the data was carried out in STATISTICA 10.0.

Results and Discussion

Characteristic of stable microclimate

Basic characteristics (mean, standard deviation, range) and optimal values of each parameter stable environment are described in Table 1. Some values of stable microclimate were not found in their optimal range. Thermo neutral zone for cows (with milk yield over 22 kg) is from 4 °C to 10 °C. At high air temperatures intake of feed is reduced, deficit in the body occurs and milk yield is reduced [8]. Relative humidity is the second major indicator of the quality of stable environment [5]. In the stable should be relative humidity in the range of 40–80 % and it should not exceed 85 % [8].

Table 1 Basic characteristic of stable microclimate parameters

Parameters of stable microclimate	**Unit**	**Mean ± SD**	**Range**	**Optimum values** [8]
Cooling power	W/m	468.68±110.91	185.21–614.30	170–500
Air velocity	m/s	0.41±0.27	0.08–1.15	0.1–0.3*
Light intensity	Lx	1270.89±1162.94	341.33–6654.33	150–200
Air temperature	°C	18.63±4.50	9.32–28.07	4–10
Relative air humidity	%	54.81±11.99	33.44–8.90	40–80

** - at high air temperature 0.5 – 1.5 m/s; SD – standard deviation*

Table 2 shows the relationship between the parameters of stable environment.

With increasing air temperature decreased cooling power ($r = -0.77$, $P < 0.001$). These results confirm Zejdová et al. [8] and they add that lower values (less than 170 W/m^2) are typical for very hot weather and negative relationship of cooling power and air temperature was caused by method of measuring.

With increasing air velocity decreased cooling power ($r = -0.52$, $P < 0.01$). The optimal value of air velocity has a beneficial effect on blood circulation and metabolism. At higher speeds and at low temperature environment occurs over cooling. Adverse air flow is known as the draught. [14]. Inadequate air ventilation increases the risk of disease of animals [15].

Insignificant correlations ($P < 0.05$) were found for relative humidity, light intensity among other microclimatic parameters. The relative humidity depends mainly on the air temperature, degree of saturation with water vapor and air flow [5].

Table 2 Relationship among stable microclimate

Correlations among stable microclimate	Cooling power	Air velocity	Light intensity	Air temperature	Relative air humidity
Cooling power	-	-0.52**	-0.07	-0.77***	0.15
Air velocity	-0.52**	-	0.31	0.29	-0.14
Light intensity	-0.07	0.31	-	0.17	-0.24
Air temperature	-0.77***	0.29	0.17	-	-0.28
Relative air humidity	0.15	-0.14	-0.24	-0.28	-

***- P > 0.01; *** - P > 0.001; unmarked - P < 0.05*

Characteristic of milk composition

Basic characteristics (mean, standard deviation, range) of each milk components are described in Table 3. Some values of minerals content were not found in their optimal range (calcium content: 0.9–1.4 g.l^{-1}; content of magnesium: 0.05–0.24 g.l^{-1}; content of chlorides 0.8–1.4 g.l^{-1}) [16].

Relationships among milk composition are described in Table 4. With increasing lactose content increased fat content ($r = 0.64$, $P < 0.01$) and protein content ($r = 0.57$, $P < 0.05$). Had also been found negative correlation between calcium content and magnesium content ($r = -0.41$, $P < 0.05$).

The composition and functional properties of cow's milk are very important [17].

Table 3 Basic characteristic of milk composition

Parameters of milk composition	Unit	Mean ± SD	Range
Content of calcium	$g.l^{-1}$	0.99±0.11	0.77–1.18
Content of magnesium	$g.l^{-1}$	0.34±0.09	0.17–0.61
Content of chlorides	$g.l^{-1}$	0.88±0.05	0.78–0.99
Content of fat	$g.100g^{-1}$	3.79±0.24	3.34–4.31
Content of protein	$g.100g^{-1}$	3.09±0.08	2.93–3.24
Content of lactose	$g.100g^{-1}$	4.64±0.11	4.38–4.85

SD – standard deviation

Table 4 Relationship among milk composition

Correlations among milk composition	Content of calcium	Content of magnesium	Content of chlorides	Content of fat	Content of protein	Content of lactose
Content of calcium	-	-0.41*	-0.03	-0.20	-0.03	0.04
Content of magnesium	-0.41*	-	-0.01	-0.09	-0.28	-0.12
Content of chlorides	-0.03	-0.01	-	-0.06	-0.09	-0.17
Content of fat	-0.20	-0.09	-0.06	-	0.32	0.64**
Content of protein	-0.03	-0.28	-0.09	0.32	-	0.57*
Content of lactose	0.04	-0.12	-0.17	0.64**	0.57*	-

** - $P > 0.05$; ** - $P > 0.01$; unmarked - $P < 0.05$*

Relationship between stable microclimate and milk composition

Table 5 presents relationship between stable microclimate and milk components content. Air temperature had a statistically significant effect on magnesium content ($r = 0.42$, $P < 0.05$) and protein content. ($r = -0.49$, $P < 0.05$). Positive correlation was found between the cooling power and the protein content ($r = 0.48$, $P < 0.05$) and a negative correlation was found between the relative humidity and the magnesium content ($r = -0.45$, $P < 0.05$) in the milk samples.

Season have a great impact on composition of bulk tank milk samples. Content of protein, fat, lactose, calcium and chlorides were the lowest in summer and the highest in winter, with intermediate values in spring and autumn [18, 19]. Some studies have shown that seasonal variation affects milk composition through several aspects, such as diets, photoperiod, and temperature [10]. High values of relative humidity have a negative effect on milk production of dairy cows [5].

Table 5 Correlations between stable microclimate and milk composition

Correlations	Content of calcium	Content of magnesium	Content of chlorides	Content of fat	Content of protein	Content of lactose
Cooling power	-0.03	-0.37	-0.01	0.23	0.48*	0.21
Air velocity	-0.07	0.02	0.00	0.25	0.10	0.29
Light intensity	-0.03	-0.17	-0.31	0.18	0.26	0.24
Air temperature	-0.17	0.42*	-0.17	-0.06	-0.49*	-0.18
Relative air humidity	0.23	-0.45*	-0.16	-0.17	-0.09	-0.35

** - $P > 0.05$; unmarked - $P < 0.05$*

Conclusion

The aim of this study was evaluated the effect of stable microclimate on composition of bulk milk samples from Holstein cows. Measured parameters were stable microclimate (cooling power, air velocity, light intensity, air temperature and relative air humidity) and milk composition (content of calcium, magnesium, chlorides, fat, protein and lactose). The research has shown that high values of air temperatures and low values of cooling power negatively affected composition of milk.

Acknowledgement
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Modification of the zona pellucida sperm-binding protein 3 (ZP3) in pigs during folliculogenesis

DENISA VILDOVA[1], MICHAL JESETA[2], MARTIN HOSEK[1], LADISLAV MACHAL[1]
[1]Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Department of Genetics and Reproduction
Veterinary Research Institute
Hudcova 296/70, 621 00 Brno
CZECH REPUBLIC

xvildova@node.mendelu.cz

Abstract: Polyspermic fertilization is still one of major problems during in vitro fertilization in pigs. The zona pellucida is an extracellular matrix surrounding mammalian oocytes, plays an important role during fertilization and forms polyspermy block after fertilization as well. The porcine zona is composed of three glycoprotein called ZP2, ZP3 and ZP4. The purpose of this study is to evaluate modifications of the zona pellucida sperm-binding protein 3 (ZP3) of oocytes with different developmental competence. This protein is involved in binding of the spermatozoa to the oocyte. Using western blot analysis, it was found no significant differences in expression of ZP3 protein in oocytes with lower developmental competence (from small follicles), in compared to oocytes with higher developmental competence (from medium follicles). But it was detected band in area > 50 kDa, which could play an essential role during fertilization of oocytes.

Key-Words: zona pellucida, polyspermic fertilization, ZP3 protein, porcine oocytes

Introduction

The zona pellucida (ZP) is an extracellular matrix that surrounds all mammalian oocytes and mediates species-specific binding of spermatozoa to the oocyte during fertilization [1]. Following fertilization, zona pellucida form the zona block to prevent polyspermy and protects the preimplantation embryo during passing down through the oviduct [2].

The porcine zona pellucida is composed of three highly specific glycoproteins modified by many posttranslational modifications including glycosylation and sulfation [3]. The glycoproteins are called as ZP2 (also named ZPA and ZP1), ZP3 (also named ZPC and ZP3α) and ZP4 protein (also named ZPB and ZP3β) [4]. ZP3 and ZP4 protein probably form long fibres of the ZP architecture, whereas ZP2 protein serves as crosslinker. The thickness of porcine ZP is 16 μm [3].

After fertilization, the ZP is modified by the contents of cortical granules and it occurs to proteolytic cleavage of ZP2 protein and to formation of disulfide bonds as well. This process is known as zona hardening and constitutes a major block to prevent polyspermy [5].

Polyspermic fertilization one of major problems during in vitro fertilization of porcine oocytes because it is lethal for embryo development. The polyspermy rate in vitro can reach 65% [6].

It has been shown that boar spermatozoa evince a high affinity for the ZP3-ZP4 heterocomplex but not for free glycoprotein subunits [7]. In study Yonezawa et al. [8] assumed the carbohydrate structures of ZP4 in the porcine ZP3-ZP4 complex are responsible for sperm-binding activity in this complex. The question of why ZP4 shows the sperm-binding activity only in the heterocomplex with ZP3 needs to be clarified in the future.

The ZP3 protein (46 kDa) belong to the ZPC subfamily and its immature form is composed of 421 amino acid (AA). The mature form of this protein is composed of 310 AA [9].

This study is aimed to modification of ZP3 protein of oocytes with different developmental competence during folliculogenesis - according to ability of oocytes to undergo normal fertilization and zona hardening. Oocytes were derived from small and medium follicles in selected phase of estrous cycle.

Material and Methods

Oocyte donors

Cycling pubertal gilts Landrace×Czech Large White crossbred were used as oocyte donors (age 8.5–11 months, weight 130–150 kg). The ovaries were recovered at local slaughterhouse and transported to the laboratory within 2 h at 39°C. In the laboratory, the ovaries were evaluated by morphological status (shape and size of follicles and of corpus luteum) and divided into the four phases of folliculogenesis: in the early (days 1–5), middle (days 6–10) and late (days 11–14) luteal phases and in the early (days 15–16) follicular phase.

Oocyte collection

Oocytes were recovered from antral follicles by aspiration (5–9 mm) and cutting of the ovarian cortex (2–4 mm), respectively. Oocytes from larger follicles (≥10 mm) were not included in this study because of the dominant follicles phase. Oocytes were divided in 6 subpopulations:

1. Early luteal phase (days 1–5), small follicles (2–4 mm) – subpopulation called S 1–5
2. Middle luteal phase (days 6–10), small follicles (2–4 mm) – subpopulation called S 6–10
3. Late luteal phase (days 11–14), small follicles (2–4 mm) – subpopulation called S 11–14
4. Late luteal phase (days 11–14), medium follicles (5–9 mm) – subpopulation called M 11–14
5. Early follicular phase (days 15–16), small follicles (2–4 mm) – subpopulation called S 15–16
6. Early follicular phase (days 15–16), medium follicles (5–9 mm) – subpopulation called M 15–16

Oocyte maturation

All healthy cumulus-oocytes complexes with dark, evenly granule ooplasm and at least two cumulus layers by Hulinska et al. [10] were used for the oocyte maturation. The oocytes were matured in 500 µl of TCM-199 medium (Earle's salts) with addition of 0.20 mM sodium pyruvate, 0.57 mM cysteamin, 50 IU ml–1 penicillin, 50 µg ml–1 streptomycin, 10% BFS (bovine fetal serum; Sigma Chemicals Co., Prague, Czech Republic) and gonadotropins (P.G. 600 15 IU ml–1; Intervet, Holland) in a 4-well muti-dish (Nunc, Intermed, Denmark) at 39°C in atmosphere of 5% CO2. The oocytes were matured for 44 h in vitro. After maturation, neither the maturate oocytes (MII) nor the groups of immature oocytes (GV) were vortexed in the presence of hyase to remove cumulus cells.

Western blot

To the samples of 25 oocytes from each subpopulation of oocytes before maturation and after maturation were added lysis buffer and 10% SDS (sodium dodecyl sulfate; Serva, Heidelberg, Germany). The samples were centrifuged and sonicated followed by addition of sample buffer (0.5 M tris-HCl, 10% SDS, glycerol, bromophenol blue, dH2O, 2-mercaptoethanol; Serva) and the samples were boiled. These samples were loaded in polyacrylamide gel (Serva) and SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) was performed in Mini-Protean Tetra System (Bio-Rad, Hercules, CA, USA). Dual Xtra was used as marker (Bio-Rad).

After SDS-PAGE, the activation of PVDF membrane by methanol was performed followed by composition of western blot sandwich and semi-dry western blotting (Mini-Protean Tetra System; Bio-Rad) for 1 h. Then the PVDF membrane was blocked using 5% powdered milk in washing buffer (100 mM NaCl, 20 mM tris-HCl, 0.075% Tween) for 1 h and the membrane was incubated with primary antibody ZP3 N-20 (1 : 1000 dilution, Santa Cruz) over night at 4°C. The PVDF membrane was washed three times for 5 min and two times for 10 min and incubated with secondary antibody Rabbit anti-Goat HRP (1 : 2000 dilution, Santa Cruz) for 1 h. ZP3 protein was visualized using ECL (Enhanced Chemiluminescence; Santa Cruz) and were processed using NIS-Elements ar 3.0 software.

Statistical analysis

The results were statistically analyzed by the T-test using STATISTICA Cz, version 10 software (StatSoft, Inc., Prague, Czech Republic). Differences at $P < 0.05$ were considered statistically significant.

Results and Discussion

Modifications of ZP3 protein during folliculogenesis

Using western blot, it was detected modifications of ZP3 protein of oocytes with different developmental competence (Fig. 1). Days 1−14 introduce luteal phase of estrous cycle and days 15−16 introduce follicular phases of estrous cycle.

Band of the subpopulation of oocytes S 1−5 was always weaker in compared to the other subpopulations and by this subpopulation does not occur band > 50 kDa, both before maturation and

after in vitro maturation. Band > 50 kDa was appeared gradually according to the days of estrous cycle and follicle size and it is located there also after maturation. Moreover, bands of individual subpopulations after maturation seemed to be shorter in compared to bands before maturation.

It was found no significant differences in expression of ZP3 protein of immature oocytes recovered from small follicles in compared to medium follicles. It was found no significant differences in expression of ZP3 protein of mature oocytes recovered from small follicles in compared to medium follicles as well. The expression of ZP3 was evaluated by the mean intensity of this protein (Tab. 1). The individual subpopulations of oocytes were evaluated as unit (small or medium follicles) because mean follicle size does no change and developmental competence of oocyte was evaluated by follicle size.

Fig. 1 Modifications of porcine ZP3 protein during folliculogenesis: (A) subpopulations of immature oocytes, (B) subpopulations of mature oocytes.

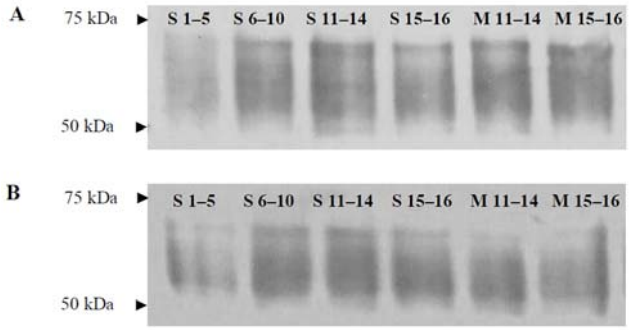

Table 1 Effect of meiotic maturation and developmental competence on expression of porcine ZP3 protein. Values with different superscripts by the same stage of meiotic maturation are significantly different ($P < 0.05$).

Stage of meiotic maturation	Follicle size	No. of measurements	Mean intensity of ZP3 protein ± SEM
GV (0 h)	S (2−4 mm)	12	31.84 ± 6.63^{a}
GV (0 h)	M (5−9 mm)	6	30.66 ± 12.29^{a}
MII (44 h)	S (2−4 mm)	8	24.41 ± 5.82^{a}
MII (44 h)	M (5−9 mm)	4	23.24 ± 6.79^{a}

Antosik et al. [11] divided oocytes in 4 groups according to their morphology of cumulus-oocytes complexes (COC) and western blot analysis was performed. It was detected increased expression of ZP3 protein in oocytes of grade I in compared to other grades (II–IV). This data suggest that not only follicle size but also COC morphology are important factors that affect the expression of ZP3 protein in pigs.

In present study are used only healthy COC with homogenous cytoplasm and at least two layers of cumulus cells.

Our results suggest that there is no difference in expression of ZP3 protein of oocytes from small and medium follicles. But in our lab was previously demonstrated [10] that oocytes recovered from small follicles have significantly higher polyspermy rate in compared to oocytes from medium follicles. The monospermy rate in oocytes from small follicles increased significantly from the early luteal to the late luteal phase and remained unchanged in the early follicular phase. A similar tendency was observed in the total efficiency of fertilization.

It is possible that band in area > 50 kDa is responsible for increasing of the monospermy rate in small oocytes and also for increasing of the efficiency of fertilization.

Conclusion

It is generally known that to polyspermy in pigs occurs more often in conditions in vitro than in vivo. In vitro fertilization of porcine oocytes and also polyspermic fertilization are therefore hot issue.

The ZP3 protein is one of proteins of the porcine zona pellucida and together with the ZP4 protein is involved in sperm-binding activity of zona. The precondition that expression of ZP3 protein varies by oocytes with different developmental competence was not correct. In this study, it was found no significant differences in expression of ZP3 protein of ocytes recovered from small follicles in compared to medium follicles, both before maturation and after maturation. However, it seems to be that before in vitro maturation occurs to modification of ZP3 protein manifested by band in area > 50 kDa. This band may play an important role during fertilization. Further studies are needed to confirm this hypothesis.

Acknowledgement

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Section – Agroecology

Greenhouse gasses emissions during maize growing for energy purposes

JAROSLAV BERNAS, JAN MOUDRY jr., ZUZANA JELINKOVA, MAREK KOPECKY
Department of Agroecosystems
University of South Bohemia in Ceske Budejovice
Studentske 13, 370 05 Ceske Budejovice
CZECH REPUBLIC

bernasj@seznam.cz

Abstract: Due to the increasing energy consumption and depletion of fossil fuels, alternative energy sources are becoming an increasingly important topic. One of the most important renewable energy sources is the energy from phytomass. Recently, also in the conditions of the Czech Republic, there has been a significant development of production of energy crops as raw material for the biogas production in biogas plants (BGP). However, farming and particularly technical processes associated with it participate in the anthropogenic emission production. This article presents the results of monitoring of emission load resulting from the cultivation of maize (*Zea mays* L.) for energy purposes. As a tool for emission load measuring (expressed in CO_2e where CO_2e = 1x CO_2 + 23x CH_4 + 298x N_2O), the simplified LCA method, respectively its climate impact category, was used. For calculation, the SIMA Pro software and the Recipe Midpoint (H) method was used. From the results, it is obvious that the cultivation of maize for energy purposes produces the greatest amount of CO_2e emissions within nitrate fertilization (0.052455 kg $CO_2e.1kg^{-1}$ of dry matter) and field emissions (0.050359 kg $CO_2e.1kg^{-1}$ of dry matter). Maize cultivation for energy purposes shows a higher emission load as compared for example with energy grasses.

Key words: maize, greenhouse gases emissions, Life Cycle Assessment, crop production

Introduction

The current situation and trends indicate the probability of irreversible effects on the world economy and particularly on the global climate. Energy demand will be growing constantly and it will drain especially irreplaceable fossil energy sources. It is an undeniable fact that fossil fuels are limited and it is necessary to look for other sources. We could say that in case of the economical land use, there will be biomass constantly available [13]. One of the possibilities is its transformation into biogas through anaerobic fermentation in biogas plants (BGP) [21]. In 2012, there were about 320 biogas plants in the Czech Republic. There will have been about 720 of them by 2020 [9]. With the increasing number of biogas plants, also the demand for suitable substrates increases while we could assume that the maize silage will still predominate. Also the current biogas production in BGP is based predominantly on the usage of maize. However, recently, there have been certain problems relating to its cultivation [23]. In terms of biomass energy utilization (in our case, specifically grown maize), it is necessary to deal with not only issues related to economic and social topics, but also environmental issues [26]. In terms of GHG emission production (in the Czech Republic, mainly N_2O, CH_4 and CO_2), it is also an important producer within agriculture, in addition to energetics and industry [18]. For example, according to Svendsen [29], this contributes by 9.2% to the total GHG emissions within the European Union. Within the trend of sustainability, however, also the agriculture should contribute to reduction of the emission load. In the literature, there is often a question of the impacts of agricultural alternative forms on reduction of environmental load discussed [11, 12]. For example, there are very often different crops, etc. compared which brings not always relevant results [28]. Therefore, for the energy crop cultivation, there is necessary to find possibilities of emission savings elsewhere than in changing of the entire farming system. To monitor specific emission load in different farming systems, The LCA (Life Cycle Assessment) analysis can be used [10]. It evaluates the environmental impact of a product based on the assessment of the impact of material and energy flows that are exchanged by the monitored system with the environment [8]. LCA is a transparent scientific tool [30] which evaluates the environmental impact on the basis of inputs and outputs within the production system [7]. On the basis of this study, it is possible to make a model of the established production system, to identify the strongest sources of emissions from particular

energy flows and to determine the total emission load within the maize cultivation.

Material and methods

The aim of this study was to develop a model of technological process of cultivation of maize and wheat and to determine the impact of the emission load on the environment through it. As a tool for calculation of the emission load, the simplified Life Cycle Assessment (LCA) method was used. It is defined by international standards - ČSN EN ISO 14 040 (CNI, 2006a) and ČSN EN ISO 14 044 (CNI, 2006b). The results of the study were related to the Climate change impact category expressed as an indicator of carbon dioxide equivalent (CO_2e = 1x CO_2 + 23x CH_4 + 298x N_2O). For calculation, the SIMA Pro software with the Recipe Midpoint (H) integrated method was used. The functional unit of the system was 1 kg of the final product (1 kg of dry matter). Technological process of cultivation of silage maize for biogas production in BGP was compiled on the basis of primary data (direct information from farmers) and secondary data (obtained from the Ecoinvent database, specialized literature and agricultural production technology standards). The database uses data geographically related to Central Europe. The range of time horizons for the primary data collection was between the years 2012 - 2014 and the years 2000 - 2014 for the secondary data. Data selected for modelling are based on the average of commonly applied technologies. To the model system, there were agrotechnical operations from seedbed preparation, seed quantity, the use of plant protection products, the production and application of fertilizers, etc., to the harvest of the main product included. In addition to the emissions resulting from the above inputs, there are so called filed emission (N_2O) released after the application of nitrogen fertilizers produced. For their quantification, the IPCC (Intergovernmental Panel on Climate Change) methodology is used [3].

Results and discussion

Climate changes are a key topic of these days. Production of greenhouse gases in the world needs to be constantly monitored and it is necessary to look for ways how to reduce their most important resources at the same time. For example, emissions from agriculture represent about 10 - 12% of the total produced GHG emissions (CO_2e) in the world representing 5.1 to 6.1 billions tones of CO_2e [20]. Within the EU-27, the total share of emissions from agriculture in total production of CO_2e is estimated at 10.1% [22] and in the Czech Republic, this share is 6.3% [6].

As stated before, results of the study were related to the *Climate change* impact category expressed as an indicator of carbon dioxide equivalent (CO_2e = 1x CO_2 + 23x CH_4 + 298x N_2O). The same concentration of different greenhouse gases has very different consequences for increasing absorption of long-wave radiation, so the certain greenhouse gases are more effective than others [19]. Nitrous dioxide (N_2O) is the most effective greenhouse gas produced by agriculture [15]. One kilogram of this gas has the same greenhouse effect as 289 kg of CO_2 [27, 15]. In addition, these gases (CO_2, N_2O, CH_4) are characterized as greenhouse gases with a direct impact on climate [14].

This paper evaluates the current model of a technological progress within the cultivation of maize for the production of biogas. Results show the amount of emission impact on the environment. Table 1 shows the values of particular system processes while the highest emission load is associated with agrotechnical operations (0.020346 kg $CO_2e.kg^{-1}$ of dry matter), N fertilizer application (0.052455 kg $CO_2e.kg^{-1}$ of dry matter) and production of N_2O field emissions released after the application of N fertilizers (0.050359 kg CO_2e.kg-1 of dry matter). Also Barros [1] states that the greatest amount of GHG emissions released into the atmosphere comes mainly from N fertilizers. Zou et al. [31] and Mori et al. [16] also state that fertilizer usage has an effect on increasing N_2O emissions from the soil.

Table 1 Production of emissions within particular system processes, own source - Bernas et al., 2014

System subprocesses	**kg $CO_2e.kg^{-1}$ of maize dry matter**
Organic fertilizers	0.003607
Mineral fertilizers N	0.052455
Mineral fertilizers P	0.007475
Mineral fertilizers K	0.002661
Total fertilizers	***0.066198***
Seed consumption	0.003203
Chemical protection	0.000763
Agrotechnical operations	0.020346
N_2O field emissions (converted to CO_2e) generating after the application of N fertilizers.	0.050359
Total production	***0.140870***

The highest CO_2e emission load comes from nitrogen fertilizer application (0.052455 kg $CO_2e.kg^{-1}$ of dry matter) and production of N_2O field emissions released after the application of N fertilizers (0.050359 kg $CO_2e.kg^{-1}$ of dry matter). On the contrary, the lowest amount of CO_2e emissions results from the use of chemical plant protection products (0.000763 kg $CO_2e.kg^{-1}$ of dry matter). This is contrary to the statement of Fott [5] who states that emissions from agricultural activities come mainly from the usage of nitrogen fertilizers and pesticides precisely. Graph 1 shows a comparison of two strongest emission sources also expressed in $CO_2e.kg^{-1}$ of maize dry matter with the emission load resulting from the remaining system processes altogether.

If we think of CO_2e production reduction within the chosen cultivation process, it is necessary to focus on the two most powerful sources (N fertilizer application and field emission arising from the application of N fertilizer). In this respect, we often deal with the question regarding reducing the dose of fertilizer and the total change of the agricultural system [4, 17]. Another way how to reduce emissions of greenhouse gases is the replacement of maize by another energy plant. Also Bellarby [2] proposes the cultivation of less loading plants as a way how to reduce (namely mitigate) GHG emissions. These may be, for example, energy grasses. These have prerequisites to lower CO_2e production during their life cycle thanks to the character of perennial plants and generally lower fertilization requirements.

Fig. 1 Network of energy flows, own source (SIMA Pro) - Bernas et al., 2014.

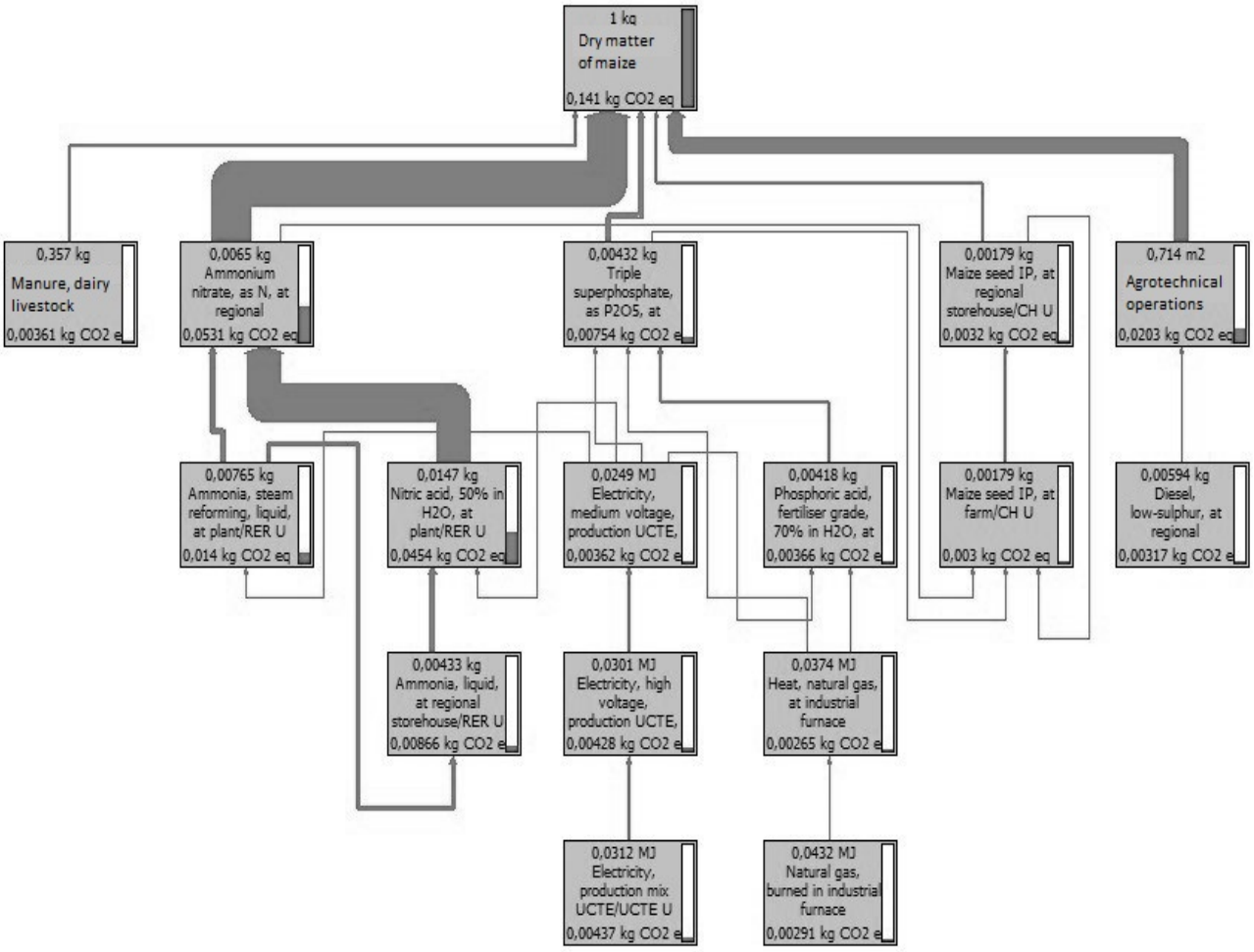
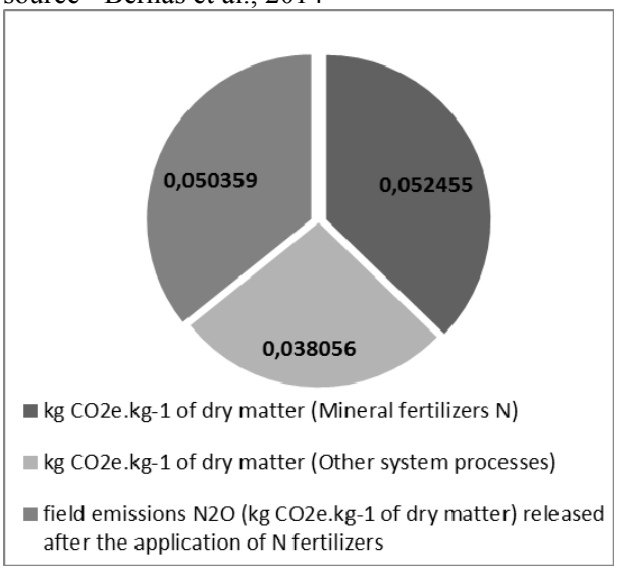


Figure 1 represents a network of particular energy flows involved in the production of 1 kg of maize dry matter. The strongest energy flow demonstrates the emission load due to the use of N fertilizers. One of the reasons why N fertilizers are the strongest producers of GHG emissions within agriculture is their constantly rising consumption. For example, Robertson and Vitoušek [25] stated that global consumption of N fertilizer increased tenfold in the period from 1950 to 2008.

Graph 1 Main sources of CO_2e emissions, own source - Bernas et al., 2014



** Among other system processes, an application of organic fertilizers, mineral P and K fertilizers, seed consumption, chemical plant protection and agrotechnical operations were included.*

Conclusion

The results show that the total emission load of the selected cultivation cycle of maize intended for biogas production represents 0.140870 kg $CO_2e.kg^{-1}$ of maize dry matter. From the system subprocesses, the largest emission load for the *Climate change* impact category is formed by nitrogen fertilizer application (0.052455 kg $CO_2e.kg^{-1}$ of dry matter) and N_2O field emission resulting after the application of N fertilizer (0.050359 kg $CO_2e.kg^{-1}$ of dry matter). The reduction of the amount of CO_2e produced within the cultivation of maize for biogas can be done by reducing the dose of fertilizer (probably at the cost of lower yields), changes of the cultivation technology or choosing another energy plants. When deciding on the introduction of another energy plants suitable for the production of biogas, it is also necessary to know the CO_2e emission load generated during its growing cycle. Based on this finding, it would be possible to carry out further evaluation and comparison.

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Radiocaesium in forest blueberries in selected location of Jeseníky protected landscape area

ANDREA CERVINKOVA, MICHAEL POSCHL
Department of Molecular Biology and Radiobiology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xcervin4@ node.mendelu.cz

Abstract: Radioactive contamination of the Czech Republic caused by fallout after the Chernobyl nuclear power plant accident continues to be actual after 28 years. Due to the event the environment of the Czech Republic is still contaminated with radiocaesium (^{137}Cs) with average surface activity (the 19th November 2014) of 4.034 kBq·m^{-2}. Trace amount of radiocaesium (^{137}Cs) can be also identified in fruits of forest plants. This study brings new findings about the content of radiocaesium (^{137}Cs) in the wild blueberries. Also the radiocaesium transfer (T_{AG}) from soil to berries was analyzed. Samples of the fruits together with the upper soils under the plants were collected on selected locations of Jeseníky protected landscape area. The ^{137}Cs activity was measured by gamma spectrometric analysis using HPGe detector. It was found that the radiocaesium activity concentrations in blueberries (fruits) ranged from 15.66 Bq·kg^{-1} to 86.54 Bq·kg^{-1}, and correlated (r = 0.93) with the ^{137}Cs activity in soil, which reached values from 184.32 Bq·kg^{-1} to 520.54 Bq·kg^{-1}. Soil-to-fruit aggregated transfer factor was 3.839×10^{-2} m^2·kg^{-1}. The factor represented very low value of the radiocaesium transport from soil to blueberry fruits, and is also comparable with published data. The determined values of ^{137}Cs in blueberries did not exceed the intervention limit specified for food (600 Bq·kg^{-1}), and do not represent health hazard for the population.

Key words: ^{137}Cs, *Vaccinium myrtillus L.*, Jeseníky PLA, aggregated transfer factor (T_{AG})

Introduction

Accident in Chernobyl nuclear power plant (NPP) was followed with contamination of wide areas of the northern hemisphere. Huge amount of radioactive material (1.2×10^7 TBq incl. 8.5×10^4 TBq of radiocaesium ^{137}Cs) was released into the environment [1]. The radioactive contaminated clouds were transferred, disseminated and subsequently the contained radionuclides were deposited in form of rain-fall to the land surface [2]. The contamination of the Czech Republic was very unevenly distributed with average surface activity of 7.6 kBq·m^{-2} [3]. The highest ^{137}Cs deposition was in the southern and northern Moravia [4]. Especially in the Jeseníky protected land area (PLA), where radiocaesium (^{137}Cs) activity of 50 kBq·m^{-2} in forest soil was found [5]. The Environment of the Czech Republic is still burdened by this anthropogenic radionuclide, because the half-life of ^{137}Cs is 30.07 years [6]. Due to the specific properties of soil the radiocaesium is retained in natural (agriculturally uncultivated) ecosystem in high concentration [7] and the natural ecosystem can be a source of secondary contamination of plants and their fruits, e.g. of forest edible berries. The wild berries are an essential part of the diet for the general population and annual consumption is 1.8 kg per capita in the Czech Republic [8]. Blueberries are called "super fruits" for their extraordinary nutritional and pharmaceutical values [9, 10]. The fruits contain a lot of vitamins, minerals and another element, which are beneficial for human organism. Blueberries are also used in modern medicine [11-15]. The forest berries, however, contain much more post Chernobyl radiocesium (^{137}Cs) compared with fruits from agricultural field production [16].

Material and methods

For the study the protected land area (PLA) of Jeseníky (northeast upland of the Czech Republic) was chosen, because this region has the highest surface contamination of post-Chernobyl radiocaesium (^{137}Cs). The locations of sampling were identified in the destined mountain area according to soil type, altitude and mutual distance. The locations of sampling were divided into four

parcels (100 m^2). Blueberry samples were collected during the fertile seasons of plants. The blueberries were taken using standard manual process (without help of comb). During the sampling of fruits, soil samples (depth 15 cm) were also picked under the blueberry plants. Preparation of samples – blueberries were deprived of impurities, stored to small boxes (volume 100 ml), weighed, identified and laid into the freezer. Soil samples were prepared according to standard procedure – they were deprived of stones and residues of plants, dried to constant weight, crushed, stored to small boxes (volume 100 ml), weighed and labeled. Content of the radiocaesium (^{137}Cs) was measured by gamma spectrometric analysis using HPGe detector, software GENIE 2000 in 100 ml of geometry, measuring time of 10 hours. Aggregated transfer factors (T_{AG}) were defined for evaluation of radiocesium transfer from soil to fruit. Aggregated transfer factor was calculated as ratio of the radiocaesium activity concentration in native fruits (Bq·kg^{-1}) to total ^{137}Cs surface activity of soil (Bq·m^{-2}). Also correlation between the activity of soil and fruit was determined. The internal radiation doses for the consumer were computed for blueberry samples, which showed the highest content of radiocaesium (^{137}Cs). The analysis of the potential health hazard risk due to the consumption of blueberries was based on estimating the effective ingestion dose (*D*) according to the equation [17]:

$$D = h(g) \times A_e \times m \ (Sv \cdot y^{-1})$$

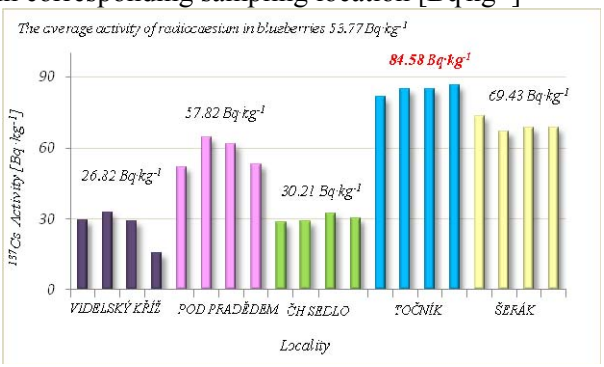
where: *h(g)* is the effective committed dose per unit uptake of the ingested radiocaesium for an individual belonging to age group (Sv·Bq^{-1}), A_e is the activity concentration of the radiocaesium that the blueberries contains (Bq·kg^{-1}), and *m* is the fresh mass of the blueberries ingested per year by the standard individual (kg·y^{-1}) in the respective area.

Results and discussion

Radiocaesium (^{137}Cs) activity concentration in blueberries

The ^{137}Cs activity concentrations in wild blueberries reached the values from 15.66 Bq·kg^{-1} to 86.54 Bq·kg^{-1}. The lowest radiocaesium concentration was found in blueberries which were taken from the forest ecosystem at an altitude 930 m.a.s.l. – area Videlský kříž. The highest activity of radiocaesium was determined in blueberries which were picked from the homogeneous scrub of blueberries at an altitude 1143 m.a.s.l. – locality Točník. Radiocaesium (^{137}Cs) is metabolically and physiologically similar to potassium [18], so it is accepted by the plant as element which is designated for plant nutrition. Due to nutrient competition in plants species, which grow in the forest ecosystem, lower concentration in blueberries can be expected [7].

Fig. 1 ^{137}Cs activity concentration in the blueberries in corresponding sampling location [Bq·kg^{-1}]



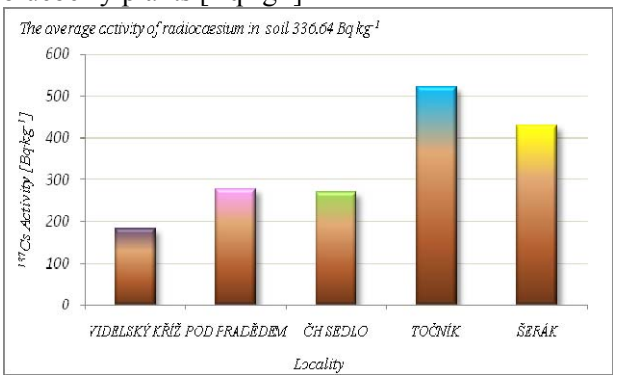
On the contrary – in blueberries, which grow in the homogeneous scrub, the radiocaesium content is higher because the element is transferred among blueberry plants via a net of roots. It conforms to results of Duff et al [19] that the blueberry plants, which grow in a higher altitude, have higher radiocaesium content than the blueberries plants which grow in a lower altitude. This fact is with high probability caused by the soils properties – the forest soils in a higher altitude have bigger content of humus and are more acidic, so allow the considerable mobility of radiocaesium. The radiocaesium is more available for plants [20]. Detected ^{137}Cs activity concentration in our blueberry samples is also comparable with published data of Solatie a Ylipieti [21]. The identified activities of radiocaesium (^{137}Cs) in blueberries were relatively high. Švadlenková et al. [22] summarized that the higher radiocaesium activities in blueberries deserve a special attention compared with another edible forest berries. Kostiainen [23] confirms this fact – the content of radiocaesium (^{137}Cs) decreases in berry fruits in this sequence: blueberries > blackberries; raspberries > strawberries > rowanberries. The ecological requirements of blueberry plants are likely reason of this fact. Acidophil blueberry plants *(Vaccinium myrtillus L.)* search acidic humus wet soils in a higher altitude where they form extensive „carpets“ of union *Vaccinion*. The plants are also bio indicator of acidic soils. The acidity of soil is the main factor which influences the availability of radiocaesium [24]. The radiocaesium uptake via roots is higher when the soil reaction (pH) is lower [25]. In addition, the radiocaesium is in negative correlation with absorption capacity of

the clay and in positive correlation with cation exchange capacity of humus [26]. A secondary contamination of blueberries with the flying of contaminated soil grain [27] or also still relatively high radiocaesium source in rooting depth [22] can be another reasons of the higher content of radiocaesium (^{137}Cs) in blueberries. Although the detected ^{137}Cs activities in blueberries were relatively high, the activities do not represent any important environmental problem and any health hazard for human. It was calculated from our results, that the T_{AG} of blueberries (3.839×10^{-2} $m^2 \cdot kg^{-1}$) shows the similar values to the artificially resulted T_{AG} category (5.5×10^{-2} $m^2 \cdot kg^{-1}$) by Howard et al. [28]. The determined values of ^{137}Cs concentrations did not exceed the intervention limit specified for food (600 $Bq \cdot kg^{-1}$) [29].

Radiocaesium (^{137}Cs) activity concentration in soils

The activity concentration of radiocesium in soil reached the values from 184.32 $Bq \cdot kg^{-1}$ to 520.54 $Bq \cdot kg^{-1}$. The lowest radiocaesium concentration was found in soil which was taken from locality Videlský kříž. The highest activity of radiocaesium was determined in soil which was picked from the locality Točník.

Fig. 2 ^{137}Cs activity concentration in soils under the blueberry plants [$Bq \cdot kg^{-1}$]



The activity concentration of radiocesium in soil reached the values from 184.32 $Bq \cdot kg^{-1}$ to 520.54 $Bq \cdot kg^{-1}$. The lowest radiocaesium concentration was found in soil which was taken from locality Videlský kříž. The highest activity of radiocaesium was determined in soil which was picked from the locality Točník. The detected activities in soil are relatively high. The forest ecosystem is the likely reason of the higher values in soils because the forest ecosystem is (compared to the agricultural ecosystem) very complicated and the ecosystem do not allow for great mobility of radiocaesium because the ecosystem contains a lot of clay minerals which can bind the radiocaesium [30]. The placement of radiocaesium (^{137}Cs) in soil profile is another likely reason. Walton [31] summarized that more than 80 % of radiocaesium (^{137}Cs) remains in upper 15 cm of surface soil. In our study, samples of soil were taken from upper 3.5 cm. The found ^{137}Cs activity in soil exceeded the intervention limit specified for soil in the Czech Republic (100 $Bq \cdot kg^{-1}$). However, this limit is determined for agricultural soils, not for natural (forest) ecosystem which represents the main reservoir of radiocaesium (^{137}Cs) in the environment of the Czech Republic.

Conclusion

It was found that the activity concentration of radiocaesium (^{137}Cs) in the blueberry fruit sampled in the Czech Republic in the PLA Jeseníky reached the maximum activity of 86.54 $Bq \cdot kg^{-1}$. If the average consumer will consume such berries (1.8 kg per year per capita), he could receive the internal radiation dose of 2.02×10^{-3} $mSv \cdot rok^{-1}$. Nevertheless, with respect to the antioxidant effects of edible forest fruit, the consumption can be considered to prevent effects ionizing radiation [32-34]. The study by Wan et al. [35] supports this fact, i.e. the presence of antioxidants in the body of the irradiated organism reduces the effect of radiation. The detected ^{137}Cs activities in soils taken under the blueberry plants were relatively high, reached values from 184.32 $Bq \cdot kg^{-1}$ to 520.54 $Bq \cdot kg^{-1}$ and correlated ($r = 0.93$) with activities in blueberry fruits. These findings correspond with characteristics of forest ecosystems, where a number of contaminants incl. radiocaesium are fixed for a long time and are conformable with earlier published data.

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Nesting preference of European Bee-eater (*Merops apiaster*) in conditions of South Moravia (Czech Republic)

RENATA DANKOVA, VLADIMIR HULA
Department of Zoology, Fisheries, Hydrobiology and Apiculture
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xdanko11@node.mendelu.cz

Abstract: European Bee-eater (*Merops apiaster*) belongs among one of the most endangered birds of the Czech Republic. It is nesting in specific conditions with barren soil. The aim of our study was investigate all nesting colonies in conditions of South Moravia. All visited sites (36), including historical ones without birds (11), were properly described via environmental conditions. The main factor involving nesting is presence of steep slope with barren surface (with different angle of slope). Other factor is heterogeneity of surrounding landscape which fit perfectly with unique habitat of agricultural terraces. This habitat is also inhabited by other interesting bird species including *Saxicola rubetra*, *Sylvia nisoria* and *Lanius excubitor*. Other habitats were sand pits, former quarries and clay pits. All sites in the Czech Republic are artificial and men made only. Based on these results we would like to design conditions, which we can use for creation of other artificial nesting sites within south Moravian countryside.

Key-Words: bare walls, agricultural terraces, nesting

Introduction

European Bee-eater (*Merops apiaster* Linnaeus, 1758) is migratory bird species. It returns during May in the Czech Republic [1, 2]. This species come back to the Czech Republic from its distant wintering areas in tropical and southern Africa. The time of leave to wintering areas is particularly changing in case of young due to smaller physical constitution than adults [3].

The main population decline of European Bee-eater (*Merops apiaster*) was in the years 1970 – 1990. Its general abundance is increasing since the 90s years of last century. The total number didn't reach yet numbers before upper mentioned decline. This species needs bare soil walls for nesting, where it digs long nesting burrows [1, 2] (Fig 1).

First sure nesting was recorded in the Czech Republic in fifties, but several observation were done before this period, but not properly documented. It nests here regularly until now [2]. Nesting sites are sandy or loamy. Nesting was observed also on the ground abroad [1]. European Bee-eater nests often in the vineyard terraces, clay pits and sand pits together with Sand Martin (*Riparia riparia*). All nesting sites European Bee-eater were created by human activity in the Czech Republic [1].

Xerothermic habitats got terraced character at the turn of the 60th to 70th years of the 20th century that could be used as example for the cultivation of the wine. Creating terraces was a big intervention to landscape. These xerothermic places have become important habitats for many species [4, 5, 6], which include the European Bee-eater [1]. European Bee-eater is reliant during nesting on enough suitable slopes for digging burrows. Barren spots created as a result of erosion or human activity cannot stay barren for long time, because succession is always present in the landscape and therefore suitable sites overgrown. In addition the suitability of the material, from which the slope is formed, continuously decreases over time. European Bee-eater is therefore dependent on human activity, with which is associated the creation of appropriate new soil walls. It is also important to keep older slopes, where European Bee-eater nests repeatedly. Also mining activity in sand pits and quarries creates such suitable habitats [1]. It is therefore important to monitor habitat conditions that change over time or habitats directly disappear.

The aim was to find suitable habitats, where European Bee-eater nests, check nesting availability and identify main environmental conditions which it needs for its nesting in southern Moravia. The reason is future possible management of such area

for support nesting this species in the Czech Republic.

Material and Methods

European Bee-eater was observed on agricultural terraces, sand pits, clay pits, quarries and slopes in the landscape of South Moravia. Suitable sites were selected before arriving European Bee-eater, and were inspected repeatable after species arrival. Site selection was carried out on the basis of suitability slopes for nesting and looking for old nesting marks from previous years. Historical nesting sites were also visited (selected based experience of local biologists).

The selected sites were visited during the nesting period. Vocalizations pointed to their presence in case of less number of breeding pairs. The birds were observed and recorded the number of breeding pairs, with a reasonable distance from nesting sites, always from the same observational point.

All monitored sites were located in the South Moravian Region. Particular sites were within following districts: Znojmo (1), Brno-venkov (8), Hodonín (8) and Břeclav (19). The research was conducted from early May to mid-August.

Category nesting sites of European Bee-eater

Vineyard terraces

This habitat presents barren slopes that form the front terraces with adjacent vineyards. There are trees and shrubs growing in surroundings of these slopes.

Terraces with other crops

This is the bare slopes that form the front terraces with adjacent crops. There are cultivated variety crops (corn, alfalfa, canola, etc.). Bushes grow predominantly around these terraces, but also trees present.

Barren slopes in the landscape

These are the slopes in the landscape that aren't overgrown by vegetation, some are newly created, some overgrown vegetation gradually. These habitats include slopes located in different type of landscape: meadows, field paths or in one case the slope is situated directly over shooting range. There are trees and shrubs.

Sand pits and quarries

Sand pits, clay pits and quarries, which are formed during the extraction bare slopes. It is a mining area where mining takes place and the barren walls of quarries. Bushes grow in the nearby.

Results and Discussion

European Bee-eater nested in 2014 nesting period (13.5.-6.8. 2014) mainly in district of Břeclav (11 sites of evidence nesting), further in district Brno-venkov (4 sites of evidence nesting) and in district Hodonín (3 sites of evidence nesting).

Vineyard terraces

This includes 13 terraces with vineyards. Nesting was observed at the six vineyards, from 2 pairs to 21 pairs per location (Fig. 4). Earlier nesting was found on 3 locations. One site obtained to European Bee-eater regular food hunting ground. No traces of staying European Bee-eater (*Merops apiaster*) were found on 4 locations.

All slopes were barren in spots holes. Slopes orientation was different to cardinal points – north, south, west and east, southwest and southeast (Fig. 3). Burrows were always in angle of 90 degrees to the slope. The nesting (historical and recent) wall, have height from 0.5 m to over 2 m (Fig. 2).

Trees and shrubs around the slope served them as an observation places. Tension pillars in the vineyards were also good such observation post. These places served also to its rest and scaling spots for feeding. The bird firstly checked a pray, before consumption. Birds feed regularly on insects living in vineyards.

Terraces with other crops

This habitat is represented by six terraced sites, on which other crops than vine grew (alfalfa, canola, corn, etc.). Three of these terraces were occupied during the nesting season by European Bee-eater. One terrace wasn't farmed, place overgrown by grass and bushes. On this habitat nested from 6 pairs to 17 pairs (Fig. 4). One terrace, where grew corn, was not occupied. Two European Bee-eaters were observed during the first visit of the site. They started burrowing there. The site was not occupied at the next visit. Traces from previous nesting were found here. Two terraces with barren slopes weren't inhabited.

Angles of slope at places of burrows were always around 90 degrees and orientation to the cardinal points was different – south, southwest, southeast and northwest (Fig. 3). In the place burrows were barren slopes. Nesting spots were between of 1 – 3 m height (Fig. 2).

As an observation spots there were trees and shrubs only. They feed on insects from surroundings fields here.

Barren slopes in the landscape

Altogether 13 different locations were studied; eight sites were used by European Bee-eater. There nested from 1 pair to 26 pairs (Fig. 4). This includes wide variety of habitats. Barren slopes are the most

common element suitable for nesting of European Bee-eater. The bird was not observed at 5 locations; old burrows were found from previous years here. One of these sites was probably visited by European Bee-eater in this breeding season (May to August 2014) too, but it was not inhabited (on the end of June).

Orientation to the cardinal points was different – north, south, west, east, northeast, northwest, southeast and southwest (Fig. 3) and the slope was always around 90 degrees. The slopes with burrows were high from 0.5 to 6 m (Fig. 2).

As an observation sites there were bushes, trees and also dead branches. The species fees here on insects on all types of habitats– in orchards, fields, gardens, vineyards, etc.

Sand pits and quarries

It includes 4 locations. Large colony of Sand Martin (*Riparia riparia*) nested at one location. European Bee-eater nested at this location also (two pairs nested here) (Fig. 4).The historical nesting was recorded on two such locations.

There were no marks of nesting at two locations. The slope, where it nested was perpendicular. The slopes to the cardinal points were different (Fig. 3). The slope was located in the sand pits with common Sand Martins (*Riparia riparia*) nesting on the southwest. The bare wall had a height of over 2 m (Fig. 2).

Various shrubs around served for observation. The birds were looking for food at surroundings fields.

There were inspected a total of 36 sites altogether. European Bee-eater (*Merops apiaster*) nested on 18 of them in nesting period of 2014 (Fig. 4). Altogether 11 historical locations were found (with empty burrows only). Seven possible sites were not occupied. Slope of walls was about 90degrees and orientation to the cardinal points was different (Fig. 3), mostly southwest. Heights of slopes were from 0.5 to 6 m (Fig. 2), but slopes heights of around 2 m were the most occupied. Various shrubs and trees planted in close proximity to the slopes an important factor.

Other kinds of birds like to use burrows European Bee-eater to its nesting [7, 8]. In our observation, it was mainly Tree Sparrows (*Passer montanus*).

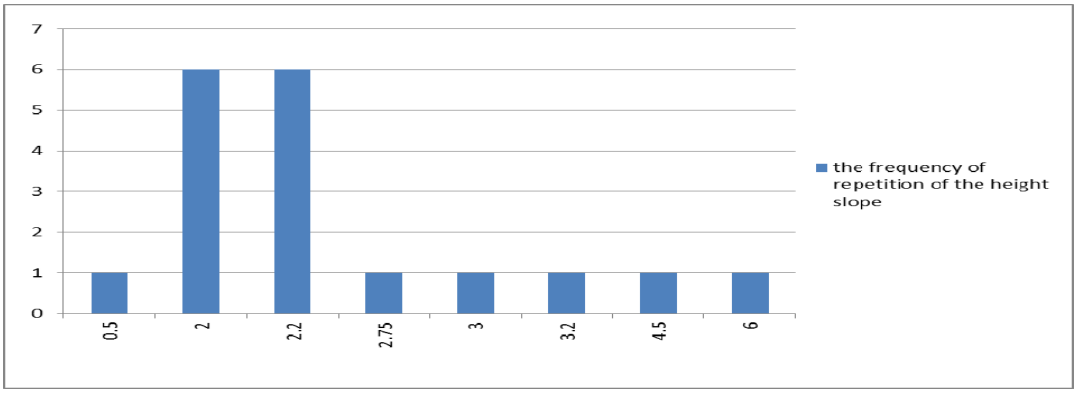
The Czech colonies of European Bee-eater are generally small and the same result has some other authors [9]. Most pairs are nesting together (the riches colony host 27 pairs, the smallest at least 2 pairs).

They prefer if they can build burrows by themself [10]. First at all they search for suitable spot at slopes and later they dig the burrow. During their search they check the substrate first, if it is not suitable, they start burrow new hole. The best substrate in conditions of Moravia is loess [11].

It digs burrow mostly at the end of May [10]. At this year the digging was observed at the first week of June.

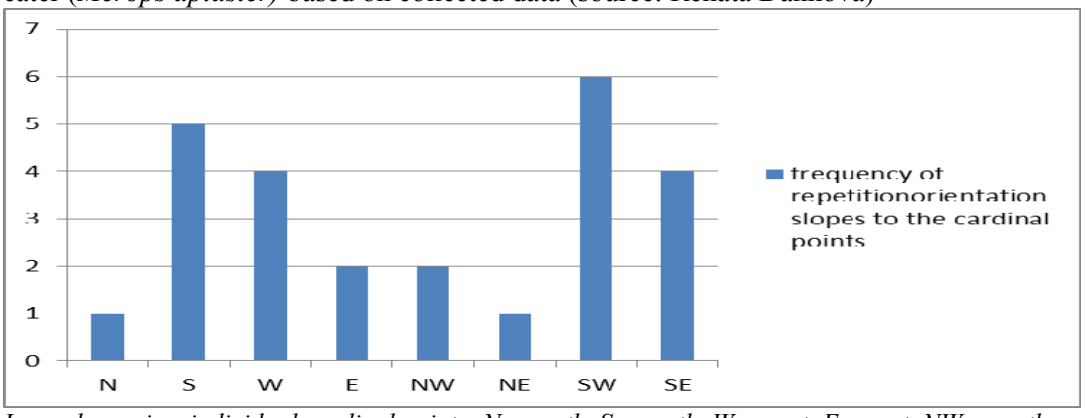
Fig. 1 Nesting site (source: Renata Daňková)

Fig. 2 Frequency of repetition heights of slopes with the presence of European Bee-eater (*Merops apiaster*) – source: Renata Daňková



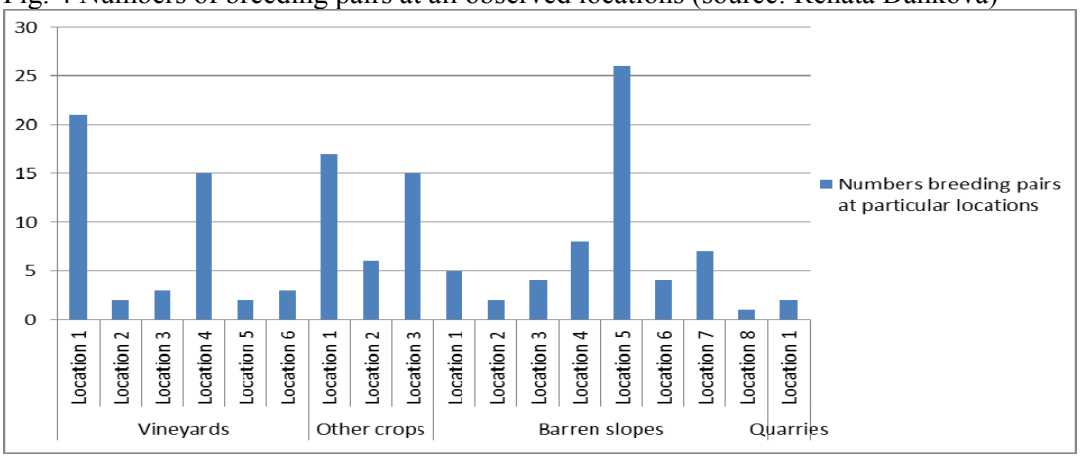
Legend: x-axis – the total height of slopes is quoted in meters, y-axis – frequency of repetition of the same height of slope

Fig. 3 Frequency of repetition orientation slopes to the cardinal points with the presence of European Bee-eater (*Merops apiaster)* based on collected data (source: Renata Daňková)



Legend: x-axis – individual cardinal points: N – north, S – south, W – west, E – east, NW – northwest, NE – northeast, SW – southwest, SE- southeast, y-axis – frequency of repetition of individual orientation to the cardinal points

Fig. 4 Numbers of breeding pairs at all observed locations (source: Renata Daňková)



Legend: x-axis – the individual locations occupied by European Bee-eaters (Merops apiaster) with their inclusion in the relevant category y-axis – the number of breeding pairs

Conclusion

Barren slopes of agricultural terraces, sand and clay pits, quarries and barren slopes in the landscape are very important for European Bee-eater at present time. They look for manmade habitats in the countryside which are the only suitable habitats of this species in conditions of Czech Republic. They nest close to human settlements and also other human activities don't disturb them to much (one place is at active sport shooting range). Its important to notice that they are strongly connected to human activities in landscape and that they cannot survived in modern countryside without these activities.

The birds choose slopes that are nearly perpendicular. If the slope isn't perpendicular, the holes are located on the most perpendicular parts of the slope.

Ravine routes with vertical slopes, which were in the vineyards, proved to be also very suitable for nesting. Even though the walls are low, it is like to use them on both sides.

The space between individual burrows can be very small (13 cm). Birds can use effectively the space of the slope because the suitable habitats are rare and scarce.

The interesting fact is that burrows are reachable for other animals including predators, but predation was not recorded.

European Bee-eater prefers for its nesting various high of slopes. However, most of the burrows are always at the highest slope, the number of nests decrease with decreasing height of the slope (Fig. 1).

The most important is of maintaining at least part of the barren slopes in which it can dig its burrows. It is not problematic for the bird, if slopes are therefore slightly covered by low and sparse vegetation, but the species usually prefers slopes without vegetation.

The preliminary results shows that the species is not affected by orientation of the slope, and that

more important is the height of the slope and its cover.

Furthermore, shrubs and trees have to be present in the vicinity of nesting sites. These are use as observation spots, but also for resting and food adjustment. Vineyard tension pillars are also used for the same reason in the vineyards.

European Bee-eater needs a lot of insects during the nesting, because the birds nest in colonies and the surroundings has to offer sufficient food supply for higher numbers of specimens locally. They feed primarily butterflies, beetles and different kind of bees. The food supply has to be supported by nature management activities, because is crucial.

Burrows constructed by European Bee-eater are also important for other bird species such as Tree Sparrow (*Passer montanus*) and Black Redstart (*Phoenicurus ochruros*).

Acknowledgement

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The comparison of microbial activity in rhizosphere and non-rhizosphere soil stressed by drought

JAKUB ELBL, JAROSLAV ZAHORA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jakub.elbl@mendelu.cz

Abstract: This work presents the analysis of the influence of drought on microbial activity in rhizosphere and non-rhizosphere soil. Microbial activity was expressed as basal respiration (BR). Three groups of the treatment (A, B and C) with different regime of irrigation were prepared. The soil water content was maintained at 70% of maximum capillary capacity (MCC) in group A and at 40% in group B. In group C, soil water regime was maintained in the range of wilting point. Each group of the experiment was divided into three variants (A1 = B1, C1; A2 = B2, C2 etc.) with three repetitions: Variants A1 (B1, C1) were a controls without addition of another fertilizer. Variants A2 (B2, C2) were fertilized with mineral nitrogen fertilizer DAM 390 (0.140 Mg of N per ha) and variants A3 (B3, C3) contained 45 g of C_p per a pot. Significant differences in BR between individual variants were found in both rhizosphere and non-rhizosphere soil. The highest BR was always measured in variants A3 where soil water regime was maintained at 70% of MCC and C_p was applied. Differences in values of BR were detected between rhizosphere and non-rhizosphere soil. Unfortunately these differences were not significant. Based on these results, we can conclude that the drought in combination of method of fertilization has direct effect on microbial activities in soil, but this effect does not cause significant changes in microbial activities of rhizosphere and non-rhizosphere soil.

Key-Words: Drought, Microbial Activity, Rhizosphere Soil, Mineral Nitrogen, Arable Soil,

Introduction

While the effects of drought are well documented, a proper working definition of drought is less clear. Drought is a complex phenomenon that is difficult to accurately describe because its definition is both spatially variable and context dependent. Drought can be classified into three categories: meteorological, agricultural, and hydrological drought [1]. The wide variety of sectors affected by drought, its diverse geographical and temporal distribution, and the demand placed on water supply by human-use systems make it difficult to develop a single definition of drought and its influence [2]. Drought should be perceived as a natural aspect of climate under all climatic regimes as it occurs in both humid and arid areas (clearly with different impacts unique to the existing ecosystems). Central Europe is not frequently thought of as being a particularly drought-prone region in the European context with the exception being the Panonian Basin that, in part, includes eastern Austria and a large part of Hungary [3]. Therefore, there are not enough scientific papers dealing with the effects of drought on soil properties in the condition of central Europe.

Drought threat has significant consequences for belowground carbon (C) and nutrient cycling (N etc.). It may affect soil processes through changes in C allocation to roots and foliage as well as C turnover in the rhizosphere [4] and thus significantly affect microbial activity in rhizosphere and non-rhizosphere soil. The rhizosphere is an ill-defined a few millimeters thick that surrounds plant roots. Healthy soil is teeming with microscopic and larger organisms that perform many vital functions including converting dead and decaying matter together with minerals as plant nutrients. Therefore microbial activity represents important indicator of healthy soil [5]. Only healthy soil is resistant to negative phenomena: depletion of soil fertility, soil degradation and soil erosion.

The rhizosphere is a living space for soil microorganisms, which are very important for soil health and fertility. Plants produce root exudates which stimulate heterotrophic growth and lead to local competition for inorganic nutrients between

roots and microorganisms [6], [7]. Conversely, non-rhizosphere soil is not affected by plants roots and root exudations. Therefore there is lower level of microbial activity and soil fertility but this part of soil is necessary for the stability of the soil aggregates and resistance of soil to negative phenomena's (soil erosion, leaching of nutrients etc.). In the present work, we focused on the comparison of effect of drought on microbial activity in rhizosphere and non-rhizosphere soil. Moreover we looked for a link between the decrease in microbial activity and an increase in loss of mineral nitrogen from soil.

These hypotheses were tested: (1) the recurring period of drought has negative impact on microbial activity in both rhizosphere and non-rhizosphere soil; (2) drought – decrease in soil water content has a greater negative impact on microbial activity in non-rhizosphere zone than in rhizosphere zone; (3) microbial activity affects the soil´s ability to retain mineral nitrogen – decrease in microbial activity results in increase in leaching of mineral nitrogen.

Material and Methods

Experimental design

The above hypotheses were tested by pot experiment, which was carried out according Elbl et al. [8]: Twenty-seven PVC tubes (see Figure 1) were used as experimental containers and located in the growth box (phytotron; see Figure. 1). During the whole experiment, all containers with indicator plant *Deschampsia caespitosa* (one plant per experimental containers) were kept in a growth box at 24°C (day temperature), 20 °C (night temperature) and 65% humidity (for all 24h) with a day length of 12 h (light intensity 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$). Three groups of experiment A, B and C with different regime of irrigation were prepared. The complete overview is shown in the Table 1.

Table 1 Distribution of the laboratory experiment

Group	WHC	Variants	Characteristics
A	70%	A1	Control
		A2	0.140 Mg N/ha
		A3	50 Mg C_p/ha
B	40%	B1	Control
		B2	0.140 Mg N/ha
		B3	50 Mg C_p/ha
C	Wilting point	C1	Control
		C2	0.140 Mg N/ha
		C3	50 Mg C_p/ha

Each group of experiment was divided into three variants (A1, B1, C1; A2, B2, C2 etc.) with three repetitions: Variants A1 (B1, C1) were controls without addition of another fertilizer. Variants A2 (B2, C2) were fertilized with mineral nitrogen fertilizer DAM 390 (one hundred liters of DAM 390 contain 39 kg of nitrogen - 1/4 of nitrogen is in the form of ammonium, 1/4 is in the nitrate form and ½ is in the form of urea). Recommended dose of Nmin for extensive grass ecosystem was applied there (0.140 Mg N/ha). Variants A3 (B3, C3) contained 45 g of Cp per pot. This dose of Cp is in accordance with ČSN EN 46 5735 representing 50 Mg/ha. C_p was applied into topsoil. Used mineral fertilizer DAM 390 and Cp are registered for agriculture use in the Czech Republic. The water content in soil was maintained at 70% of soil Water Holding Capacity (WHC) in group A, at 40% in group B. WHC was determined for top soil and subsoil according Dykyjova [9]. After filling and achieving the required values of WHC, experimental containers were weighted. Subsequently measured weight was maintained throughout the experiment by irrigation. Soil water regime was maintained in the range of wilting point in group C. Indicator plant was supplemented by salad (*Lactuca sativa* L.): one indicator plant and salad per one experimental container. The soil water content (in this group) was maintained at 70% of WHC (containers have the same weight as in group A) at the beginning of the experiment. Subsequently, these containers were not irrigated until plants (salad) began to wilt. After reaching the point of wilting, plants were irrigated by one-off dose of demineralized water at the same weight as in the group A and again, these containers were not irrigated before reaching the wilting point.

Fig. 1 Experimental containers in a growth box

Determination of CO_2 production

Basal respiration (BR) was determined by measuring the CO_2 production from soils incubated in serum bottles for 24 hours. Field moist soil (15 g) was weighed into each of three 120 cm^3 serum bottles. Bottles were sealed with butyl rubber stoppers and incubated at 25°C. After 3 and 24 hours 0.5 cm^3 sample of the internal atmosphere in each bottle was analyzed by gas chromatography (Agilent Technologies 7890A GC System equipped with a thermal conductivity detector). Respiration was calculated from the increase in CO_2 during the 21 h incubation period. At the end of the measurements, the total headspace volume for each replicate bottle was determined by measuring the volume of water required to fill the bottle. The results are expressed per gram of dry soil and hour [10]. BR was measured in soil sample which were collected from rhizosphere and non rhizosphere zone.

Determination of mineral nitrogen leaching

The loss of mineral nitrogen (N_{min}) was measured according Elbl et al. [8] and published in Elbl et al [8]. These results were used for reference purposes only: examination of a link between the decrease in microbial activity and formation of N_{min}.

Statistical analysis

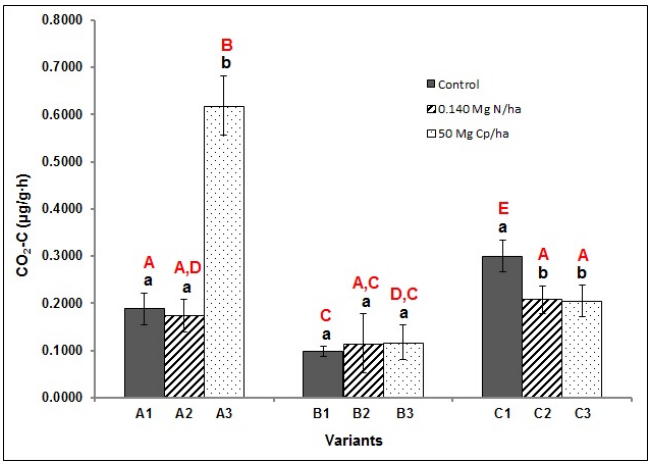
The potential differences in microbial activity between individual variants and groups of experiment were analysed by one-way analysis of variance (ANOVA; $P<0.05$) in combination with post-hoc Tukey`s HSD test ($P<0.05$). The potential differences in microbial activity between rhizosphere and non-rhizosphere soil were tested by t-test ($P<0.05$). The relationship between level of microbial activity and loss of Nmin was tested by regression analysis. All data were analyzed in the Statistica CZ 10 software. Graphic processing of measured data was performed in Microsoft Excel 2010.

Results and Discussion

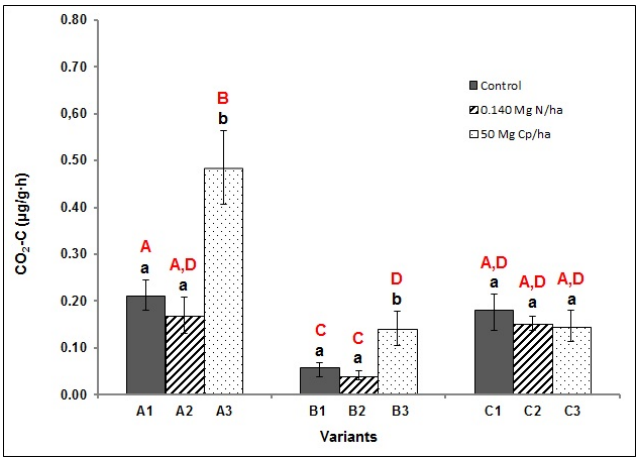
In soil microbial ecology, the actual (basal respiration, BR) and the potential microbial respirometric activity (substrate-induced respiration, SIR) are well-established and widely used method for measurement of microbial activity [12]. Soil respiration is one of the most important indicators of microbial activity in the soil. During the experiment, two types of respiration were measured: basal and substrate indicated respiration. The only values of BR are presented in this work.

Fig. 2 Basal respiration (mean ± SD, n = 3)

A) Rhizosphere soil



B) Non-rhizosphere soil



Comment for the Fig. 2: different small letters indicate significant differences ($P<0.05$) between individual variants within the same group and different uppercase letters indicate significant differences between all individual variants (regardless groups).

Microbial activity in rhizosphere and non-rhizosphere soil

Soil microbial communities play a critical role in ecosystem processes, such as carbon cycling, nutrient turnover, or the production of trace gases [12]. The BR was chosen as microbial indicator because it represents metabolic activity of soil microbes and thus their activity. The influence

of plants on the soil microbial community can be especially important in agricultural systems in which cash and cover crop selection can vary from short rotations (2-3 years) that might include one or two crop species to longer rotations (6-10 years) which might include six or eight species including both annual and perennial crops [13]. Therefore, we focused on microbial activity in zone with or without influence of plant roots.

Figure 2 presents significant differences in microbial activity in rhizosphere and non-rhizosphere soil between individual variants and all variants of experiment. The significant ($P<0.05$) highest microbial activity was found in variant A3 for both soil types. Conversely, the significant lowest microbial activity was observed in variants B for rhizosphere soil and in variant B2 at no-rhizosphere soil. The optimal water content in soil at respiration is from 50 to 70% of the soil WHC. This fact, together with the content of C_{org} in variants A3 is the main reason for the differences in BR between individual groups. Positive effect of C_p addition on microbial activity in soil was confirmed by [14][15].

The results indicate that the drought in combination with precipitations and type of fertilization (mineral/organic type) can affect microbial activity. Influence of method fertilization and soil properties (pH, soil water content, soil type, soil organic matter content, etc.) on soil microbial activity in rhizosphere and non rhizosphere soil was confirmed by [16], [17], [18].

Comparison of microbial activity in rhizosphere and non-rhizosphere soil

The rhizosphere is the plant root–microorganism interactional site. The rhizosphere is technically difficult to be defined for studying the root-induced physical–chemical, microbial properties in this zone. Generally, the rhizosphere has a thickness of 1–2 mm, but functionally, the rhizosphere can be defined as the soil physically and chemically influenced by growth and activity of the root [19].

Table 2 Comparison of microbial activity in rhizosphere (RH) and non-rhizosphere soil (NH)

Group	Variants	BR	Mean differences	t	p
A	A1	RH NR	-0.0235	-1.9742	0.1870
	A2	RH NR	0.0041	0.1215	0.9143
	A3	RH NR	**0.1333**	6.0836	0.0251
B	B1	RH NR	0.0408	1.6155	0.2476
	B2	RH NR	0.0734	1.3811	0.3013
	B3	RH NR	-0.0245	-0.7684	0.5225
C	C1	RH NR	**0.1204**	11.4530	0.0075
	C2	RH NR	0.0548	1.3754	0.3028
	C3	RH NR	0.0591	3.5260	0.0718

Comment for the Tab. 2: The means of differences are significant at the level 0.05. These differences are shown in bold. The microbial activity in RH and NH was always compared for one experimental site.

The Fig. 2 and Table 2 show complete overview of basal respiration in rhizosphere and non-rhizosphere soil. Significant differences in microbial activity were found between individual variants of experiment either in the rhizosphere or in the non-rhizosphere soil. Significant differences between rhizosphere and non-rhizosphere soil were detected only in variants A3 and C1. The microbial activity was higher in rhizosphere zone except variants A1 and B3 but these values were not significant. These situation was caused by root exudes which have direct impact on soil microbial communities.

Positive effect of root exudes on microbial activity in rhizosphere soil was confirmed by Maul et al. [13] Bloem & Hopkins [17] and Wenhao et al. [19].

Relationship between level of microbial activity and leaching of mineral nitrogen

Microbial communities in soil consist of a great diversity of species exploring their habitats by adjusting population abundance and activity rates to environmental factors. Soil microbial activities lead to the release of nutrients available for plants, and are of crucial importance in biogeochemical cycling [17].

Geisseler [20] state, that nitrogen is essential for the growth and activity of plants and soil microorganisms. Soil microorganisms can use a wide range of N compounds. These include inorganic compounds such as ammonium-N and nitrate-N, as well as organic molecules such as amino acids and small peptides.

Consider data in the Table 3 and 4 and data which are presented in the Figure 3. These data indicate the significant influence of microbial activity on leaching of N_{min}. In accordance to our hypothesis, the increase in microbial activity causes a reduction in nitrogen leaching and conversely. Relationship between microbial activity and utilization/leaching of N_{min} from soil was studied and confirmed by Geisseler [20] and Turner [21]. The significant differences in influence of microbial activity were not found between rhizosphere and non-rhizosphere soil.

Table 3 Regression analysis of the relationship between the level of microbial activity in rhizosphere soil and leaching of mineral nitrogen

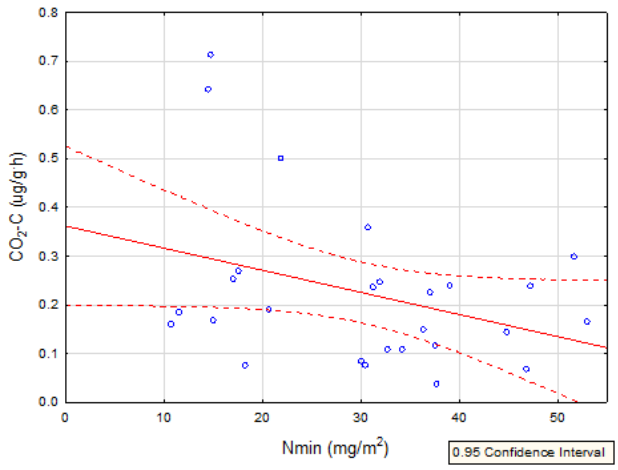
Parameter	Value
Multiple regression	0.34828
R-squared value	0.12130
Adjusted R-squared	0.08615
Statistical power	3.45119
Probability	0.07502
Standard error	12.06465

Table 4 Regression analysis of the relationship between the level of microbial activity in non-rhizosphere soil and leaching of mineral nitrogen

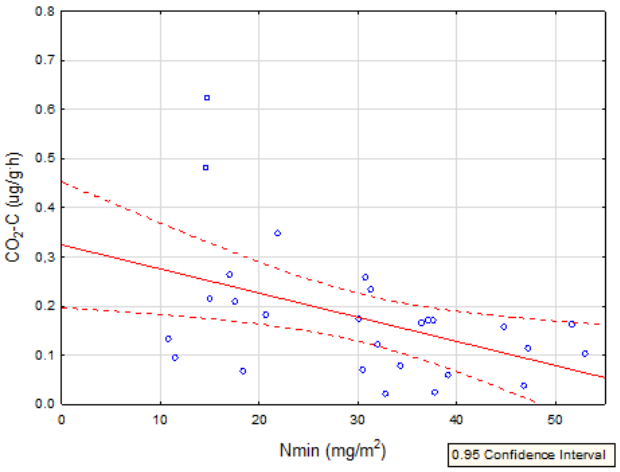
Parameter	Value
Multiple regression	0.45873
R-squared value	0.21043
Adjusted R-squared	0.17885
Statistical power	6.66296
Probability	0.01610
Standard error	11.43640

Fig. 3 Graphic processing of obtained data from regression analysis

A) Rhizosphere soil



B) Non-rhizosphere soil



Conclusion

In conclusion, our results suggest the influence of drought on microbial activity in soil and relationship between microbial activity and leaching of N_{min}. We assume that the decrease in microbial activity causes a loss of mineral nitrogen from soil. The authors stress that the experiment was conducted in specific laboratory conditions and it should be repeated as a field experiment.

Acknowledgement
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Forms of carbon in the soil and their influence on soil quality

MAGDALENA HABOVA, LUBICA POSPISILOVA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xhabova1@node.mendelu.cz

Abstract: Labile and stabile carbon forms were determined in *Haplic Cambisol* (Malonty, arable soil) and *Haplic Cambisol* (Náměšť, arable soil). We assessed total carbon content, water extractable carbon content, humic substances content and microbial biomass content. Dozens of compost were as follows: Malonty – D1 = 30 t.ha-1 and D2 = 38.7 t.ha-1, Náměšť – D1 = 93 $t.ha^{-1}$ and D2 = 158 $t.ha^{-1}$. First year of experiment increasing priming effect was observed. Second year of experiment (locality Malonty) and fifth year of experiment increasing of humic substances, water extractable carbon and microbial biomass carbon was determined. UV-VIS absorbance of humic substances was higher after compost application. Correlation between microbial carbon content and water extractable carbon was found (r_{krit} = 0.159).

Key-Words: soil organic matter, carbon forms, humus fractionation, water extractable carbon, UV-VIS spectroscopy, microbial biomass

Introduction

Soil organic matter is the main component of soil which directly influence soil fertility. It is an important source of nutrients and take a part in many chemical, physical and biological soil processes. Humic substances (HS) are essential for the stabile aggregates formation, good chemical and biological properties. It directly influences soil water regime and aeration. Chemical composition of humic substances depends on plants residues, microbiological composition (1, 2). Further is chemical composition of HS influenced by anthropogenic factors (fertilizing, crop rotation, liming and others). Soil organic carbon is represented by ***stabile***, ***labile, and black carbon*** forms. Stable carbon forms are resistant against mineralization and biodegradation. They are represented by carbon of humic acids (HA) and fulvic acids (FA) - see (3, 4). Labile carbon is soluble and easily mineralized by soil microorganisms. It is determined as water extractable carbon –see 5. Quality of humic substances is given by HA/FA ratio and by spectral properties (UV-VIS absorbance) – see 6. Labile carbon is responsible for temporary stability of soil humus. It mainly consists from non-specific organic components and its decreasing caused loss of soil humus (7). Black carbon has not been studied very well. As it is known black carbon takes part in the global carbon cycle.

Aim of our study was to evaluate content and quality of humic substances after compost amendment. Object of our study were intensively cultivated *Cambisols*, the main soil type in the Czech Republic.

Material and Methods

Haplic Cambisol (locality Malonty) is situated 10 km south-east from Kaplice (region Český Krumlov). Average temperature is 6 °C, average precipitation 650 mm, and altitude 680 m. a. s. l. Long-term field experiments were established in spring 2012. Compost was made from grass, manure and slurry. Application dozens of compost were D1 = 30 t.ha-1and D2 = 38.7 t.ha-1. Soil type was classified according to Němeček et al. (2011) as *Haplic Cambisol* and soil profile was located at N: 48.68871°, E: 014.57433 ° (8, 9, 10).

Haplic Cambisol (locality Náměšť) is situated in the east part of Třebíč region (Czech-Moravian Highland). This region is warm and wet, with average precipitation 550 – 700 mm, average temperature 7 – 8 °C, and altitude 430 m. a. s. l. Long-term field experiments were established in spring 2008.

Compost was made from grass. Application dozens of compost were D1 = 93 t.ha-1and D2 = 158 t.ha-1 . Soil type was classified according to Němeček et al. (2011) as *Haplic Cambisol* and soil profile was located at N: 49°12,808´, E: 16° 09,757´ (8, 9, 10).

Basic soil properties are given in Tab. 1 – see (11). Basic soil properties were determined by standard methods. As it is evident *Cambisols* varied in soil reaction, conductivity, and clay percentage. Total organic carbon (C_{org}) content was determined by oxidimetric titration (12). Fractional composition of HS was made by short fractionation method (13). UV-VIS spectroscopy was performed by Varian Cary 50 probe spectrometer with optical fibre. Water extractable carbon content was determined by analyser Shimadzu TOC-VCSH with CO_2 detection in infrared spectral region. Carbon of soil microbial biomass was determined by fumigation-extraction method according to (14).

Table 1 Basic soil properties

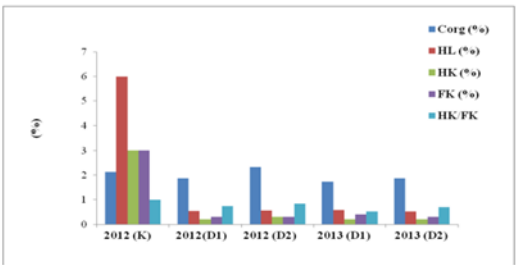
Soil type	Horizon (m)	pH/H_2O	pH/KCl	Conductivity (mS/cm)	Clay particles content (%)
1	2	3	4	5	6
KAm (Malonty)	0 – 0.2	6.26	5.06	0.06	24.04
KAm (Náměšť)	0 – 0.2	5.10	4.00	0.14	29.00

(1) Soil type, (2) horizon, (3) active soil reaction, (4) exchangable soil reaction, (5) conductivity, (6) clay particles content

Results and discussion

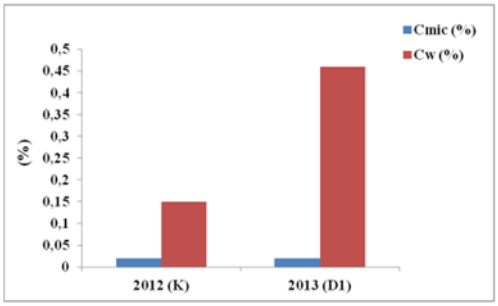
Haplic Cambisol (Malonty) –maximum of total C_{org} was determined second year after compost application (variant D1 = 30 t.ha-1), and reached 2.33% (2013). Average values of total C_{org} in 2012 was 1.88% – see Fig. 1.

Fig. 1 Total organic carbon content and fractional composition of HS in *Haplic Cambisol* (Malonty)



Content of water extractable carbon was 4.9% from C_{org} content. Microbial biomass represented 2% from total C_{org}. Results showed, that compost was used for both stabile and labile carbon formation. Only small part was used for microbial biomass formation and respiration. Increasing of HS was also confirmed by absorbance of HS in UV-VIS spectral range. HS absorbance was higher after compost application (variant D1 = 30 $t.ha^{-1}$) to compare with control. Higher dozen of compost (D2 = 38.7 $t.ha^{-1}$) caused also maximum C_{org} increasing in 2013 (2.33%). Average value in 2012 was 2.13%. Content of water extractable carbon was 4.62% from total C_{org} content – see Fig. 2. Amount of water extractable carbon was higher to compare with variant D1. We can conclude that high dozens of compost were not very effective, because they were mainly used for labile carbon formation, microbial biomass, and respiration. Absorbance of HS in UV-VIS spectral range was comparable with variant D1. This could be explain by the similar HS content on both variants – see Fig. 1. Linear correlation between microbial biomass and amount of dissolved organic carbon was found ($r = 0.21552$, $n = 27$, $\alpha = 0.05$, $r_{krit} = 0.159$).

Fig. 2 Microbial carbon and water extractable carbon content in *Haplic Cambisol* (Malonty)



Haplic Cambisol (Náměšť) – maximum of total C_{org} was determined five years after compost application (variant D1 = 93 t.ha-1). In 2008 was average C_{org} content 1.32%. In 2012 was average C_{org} content 2.4% – see Fig. 3. Content of water extractable carbon was 4.5% from total C_{org}. Microbial biomass represented 2% from total C_{org} – see Fig. 4. Results showed, that compost was used for both stabile and labile carbon formation. This was also confirmed by absorbance of HS in UV-VIS spectral range. Absorbance was higher after compost application to compare with control. Very high dozens of compost (D2 = 158 t.ha-1) caused increasing of water extractable carbon and microbial biomass. Maximum of total C_{org} (2.6%) was five years after compost application (2012) – see Fig. 3. Content of water extractable carbon was 5.4% from total C_{org}. Amount of water extractable carbon was higher to compare with variant D1 – see Fig. 4. We can conclude that high dozens of compost were not used effectively, because of increasing the labile carbon forms and respiration. Absorbance of HS in UV-VIS spectral range (variant D2 = 158 t.ha-1) was comparable with variant D1 = 93 t.ha-1.

Fig. 3 Total organic carbon content and fractional composition of HS in *Haplic Cambisol* (Náměšť)

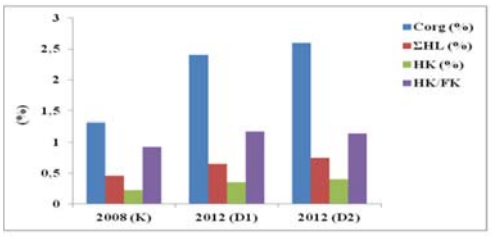
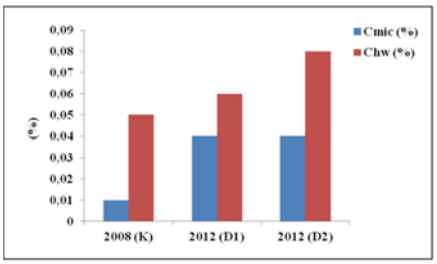


Fig. 4 Microbial carbon and water extractable carbon content in *Haplic Cambisol* (Náměšť)



Conclusion

High dozens of compost (D2 = 158 t.ha-1) were not used effectively. Increasing of water extractable carbon and microbial biomass was determined.

Formation of stabile humic substances (HA, FA) was lower. Our results showed that the carbon sequestration is directly influenced by intensive cultivation.

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Monitoring of the initial succession of zooplankton communities in newly created ponds within the Territorial System of Ecological Stability

LENKA HADASOVA, RADOVAN KOPP
Department of Zoology, Fisheries, Hydrobiology and Apiculture
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

Lenka.hadasova@mendelu.cz

Abstract: The ponds represent the interesting environment suitable for studying ecological relationships among particular compounds of freshwater habitats thanks to their easily defined borders and relatively simple food-webs structure. Zooplankton communities as representatives of freshwater organisms can provide an excellent model for assessing the success of restoration or colonization of newly created ponds in cultural environment. Within one year, initial colonization by zooplankton of two newly built ponds in TSES was monitored. Sampling was realized monthly from September 2013 to September 2014. Zooplankton identification and enumeration was carried out. Very first colonists of ponds were rotifers which occurred immediately after flooding in relatively high abundances, followed by cladocerans. During the first growing season rotifers were most abundant group of zooplankton at all. Copepods came as the last ones. In many samples predominated big species of cladocerans in both ponds. 11 taxa of rotifers, 6 taxa of cladocerans and 9 taxa of copepods were identified. The biggest impact on zooplankton communities in the first year of succession had the size of the pond, the presence of predators and the weather conditions.

Key Words: cladocerans, colonization, copepods, restoration, rotifers

Introduction

Freshwater biodiversity has declined in recent decades faster than the terrestrial or marine biodiversity. Increasing demands on freshwater resources, climate change and the impact of alien species will probably lead to continuing loss of biodiversity in the future [1]. Creation of new freshwater ecosystems or restoration of damaged ones are now common measures for conservation of aquatic biodiversity [2]. Ecological survey of ponds and of other small lentic ecosystems came in awareness in last 20 years and the attention is still rising. In spite the present trend of creating new ponds in cultural landscape, their ecological role, importance for evolutionary biology and biodiversity protection come rarely fully to realize [3]. In the past times these habitats became relatively rare due the human intervention into water regime of landscape [4]. In spite their small size and irregular water regime, ponds substantially increase biodiversity in the area and can provide suitable habitat even for very uncommon species [5]. Not only for that The Ramsar Convention on Wetlands was found to set the rules for preservation and wise using of all of the wetlands types all over the world (The Convention on Wetlands, Ramsar, Iran, 1971). The colonization of newly created ponds is realized very quickly [2] and the structure of population is changing due the water regime and the level of succession [6]. Usually, zooplankton belongs to the first colonists of new niche [7]. Its role is mainly in supporting the self-cleaning ability of water and as one of the first levels in food webs they serve also as the food for predators [8]. The abundance and species composition of zooplankton depend on many factors of the environment which are the subjects of the studies and complex monitoring of stated biotope. Even more, based on the zooplankton species composition the whole condition of ecosystem and its potential development can be evaluated [9]. In this project, the initial colonization and subsequent succession of cladocerans, copepods and rotifers, as main representatives of freshwater zooplankton in newly built ponds was monitored.

Material and Methods

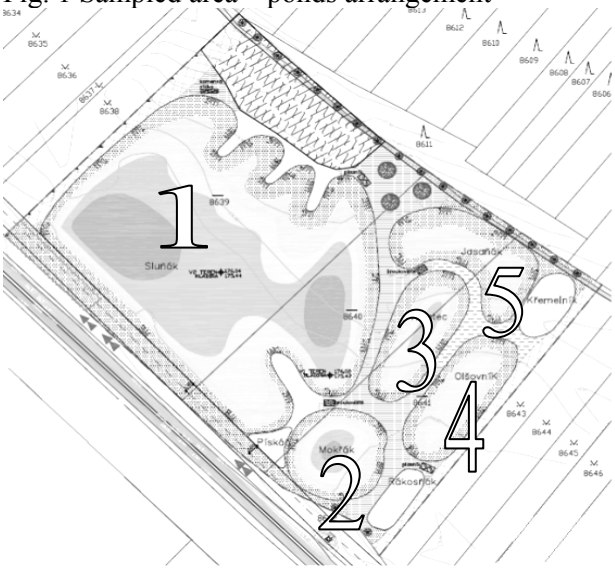
Characterization of studied site

In 2013 building plans of creating new ponds with tree planting were realized in the area near Šardice village. These lands were registered as meadows and pastures and also as the Territorial System of Ecological Stability (TSES), recently used as agriculturally used land.

The building of ponds extended the regional biocentre 12 "Díly za rybníkem" for non-forest parts in intervale of Šardický brook and connected a local and regional TSES localities bounded to local biocorridor "Šardický potok". The purpose of the construction was to increase the ecological stability of the land area and to create suitable conditions for array of species which vanished due the intensification of land use in this area.

Two newly built ponds are compared within this project. Pond no. 1 (P1) with the area of 4796 m^2 and maximum depth 2m, with indented shores and both slow and steep slopping. One side was planted by reed. Pond no. 4 (P4) with the area of 421 m^2 and maximum depth 1m, with regular shape and slow slopping of all shores. Both ponds were partially overgrown by submerse vegetation in growing season 2014. Both are flooded by groundwater and precipitations only.

Fig. 1 Sampled area – ponds arrangement



Sampling

Sampling was carried out monthly from September 2013 to September 2014, starting immediately after flooding. Always at the same time at 8 AM. Together with zooplankton samples also phytoplankton, zoobenthos and samples for water analysis were taken. Basic physicochemical parameters (temperature, dissolved oxygen, pH, conductivity, transparency) of water were measured on site. Also the actual weather conditions were noted.

Zooplankton samples for identification were taken by throwing planktonic net with mesh size 40 μm. All of the habitat types of pond were sampled to cover full spectrum of present species. Samples for enumeration were taken by planktonic tube and filtered through the 40 μm mesh. In total 15 L of water was filtered, taken from various parts of pond to get homogenous sample from the whole pond. All samples were fixed by 4% formaldehyde solution on site and transported into the hydrobiological laboratory of the department of zoology, fisheries, hydrobiology and apiculture where were subsequently processed

Results

Zooplankton colonization

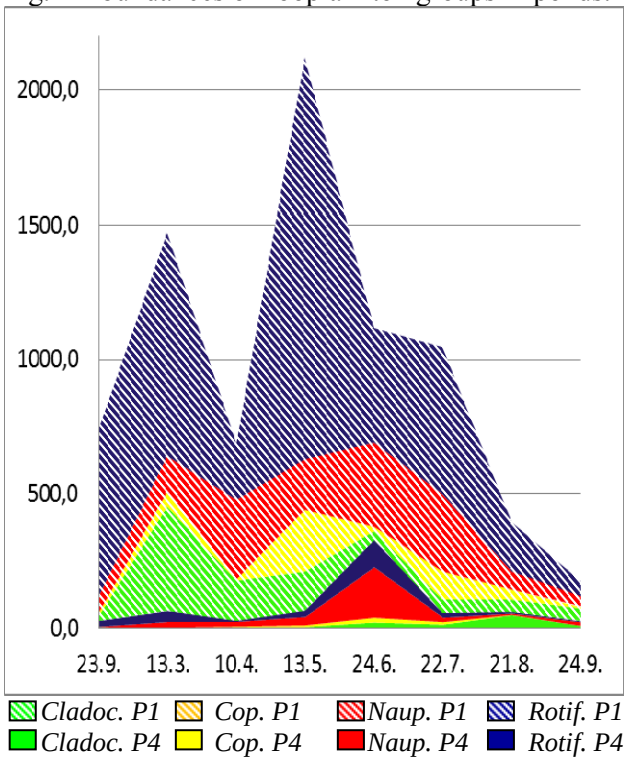
In September 2013, the pond P1 was immediately colonized after flooding, mainly by rotifers and a few cladocerans and copepods. Also some nauplium stages of copepods were present. In the first spring sample rotifers predominated again, but cladocerans showed considerable growth. From the very beginning, big individuals were present. Most of the copepods were in the nauplium stage. In April, rapid decrease of abundances of all three groups occurred. Big individuals of cladocerans were predominant anyway, most of the copepods were still in the naplium stage. In May, maximum increase of all three groups occurred. Big cladocerans and copepods species were present. Permanent eggs (ephipia) of Daphnia species started to occur and were present till the end of the monitoring. In June and July, rotifers were predominant, copepods were present in all stages (nauplium, copepodit, adult) but mostly as nauplii. Bigger species had higher abundances than smaller. Cladocerans were present in various sizes (0.5 – 4 mm) equally. At the end of the monitoring, number of rotifers and nauplii decreased, big individuals of cladocerans became predominant.

Pond P4 was in the September 2013 immediately colonized by rotifers. Cladocerans and copepods were present as well, but most of the copepods were in the nauplium stage. In March 2014, rotifers were significantly predominated again, but cladocerans showed considerable growth as well. From the very beginning, big individuals were present. Most of the copepods were in the nauplium stage again. From April to May, continual growth of all three groups was noted. Most of the copepods were in nauplium

stage. Big cladocerans became predominant. In June, rapid increase of all groups occurred. Big individuals of cladocerans and copepods were predominant, but most of the copepods were in nauplium stage. Cladocerans were present in various sizes (0.5 – 4 mm) In July, the abundances of rotifers and nauplium stages of copepods rapidly decreased, big cladocerans were predominant. In September, abundances of all groups decreased, big cladocerans were predominant.

Comparison of abundances of zooplankton in both monitored ponds is depicted in Fig. 2.
Besides the three main zooplanktonic groups were also present representatives of Ostracods and *Chaoborus sp*. Due they rare occurrence and mainly benthic lifestyle, Ostracods were not included into evaluation. The presence of *Chaoborus* is discussed in chapter Discussion.

Fig. 2 Abundances of zooplankton groups in ponds.



Zooplankton species

Within the one year sampling 11 taxa of rotifers, 6 taxa of cladocerans and 9 taxa of copepods were identified in both ponds (Tab. 1). Due the fixation by formaldehyde, it was not possible to identify Bdelloidea into lower taxon.

Table 1 List of taxa found in new ponds P1 and P4.

Cladocerans	Copepods	Rotifers
Alona sp.	Calanoida copepodids	*Ascomorpha sp.*
Bosmina longirostris	Calanoida nauplii	*Asplanchna priodonta*
Chidorus sphaericus	Cyclopoida copepodids	*Asplanchna sieboldi*
Daphnia galeata x cucullata	Cyclopoida nauplii	Bdelloidea undet.
Daphnia magna	Cyclopoida undet.	*Brachionus caliciflorus*
Scapholeberis sp.	*Eudiaptomus gracilis*	*Brachionus budapestinensis*
	Macrocyclops albidus	*Hexarthra mira*
	Microcyclops bicolor	*Keratella cochlearis*
	Thermocyclops sp.	*Keratella quadrata*
		Lecane luna
		Polyarthra dolichoptera

Discussion

Ponds generaly represent the interesting environment suitable for studying ecological relationships among particular compounds of freshwater habitats thanks to their easily defined borders and relatively simple food-webs structure [10]. Zooplankton communities as representatives of freshwater organisms can provide an excellent model for assessing the success of restoration or colonization of newly created ponds in cultural environment [11]. Such habitats are quickly colonized by organisms with high dispersion ability, as adult individuals or as other resistant stage. Thanks to their adaptation, dormant stages of water invertebrates are very suitable for dispersion [12].
Similar colonization trend of newly built ponds, first fast increase of rotifers immediately after flooding, is presented by a few authors [7, 8]. Similar colonization trend of newly built ponds, first fast increase of rotifers immediately after flooding, is presented by a few authors. On the contrary, initial colonization mainly by cladocerans was presented by Vondrák [13] and Louette and De Meester [14,¨15]. I suspect that in our case were rotifers first because of initial flooding the ponds in very early autumn so the cladocerans community couldn´t

growth enough. Presence of big individauls, namely *Daphnia magna* and *Eudiaptomus gracilis* or *Macrocyclops albidus* during the whole period can be explained by no presence of predators such a fish in the whole pond system [16]. There was the catch by electric aggregate carried out in the locality with no proving of presence of any fish species. The question is how long will last such a condition? On the contrary, the presence of predator glassworm Chaoborus sp. which was present regularly is contradictory. E.g. Mackay et al. [17] describe the strong influence of predatory pressure of glassworm on zooplankton, but it´s abundance in our ponds did not apparently correlate with fluctuation of other groups. Anyway, it would be worthy to test this influence in further studies. Important factor influencing the hierarchy of colonization is, based on our data, the size of the pond. Pond P1 is approximately ten times bigger than pond P4, which provided most likely more space for all of the main zooplankton groups and provided more variable habitats and shelters, so the growth of community was more rapid than in much smaller pond. This finding supports also Frisch et al. [18]. Despite the other factors, the biggest fluctuation in relatively fluent course of colonization is caused by weather conditions. As Jenkins and Underwood [19] present, duet he small body size and relativelly low body density, zooplankton is vulnerable to windy or rainy weather as can be seen in the sample from April. This influence is even stronger if the area of pond is larger.

Conclusion

Within one year, an initial colonization by zooplankton of two newly built ponds in TSES was monitored. Sampling was realized monthly from September 2013 to September 2014. Zooplankton identification and enumeration was carried out. Very first colonists of ponds were rotifers which occurred immediately after flooding in relatively high abundances, followed by cladocerans. During the first growing season rotifers were most abundant group of zooplankton at all. Copepods came as the last ones. In many samples predominated big species of cladocerans in both ponds. 11 taxa of rotifers, 6 taxa of cladocerans and 9 taxa of copepods were identified. The biggest impact on zooplankton communities in the first year of succession had the size of the pond, the presence of predators and the weather conditions.

Acknowledgement

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The detailed characterization of land threatened by water erosion with soil type chernozem on loess at Dambořice

ANNA HAMMEROVA, JIRI JANDAK, BEATA HRABOVSKA, JANA SIMECKOVA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

anna.hammerova@mendelu.cz

Abstract: In the Czech Republic chernozems are among the most fertile soils and are intensively farmed. It turns out that when using intensification measures, enlarging fields, using substandard farming practices and cultivating wide-row crops, water erosion becomes a major problem. The result is degradation of physical properties, compaction and depletion of the most fertile part of the soil - topsoil.
For wider pedological characteristics a plot with chernozem on loess affected by water erosion has been selected. Three probes were dug and described at three places of the slope and physical characteristics of topsoil and subsoil were conducted along with grain size analysis with the determination of carbonates in the topsoil, subsoil and in the loess cover to the depth of one meter.
Physical analysis showed that the eroded slope suffers a serious problem of soil compaction. Porosity in the topsoil and subsoil is around the critical value of 45% or below. Minimum air capacity indicates critical condition of most horizons. On average, the topsoil and subsoil classifies as loamy soil type. The grain size is dusty loam. Loess exhibits relatively high homogenity which does not dramatically change with depth, not even on the plots with the risk of land erosion. The highest carbonate content in the topsoil and subsoil is on the hillside, where occures mixing of the loess to the topsoil horizon. In the loess, the contents of CaCO3 are highly variable. Mean values range from 13.2 to 17.9% of the carbonates with a standard deviation of 2.99.

Key-Words: chernozem, loess, water erosion

Introduction

For a long time farmers are troubled by water erosion and soil degradation, which often occurs on the most fertile soils due to intensification of agriculture, the use of substandard farming practices and cultivation of wide-crops in places where there is a high risk of erosion.

The method of farming is one of the factors that create the conditions for further development of soil processes. According to [1] it has been shown that the intensification measures were not sufficiently sophisticated and compensated, so their side effects on soil created new problems related to the degradation of formerly fertile soils. This leads to degradation of soil properties, compaction, accelerated erosion, etc.

The occurrence of water erosion in the landscape is a destabilizing element that damages the soil and water, which are the two most valuable components of the natural environment. According to [2] there is about 50% of arable land in the Czech Republic which is threatened by water erosion. On the vast majority of the endangered area no measures that would prevent loss of the soil are implemented.

From the perspective of pedology, the soils on loess belong to the most fertile in the Czech Republic. Chernozems were formed under steppe and forest steppe vegetation on mostly forest-free areas. Soils that formed in loess cover, were quickly adopted by man who, by his activities, prevented the forest to expand to the areas that were used by him.

Loesses as a eolian sediments have characteristic grain composition. Grain composition is one of the properties by which we can sort loess covers and describe them. As the rocks they are composed of three main parts: the clay particles, dust particles and calcium carbonate [3].

Another characteristic, that is considered to be one of the basic distinctive ones for loess, is content of CaCO3, that is, according to [3] supposed to be 10% or more. Along with typical structural characteristics of true loesses (porosity, non-stratification, vertical cleavage)

Carbonate compounds occure in the loess in various forms. Fine dispersed carbonates usually

bind mineral grains. The most common are secondary precipitated carbonates, which occur in the form of e.g. pseudomycelium, coatings on seperable surfaces or in the form of concretions [4].

Material and Methods

For a detailed pedological characteristics we have selected a plot close to Dambořice with soil type of chernozem on loess.

Three soil probes were dug, described and sampled - on the top of the hill (A), its slope (B) and foot (C). To determine the physical properties, undamaged soil samples were taken using Kopecky cillinders from the of topsoil (currently usually from depth of 0-0.2 m) and subsoil.

For grain size distribution and the determination of content of carbonates, damaged samples were taken from topsoil, subsoil and from loess, where the samples were taken using a hollow drill with Edelman head from the depth of 0-0.1 m, 0.1-0.2 m and 0.2 m to 1 m of loess layers.

Sampling was performed according to [5]. Samples were collected according to the methodology for determining the physical properties and characteristics of water and air soil regimes. According to the methodology by [6] density, minimum air volume weight capacity and porosity were established.

To determine the grain size the sedimentation, pipetting techniques were used [7].

Determination of $CaCO_3$ content was carried out according to the methodology [5], by the Janko's lime meter.

Results and Discussion

Description of soil profiles

In order to select the location, at least partly preserved original profile was important. The profile at the top of the hill thus had to meet the requirements of the chernozem soil type, ie. more than 30 cm Ac horizon. On the slopes of the hill there were some eroded areas, where occures mixing of topsoil with shallowly deposited loess material. These cases are classified as carbonate regosoil. At the foot of the hill there is colluvium which stores erosion silt from the higher elevations of the hill. Soil type of these positions is colluvial soil modal.

Top of the hill (A)

According to the structure we can specify a segment of the original topsoil horizon (0-0.3 m), which is currently only subject to disking to a depth of 0-0.2 m. In the disked horizon, the structure is crumbled and weakly developed. In the former topsoil horizon, now at a depth of 0.2-0.3 m, the structure is polyhedral. We can find a medium recovery and weak rooting. At a depth of 0.3-0.4 m is a horizon Ac, which differs from the above-lying by a strongly polyhedral structure and a weaker rooting. The transitional horizon is at a depth of 0.4-0.47 m with a polyhedral structure and from 0.47 m there is loess with ocher color. It is dry, hard, without a clear structure with the appearance of pseudomycelium.

Hillside (B)

In the middle of the slope it was already evident according to the color that the surfaces were and currently still are experiencing water erosion, where the original Ac horizon is taken away and its remnants are gradually mixed with loess. Also here the Ap horizon is divided into two layers, while up to 0.13 m the structure is moderately developed, crumbled and medium rooted and from 0.13 to 0.27 m the structure is then developed, polyhedral with weak rooting. Throughout the horizon, there are loess nodules. After a sharp transition caused by plowing there is the mother substrate in our case loess Ck. Abundant occurrence of loess nodules decreases with depth.

Foot (C)

In locations under the hillside alluvial material gets accumulated, that buries the original humus horizon. Ap horizon extends to a depth of 0.34 m. To 0.13 m, the structure is crumbled and lumpy, moderately or well developed. In 0.13-0.34 m the structure is lumpy to polyhedral, moderately developed. Infrequent occurrence of loess nodules. Biological activity and rooting – moderate to 13 cm and weak from 0.13 to 0.34 m. Below that is the horizon AZx - formed by accumulative deposition of materials of humic horizons of colluvial soils that extends to a depth of 0.73 m. The horizon has a weakly to moderately developed checked structure with weak rooting and biological activity. Transition to loess gradual in 0.73 m. In the depth of 0.73-0.93 m we can find original Ac horizon with crumbled structure and medium biological activity. The transition to the loess is slow.

In all three probes the soil content of carbonates was in the whole depth of the profile.

Fig. 1 Top of the hill (A)

Fig. 2 Hillside (B)

Fig. 3 Foot (C)

Physical properties

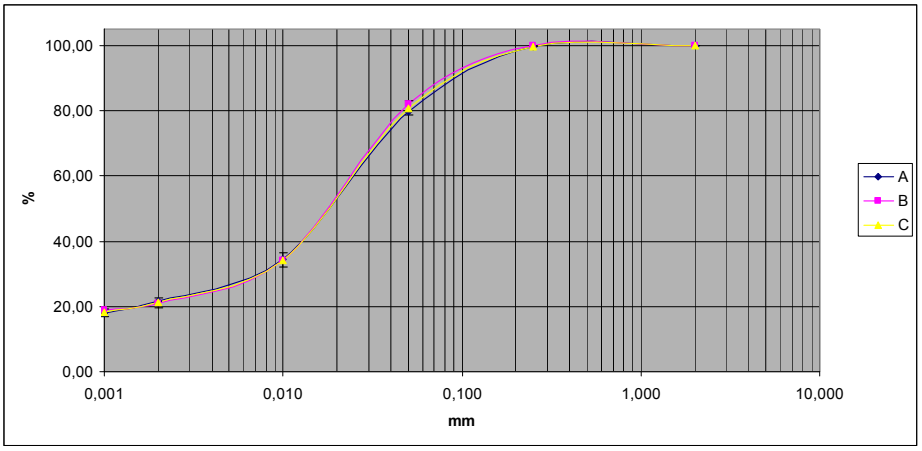
The critical value of porosity is 45%. The topsoil in position B and subsoil in all slope positions are below this value. Density indicates looseness or compactness of the soil. The measured values ranged from 1.23 to 1.6 g.cm-3 and correspond to the detected values of porosity.

According to [8] when the minimum air capacity is less than 10% the subsoil or topsoil is in critical condition. The minimum air capacity is below the critical value of 10% in the topsoil at the upper and lower position, on the slopes it reaches 17.82%. Subsoil is below the critical value at all positions.

Grain size analysis

Topsoil and subsoil on the plots contains 33-38% percent of the clay particles. According to Novak's grain size classification, it is therefore classified as moderately heavy, loamy soil.

Fig. 4 Grain curve of loess in the depth of 0-1 m of the slope in positions A, B and C



Variation range of the dust fraction content (from 0.05 to 0.01 mm) of all samples of loess ranges from 40.88 to 53.34% with the average value of 46.58 ± 0.76%. According to [3] central europian loesses contain 45-60% of dust particles.

When averaging the values of loess grain size of individual positions, the grain size curve has very similar shape in all three positions. Granulometric curves are of convex-concave shape corresponding to the loamy soil. The standard deviation is the lowest in grain size of 0.25-2 mm and it is 0.05-0.09. It is highest in the content of dust particles - its value ranges from 0.57 to 4.0 %.

Topsoil, subsoil and loess of all the plots belong, according to the triangular diagram, to the dusty loam grain size class.

Carbonates

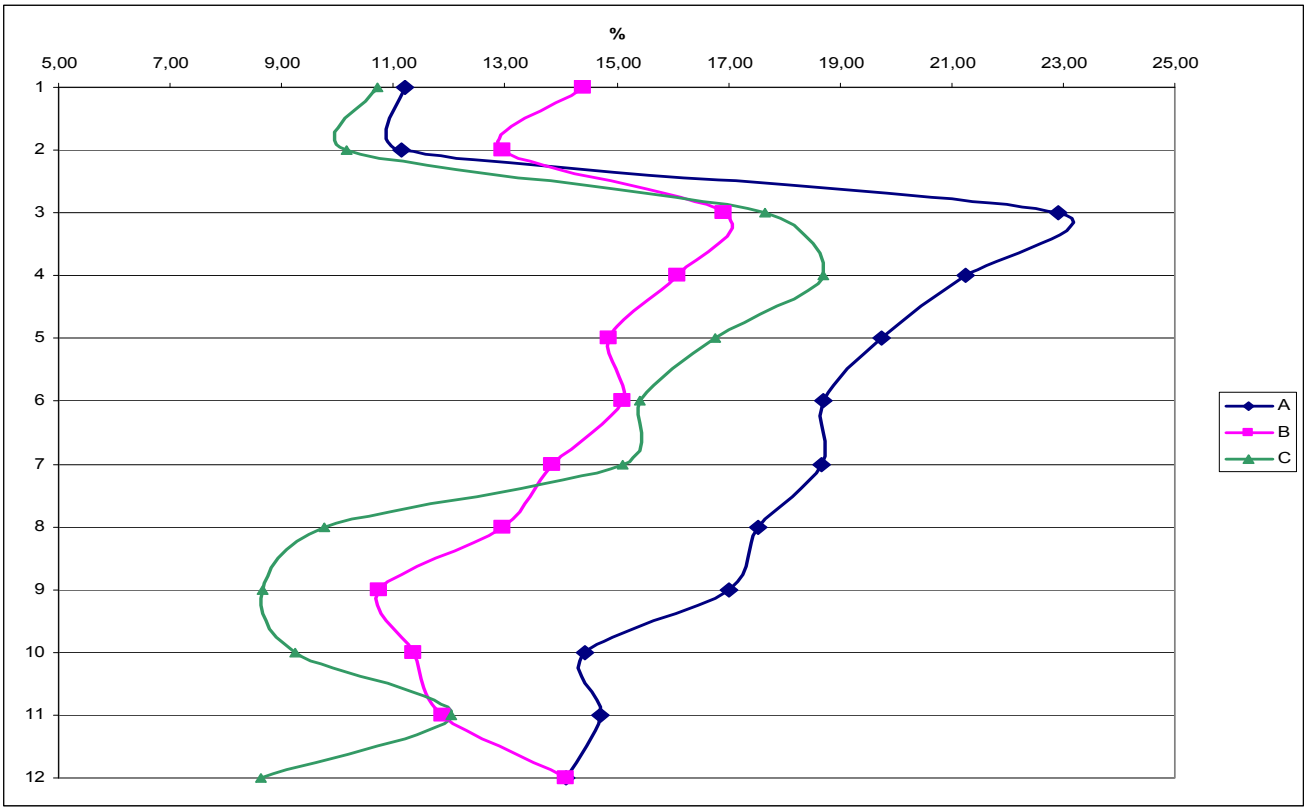
According to [4], certain amount of caution is necessary in the evaluation of carbonates, as their contents may be quite variable. The hallmark of values is considerable variance caused by the occurrence of specific forms of carbonates in loesses.

Carbonate content on top of the hill in the topsoil and subsoil are around 11% in the loess is the average content of 17.9 ± 0.94%, with a minimum of 14.08% and a maximum of 22.9%.

On the slopes of the topsoil it is 14.4% and 12.9% in the subsoil. Average value of loess is 13.79 ± 0.65%, with a minimum of 10.7% and a maximum of 16.9%.

At the foot of the slope the value of topsoil and subsoil is slightly over 10%. In the loess, the carbonate content is similar to the value on the slope with the average of 13.2 ± 1.26 with a minimum of 8.6% and a maximum of 18.7%.

Fig. 5 Carbonate content in the profiles of individual positions of the slope



Axis Y; 1 Topsoil, 2 Subsoil, 3 Loess 0-0.1 cm, 4 Loess 0.1-0.2 m, 5 Loess 0.2-0.3 m, 6 Loess 0.3-0.4 m, 7 Loess 0.4-0.5 m, 8 Loess 0.5-0.6 m, 9 Loess 0.6-0.7 m, 10 Loess 0.7-0.8 m, 11 Loess 0.8-0.9 m, 12 Loess 0.9-1 m

Conclusion

For pedological characterization we selected a plot with soil type chernozem on loess, which is threatened by water erosion and which is located in the cadastral Dambořice. The effect of water erosion was evident at the first sight, by the soil moving down the slope to its foot. At the slope of the hill the soil gets mixed with loess and is gradually fertilized. Physical analysis showed that the eroded slope suffers a serious problem of soil compaction. Porosity in the topsoil and subsoil is around the critical value or below, and in the subsoil it is below the critical value. Minimum air capacity indicates the critical condition of most horizons.

On average, the topsoil, subsoil and loess of all the positions classifies as loamy soil type. Regarding the grain size it is dusty loam. As for the loess, the particle analysis showed that it exhibits relatively high homogeneity and it does not dramatically change with depth, not even on the plots with the risk of land erosion. The content of

each fraction varies always in the span of a few percent.

The highest carbonate content in the topsoil and subsoil is on a hillside, where there occures mixing of the loess to the topsoil horizon. In the loess, the contents of CaCO3 are highly variable. Mean values range from 13.2 to 17.9% of the carbonates with a standard deviation of 2.99.

Acknowledgement

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Differences in spider species diversity on grapevine plants on terraced and plain vineyards depending on the type of management

LUCIE HAVLOVA[1], VLADIMIR HULA[1], JANA NIEDOBOVA[1, 2], RADEK MICHALKO[3]
[1]Department of Zoology, Fisheries, Hydrobiology and Apiculture
Faculty of Agriculture
[2]Department of Forest Protection and Wildlife Management
Faculty of Forestry and Wood Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[3]Department of Botany and Zoology
Faculty of Science
Masaryk University, Brno
Kotlarska 2, 611 37 Brno
CZECH REPUBLIC

lucie.havlova@mendelu.cz

Abstract: In recent years, agricultural ecosystems are considered as very problematic habitats with respect to biodiversity, and this trend is continuously decreasing, however, some agroecosystems, especially some types of vineyard, can host remarkable species and contain a high diversity of invertebrate fauna, comparable with areas under nature protection. The aim of this study was to focus on two types of vineyards: terraced and non-terraced (plain) vineyards, and investigated of araneofauna living on plants of vine grapes. The study analyses spider species spectrum and spider abundance on the grape vine plants. The spectrum of spiders found is relatively poor; the most common spiders were: *Synageles venator* (Lucas, 1836), *Salticus zebraneus* (C. L. Koch, 1837), *Nuctenea umbratica* (Clerck, 1757), *Dictyna uncinata* (Thorell, 1856), *Dictyna arundinacea* (Linnaeus, 1758), *Micaria subopaca* (Westring, 1861), *Marpissa nivoyi* (Lucas, 1846), *Agalenaetea redii* (Scopoli, 1763), but we were not able to identify juveniles up to the specific level (710 specimens). It can be assumed, that some of the observed species and their presence can affect the incidence of grapevine pests significantly. Several interesting species observation were also made (e.g. common occurrence of *Synageles venator*).

Key-Words: biodiversity, araneae, vineyard

Introduction

Impact of landscape heterogenity on different animal groups is well studied topic. Landscape heterogenity is widely discussed issue, because the homogenization is one of the greatest threats to biodiversity [1]. It is known, that sites surrounded by heterogeneous landscapes contained more species than homogenous ones [2]. It is also known, that the recession in farmland biodiversity is related to changing farming practices. Agricultural intensification has been a main cause of farmland biodiversity losses and it has led to a wide degradation in farmland biodiversity [3]. There are few interesting studies which describe influence of landscape diversity and agricultural practices on spiders [4, 5, 6]. We assume that terraced vineyards host richer fauna [7], because it's more heterogeneous and contains not only vineyards itself, but also frontal part of terrace.

From a certain point of view we can draw some conclusions concerning the potential use of spiders as bioindicators [8, 9].

The aim of this study is to focus on two types of vineyards: terraced and non-terraced (plain) vineyards, and concentrate on their araneofauna. Our research was to find out whether vine plant spider fauna on terraced vineyards is richer than vine plant spider fauna in plain areas. So far we only have data about overwintered and early spring spiders.

Material and Methods

Collection and evaluation of materials

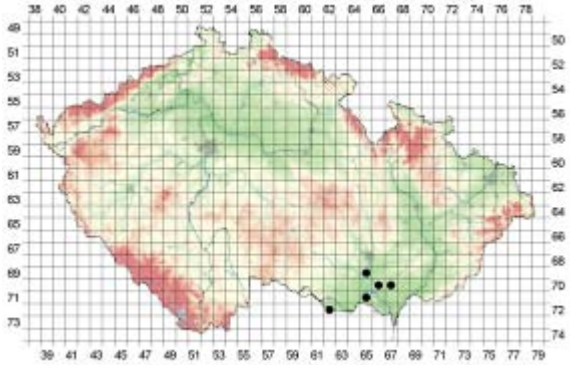
We investigated araneofauna of vine plants on terraced and non-terraced areas across South Moravian region (Czech Republic) by using of cap-board traps. These traps were devised and tested in Torino first in research which purpose was to examine their efficiency. The results of this study shows, that bubble wrap is more effective for trapping bigger spiders and cardboard itself is great for smaller species [10]. We had combined these materials to overtake the widest possible range of spiders captured. Altogether we investigated six locations (240 traps per variant).

Traps were placed and collected monthly. We began at autumn 2013, and traps were collected since the early spring of 2014. On each location two representative sites were selected: one on the terraced vineyard and the other on the non-terraced (plain) vineyard. We placed 20 cardboard traps on the edge of vineyard and in the central part (40 traps per location). Each trap was collected separately into a plastic bag and replaced immediately by a new one (see fig. 4, 5). Material from traps was collected in laboratory conditions. All individuals were determined to species (adults) or families (juveniles). For estimation of species richness we used number of families and number of species.

Localization of studied sites

The study area falls within the South Moravian Region: districts of Znojmo and Břeclav. Particular study sites were: Peklo, Výhon, Morkůvky, Růžový vrch, Gotberg and Nosislav (see fig. 1, 2, 3).

Fig. 1 Localization of study sites



Statistical analyses

The statistical analyses were performed within R environment [11]. We used species density (species per plot, see [12]), spider abundances to compare studied plots. We studied species diversity and abundances in two vineyard types (terraced x plain). For estimation we used Generalized Estimating Equations (GEE) with Poisson error structure (GEE-p) with log link function and "ar1" correlation structure [13, 14].

Fig. 2-7 Examined vineyard terraces

Legend: non-terraced vineyard at Šatov (Znojmo district) (2), terraced vineyard at Blučina (Brno-venkov district) (3), experimental plot on Nosislav (Brno-venkov district) (4), collected cardboard traps (5), Synageles venator (6), Pseudicius encarpatus (7)

Results

Until now we only calculated data from the winter and early spring time (February to April).

The spectrum of spiders was relatively poor; the most common spiders were: *Synageles venator* (Lucas, 1836), *Salticus zebraneus* (C. L. Koch,

1837), *Nuctenea umbratica* (Clerck, 1757), *Dictyna uncinata* (Thorell, 1856), *Dictyna arundinacea* (Linnaeus, 1758), *Micaria subopaca* (Westring, 1861), *Marpissa nivoyi* (Lucas, 1846), *Agalenaetea redii* (Scopoli, 1763), but we were not able to identify juveniles up to the specific level (710 specimens). We found out, that the most common spider there is salticid *Synageles venator*, which usually is not found as such common species. The species did not overwinter on vine plants, but rather occurred there as common during first spring month (1 ex during overwintering, 141 during March 2014). Most of overwintering specimens were not adults (only one *Synageles venator* and *Nuctenea umbratica* was found), the spectrum was dominated by juveniles of genus Philodromus and Salticidae (mainly *Pseudicius encarpatus* (Walckenaer, 1802), *Macaroeris nidicolens* (Walckenaer, 1802) and Salticus spp.). There are big differences between sites, but no significant differences between both types.

Species composition

During the winter time, we found only adults of *Synageles venator* (Lucas, 1836) and *Nuctenea umbratica* (Clerck, 1757). The first species became dominant during spring time in really high abundances (see fig. 9). Other species came later in the season (and they were not previously recorded as juveniles).

Impact of habitat structure

The species composition and total abundance differed significantly between terraced and plain vineyards. In terms of abundance, the only significant results were for adults only (GEE-p, X21 = 56.3, $P < 0.0001$) in case of total abundance for adults (GEE-p, X21 = 26.9, $P < 0.0001$) as well as juveniles (GEE-p, X21 = 9.2, $P = 0.0025$).

Faunistics

Nomenclature and arrangement of families, genera and species follow the most recent version of the World Spider Catalog 15.0 [15]. A total of 285 adult spiders representing four families, and 294 juveniles of 10 families were collected and identified. Considering adults, the largest portion of individuals belonged to the family Salticidae (263 adults) and Dictynidae (14 adults). An interesting finding was the high presence of spider *Synageles venator* (Lucas, 1836) (fig. 6 and 8). It was the dominant species at all studied locations during both spring months.

From the point of view of presence of juveniles, Salticidae was also the richest family (115 juveniles), but there were significant numbers of Philodromidae (96 individuals, see fig. 8) and Dictynidae (34 individuals) as well (fig. 8). Also, juveniles of other families were demonstrably more represented than adult ones. This indicates that species richness will increase in subsequent months.

Discussion

This study analysed the species spectrum and abundance of spiders living on vine plants. We support the hypothesis about importance of vineyard terraces as a source of biodiversity [4], but also we found out that an interesting spider fauna lives there.

In comparison, there were found same several common species as on faces of vineyard terraces published by Kosulic in 2014 (fig. 11), like *Dictyna arundinacea* (Linnaeus, 1758), *Drassodes lapidosus* (Walckenaer, 1802), *Heliophanus auratus* (C. L. Koch, 1835), *Marpissa nivoyi* (Lucas, 1846), *Salticus scenicus* (Clerck, 1757) and *Synageles venator* (Lucas, 1836) [16]. There were found no species listed as critically endangered, endangered and vulnerable spider species according the Red List of threatened species in the Czech Republic [17].

It is understandable that the first spring months, as mentioned above, rapidly increased abundance of some species of spiders, especially to the individuals in the family Salticidae. Species such as *Synageles venator* (Lucas, 1836), *Pseudicius encarpatus* (Clerck, 1757) or *Salticus scenicus* (Clerck, 1757) - tree dwelling active hunter predators, began to be active with the spring and started invade into installed traps. Usually, glued tapes were filled up by high numbers of caught ants and springtails. It is therefore conclusive, that the first spring spiders invaded in the traps not only for the purpose of hiding, but also for prey.

One of the most interesting findings, as mentioned earlier, was the enormous presence of salticid spider *Synageles venator* (Lucas, 1836). It is question which environmental factor allowed this extension. In study by Chong Chee-Seng [18] was pointed out the significant effect of increase of spider abundance by presence of ant. In this case it was Linyphiidae spiders and these spiders were positively associated with *Lasius* ants. This finding may lead us to a possible analogy between ants found (mostly sticked on sellotape) and *Synageles venator* (Lucas, 1836). It is certain, that the interactions between ants and other arthropods are complex and variable. Because these findings are too speculative, it will be necessary to confirm it by future experiments and further researches. The roles of native ants in vineyards will certainly run out some interesting findings.

Fig. 8 Total abundances particular families of all recorded juvenile spiders

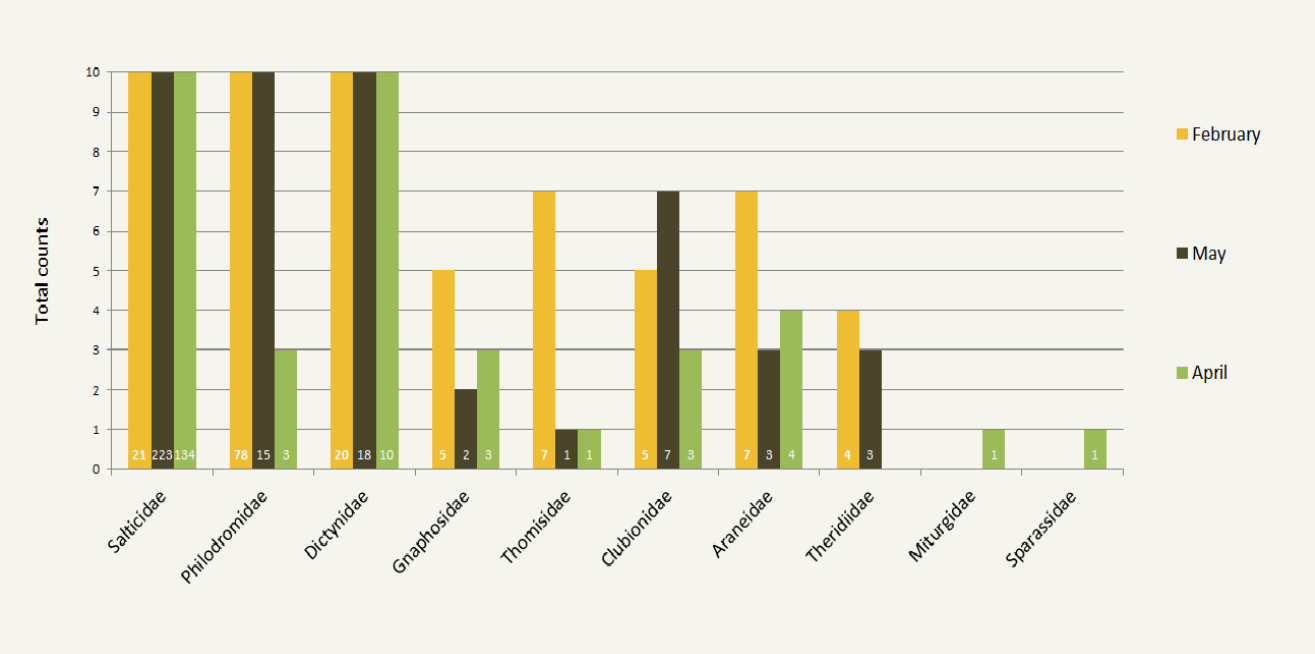
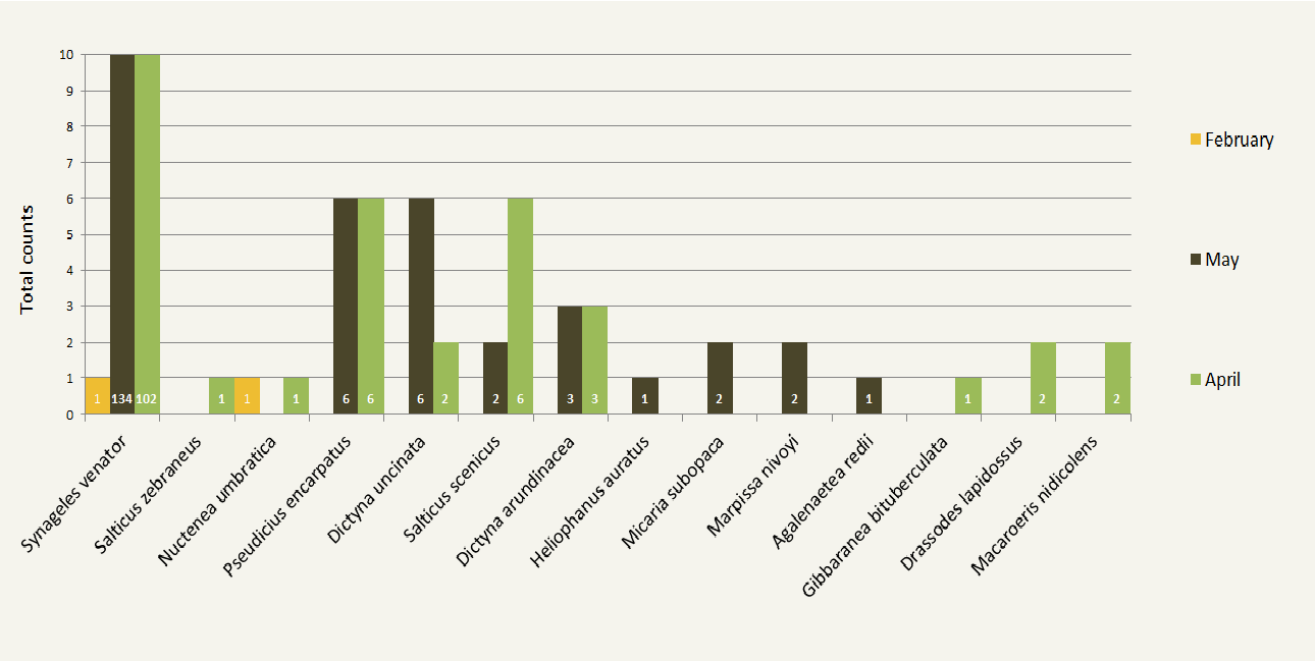


Fig. 9 Total abundances of adult specimens and grid map of the Czech Republic with the studied localities marked



Conclusion

Against the common expectations that intensive agricultural land is species poor and that particular plants did not host so many species (specimens), we found the opposite – vine plants host during winter rich and valuable fauna. The difference between different types of sites (terraced vs. plain) was found. The species spectrum contained several very interesting species (e. g. *Marpissa nivoyi* (Lucas, 1846), *Micaria subopaca* (Westring, 1861), *Pseudicius encarpatus* (Walckenaer, 1802) (fig. 7, 9) and *Synageles venator* (Lucas, 1836). Most interesting was the discovery of very common occurrence of *Synageles venator* – the widely distributed species with very low abundances, with unknown life history and with positive association

with ants. This species needs a special focus in future study.

In the future, we can think about the importance of spiders in vineyards as a bioindicators of environmental cleanliness – there raises the question "Whether and how are spiders affected by treatment on vineyards?" Given that so far there are no scientific studies on that topic, it would be interesting to see lethal and sublethal effects of pesticides, fungicides and other chemicals used in vineyards during the season.

We believe, that this research will help to define the potential of vineyard spiders and decode the questions, like biocontroling and bioindicating, which we have mentioned earlier.

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Soil aggregate stability and soil organic matter on Chernozems of South Moravia

BEATA HRABOVSKA, ANNA HAMMEROVA, JIRI JANDAK, JANA SIMECKOVA, VITEZSLAV VLCEK
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

beatahrabovska@gmail.com

Abstract: Soil organic matter positively affects the development of structural elements and therefore it is important to supply organic matter to the soil. In terms of soil protection before water erosion are important structural aggregates. Soil structure is defined as the arrangement of particles and associated pores in soils across the size.
The aim of this paper is to highlight the impact of water erosion on soil structure and soil organic matter Chernozem on loess in southern Moravia, and process the correlation between soil organic matter and macroaggregates.
Soil organic matter was determined by determining the total carbon content on the wet road method Walkley - Black. The structure was determined by a modified method according to Novák.
The stability of soil structure in the arable layer was highest at site Domanín u Bzence in the top position, which reaches 89%. The lowest coefficient stability is of site Svatobořice-Mistřín, where it reaches only 15% in the middle of the slope.
There is a positive correlation between the organic matter content of the soil and aggregation. Positive Spearman correlation relationship macroaggregates and humus content, we managed to prove only one case at the site Domanín u Bzence.

Key-words: macroaggregates, soil, Chernozem, water erosion, soil structure, soil organic matter

Introduction

Interest in the land in recent decades increased significantly, not only because of food security, but also in connection with environmental problems, and protection of human health. [1] Therefore, the function of man, in the relation to the land ceases, is not more only as the user, but increasingly, it is role as guardian, trustee, and directing the development of soils.

The soil structure is an important feature of the soil. Soil structure is defined as the mutual arrangement of the primary mineral and organic soil particles into larger formations (aggregates) of various shapes and sizes [2]. The stability of the structure of the soil must be added to this definition because the structure is not static, and changes with water content, and other agencies of stress, which may be used to the system [3]. The structure is conditional ability to associate solid phase particles or disaggregated larger units the soil matter and thus creating structural aggregates. [4] The formation of soil structure involves the physical forces of shrinking and swelling created by changes in water status of soils, freezing and thawing, tillage, or by movement of the larger biota in soils. Thus changes of structural organization are maximal in clays. The basic unit of the soil structure is soil aggregate (PED) [5, 3]. Primary peds are relatively stable aggregates that are separated from each other pores or places weakened in links of mutual. They are the simplest form of the existence of soil materials. Next, it is naturally not divided into smaller units of soil. On the contrary, they can be further grouped into larger units - aggregates higher order [5]. Most preferably the aggregates for favorable porosity are aggregates from 1 up to 10 mm [6, 7]. To soil quality are best round, crumb formation, and polyhedral structural aggregates [8]. Water-stable aggregates should be porous. The pores between the aggregates should be large enough to allow rapid infiltration [7]. In soil protection against water erosion has important role soil infiltration

properties. Rain drops fallen on the soil surface can infiltrate or runoff. Decreasing of soil infiltrability in the combination of the high intensity precipitations leads to the increasing of the surface, and run off negative phenomena as water erosion [9]. Quality of soil structure is considered beneficial earthworms [10]. Earthworms may ingest substantial quantities of soil materials which are then cast on the surface or in earthworm burrows. When earthworms are plentiful, their burrows are large enough to dominate the macroporosity in the soils [3].

Several ways to aggregate soil particles using plants and plant residues. Plant roots (particularly root hairs) and fungal hyphae grow through the crumb, wrap them, secrete polysaccharides and other organic compounds and also create a mucilaginous mass which stick together individual particles and microaggregates into larger units - macroaggregates. Annual plants have a lesser ability to penetrate into strong soil, even thought plants with tap roots have capabilities to penetrate strong layers to depth. The old root channels then become a thoroughfare for new roots [3]. It is necessary to retain crop residues in the soil. On the one hand are a valuable source of nutrients and organic matter, but also protect the soil from the effects of water erosion. In most soils organic matter is the major factor in the formation and stabilization of crumbs and globular structural aggregates. Organic matter provides energy and substrate, which used to drive bacteria, fungi and soil animals [11]. Making of structural aggregates means the protection before humus mineralization. The structural aggregates are formed by the action of the most common mineral humus substances to share with the formation of the organic-compounds of different types - primary (microaggregates) and multiple-aggregates (macroaggregates) [12]. During the aggregation process occurs for coating mineral particles of soil (clay and dust) and pieces of organic matter decomposition of plant residues [11].

Materials and Methods

In the spring of 2013, in the framework of the field survey were identified above four sloping land with arable land - modal calcareous Chernozems on loess [13] on which the erosion either directly visible or has a high probability of its occurrence.

Sampling was conducted at the end of the summer in 2013 on the slopes near the village Domanín u Bzence, Svatobořice-Mistřín, Klobouky u Brna and Stavěšice. On every slope were selected three sampling site. The first place was at the top of the slope (instead of likely preserve the original profile), the second place was in the middle of the slope (instead of the expected erosion of materials) and the third place was at the bottom of the slope (instead of the likely accumulation materials away).

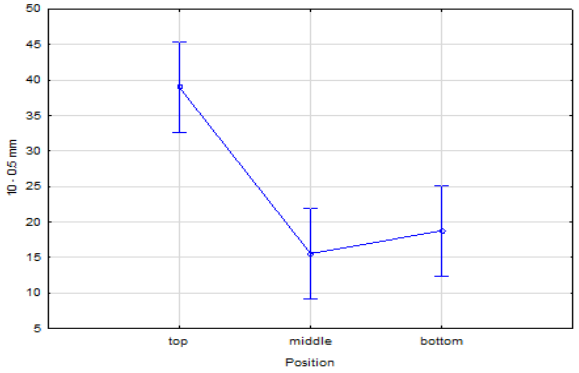
The soil organic matter was determined by the total carbon content in the wet road method Walkley – Black [14]. The soil texture was determined by the modified method of Novak [15]. Subsequently, the stability coefficient determined according Fulajtár [2].

Results and Discussion

Position in the top soil is high humus content 2.93% in the village Stavěšice and the lowest 1.90% in the village Domanín u Bzence. The average value for the selected four plots reaches 2.49%. In the middle of the slope is high humus content of 1.58% in the village Klobouky u Brna and the lowest 1.42% in the village Domanín u Bzence. The average value reaches 1.49%. At the bottom of the slope is high humus content of 2.54% in the village Svatobořice-Mistřín and the lowest 1.63% in the village Stavěšice. The average value reaches 1.91 %.

For the statistical evaluation of the content of humus was used single-factor analysis of variance (ANOVA). The statistical evaluation using Tukey's test found a statistically significant difference between the humus content in the topsoil between all of the slope at the site Domanín u Bzence.

Fig. 1 Statistical evaluation of soil aggregates has sized from 10 to 0.5 mm of arable layer in Domanín u Bzence



According to the statistical evaluation of the site Domanín u Bzence, the macroaggregates size is between 10 to 0.5 mm in the topsoil statistically demonstrable difference between the

top and middle part, and the top and bottom of the slope.

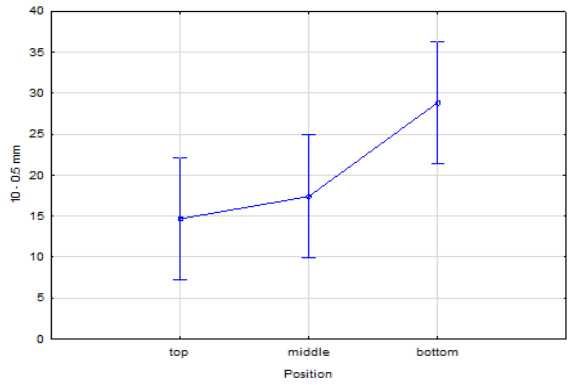
Table 1 Results of ANOVA humus content of the arable layer Domanín u Bzence

Number	Tukey´s HSD test; variable Humus			
	Position	{1} 1,8955	{2} 1,4223	{3} 1,7016
1	top		0,000227	0,000228
2	middle	0,000227		0,000227
3	bottom	0,000228	0,000227	

Table 2 Results of ANOVA soil aggregates of size 10 to 0.5 mm of arable layer Domanín u Bzence

Number	Tukey´s HSD test; variable 10 - 0,5 mm			
	Positon	{1} 38,969	{2} 15,531	{3} 18,751
1	top		0,001908	0,003867
2	middle	0,001908		0,675284
3	bottom	0,003867	0,675284	

Fig. 2 Statistical evaluation of soil aggregates has sized from 10 to 0.5 mm of arable layer in Klobouky u Brna



Location Klobouky u Brna is characterized by a high degree of erosion already in the top position.

Table 3 Results of ANOVA soil aggregates of size 10 to 0.5 mm of arable layer Klobouky u Brna

Number	Tukey´s HSD test; variable 10 - 0.5 mm			
	Position	{1} 14,672	{2} 17,402	{3} 28,846
1	top		0,809339	0,038894
2	middle	0,809339		0,084906
3	bottom	0,038894	0,084906	

Table 4 Results of ANOVA for the content of humus arable layer Klobouky u Brna

Number	Tukey´s HSD test; variable Humus			
	Position	{1} 2,4670	{2} 1,5813	{3} 1,7585
1	top		0,000227	0,000227
2	middle	0,000227		0,000228
3	bottom	0,000227	0,000228	

According to the statistical evaluation of the location Klobouky u Brna macroaggregates size is between 10 to 0.5 mm u topsoil statistically demonstrable difference between the top and bottom of the slope.

The statistical evaluation was found statistically significant difference between the humus content in the topsoil sites Klobouky u Brna between all parts of the slope.

Fig. 3 Statistical evaluation of soil aggregates has sized from 10 to 0.5 mm of arable layer in Stavěšice

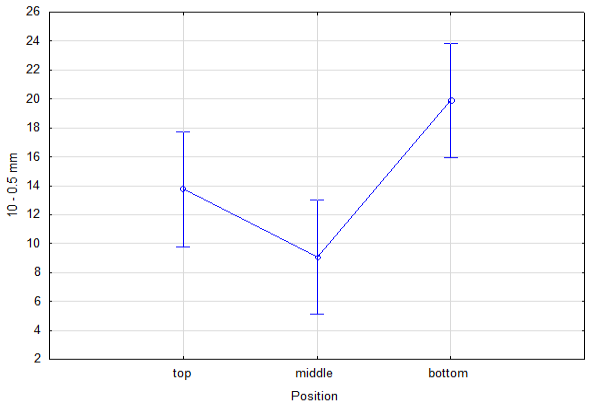


Table 5 Results of ANOVA soil aggregates of size 10 to 0.5 mm of arable layer Stavěšice

Number	Tukey´s HSD test; variable 10 - 0.5 mm			
	Position	{1} 13,754	{2} 9,0738	{3} 19,893
1	top		0,181033	0,080295
2	middle	0,181033		0,007749
3	bottom	0,080295	0,007749	

Table 6 Results of ANOVA for the content of humus arable layer Stavěšice

Number	Tukey´s HSD test; variable Humus			
	Position	{1} 2,9286	{2} 1,4326	{3} 1,6318
1	top		0,000227	0,000227
2	middle	0,000227		0,000454
3	bottom	0,000227	0,000454	

According to the statistical evaluation of the site Stavěšice macroaggregates is between size 10 to 0.5 mm of topsoil statistically demonstrable difference between the middle and the bottom of the slope.

The statistical evaluation was found statistically significant difference between the humus content in the topsoil site Stavěšice between all parts of the slope.

Fig. 4 Statistical evaluation of soil aggregates has sized from 10 to 0.5 mm of arable layer in Svatobořice–Mistřín

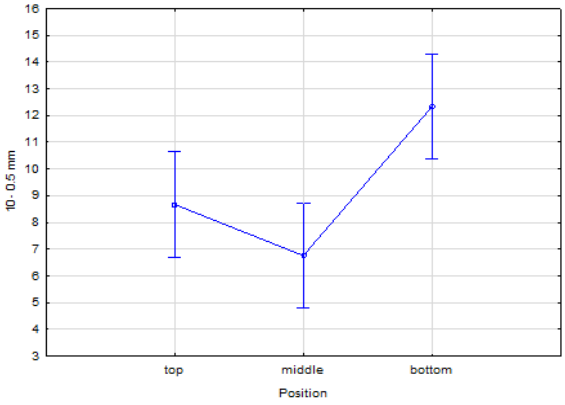


Table 7 Results of ANOVA soil aggregates of size 10 to 0.5 mm of arable layer Svatobořice–Mistřín

Number	Tukey´s HSD test; variable 10 - 0.5 mm			
	Position	{1} 8,6698	{2} 6,7567	{3} 12,322
1	top		0,284558	0,041624
2	middle	0,284558		0,006582
3	bottom	0,041624	0,006582	

Table 8 Results of ANOVA for the content of humus arable layer Svatobořice-Mistřín

Number	Tukey´s HSD test; variable Humus			
	Position	{1} 2,6830	{2} 1,5413	{3} 2,5407
1	top		0,000227	0,000784
2	middle	0,000227		0,000227
3	bottom	0,000784	0,000227	

According to the statistical evaluation of the site Svatobořice-Mistřín is between macroaggregates size from 10 to 0.5 mm of arable layer statistically demonstrable difference between the top and bottom of the slope, and between the middle and the bottom of the slope.

The statistical evaluation was found statistically significant difference between the humus content in the arable layer site Svatobořice-Mistřín between all parts of the slope.

The findings from the statistical compilation are confirmed by the determination of water resistance structure as Fulajtár [2], which provides Kv (coefficient of stability) by:

$$K_v = A/B$$

where:

- K_v is the coefficient of stability,
- A is the weight of water-resistant aggregates of sizes 10 - 0.25 mm,
- B is the mass of aggregates smaller than 0.25 mm.

The rule is that the larger the value of K_v, the lower its stable structure.

Table 9 Evaluation of the structural properties of the soil [2]

The content of aggregates of 10 mm - 0.25 mm in % (K_v)	Status structural and water-stable aggregates
≥ 70	excellent
70 – 55	good
55 – 40	satisfactory
40 – 20	unsatisfactory
≤ 20	inconvenient

Table 10 Results of the observation plots K_v

	Domanín u Bzence		Klobouky u Brna	
	K_v	v %	K_v	v %
AL A	0.89	89	0.28	28
AL B	0.39	39	0.35	35
AL C	0.33	33	0.64	64
	Stavěšice		Svatobořice-Mistřín	
	K_v	v %	K_v	v %
AL A	0.30	30	0.19	19
AL B	0.19	19	0.15	15
AL C	0.41	41	0.23	23

(Legend: K_v - coefficient stability, AL – arable layer, A top slope, B middle slope, C bottom slope)

Stability coefficient is significantly higher for sites Domanin u Bzence in the top position, which reaches 89%. By Fulajtár [2] is a very good condition. The lowest coefficient of stability is on site Svatobořice-Mistřín, where it reaches only 15% in the middle of the slope. By Fulajtár [2] the value is inconvenient.

Basic step was to determine the normality of data. For this test, we selected the Shapiro - Wilk

W test, used for the scanning of less than 50 variables (n < 50). Such tests showed that the most of the observed variables are without a normal distribution (p < 0.05). Based on this result, we chose to nonparametric statistics Spearman correlation coefficient R.

Table 11 Correlation humus content with the size of aggregates from 10 to 0.5 mm in the arable layer of site Domanín u Bzence

	Spearman correlation	
Variable	Humus	10 - 0.5 mm
Humus	1,000000	0,813676
10 - 0.5 mm	0,813676	1,000000

Table 12 Correlation humus content with the size of aggregates from 10 to 0.5 mm in the arable layer of site Klobouky u Brna

	Spearman correlation	
Variable	Humus	10 - 0.5 mm
Humus	1,000000	-0,133333
10 - 0.5 mm	-0,133333	1,000000

Tisdall and Oades [7] wrote that there is a positive correlation between the organic matter content of the soil and aggregation. Their opinion we managed to check in only one case near the village Domanín u Bzence.

Table 13 Correlation humus content with the size of aggregates from 10 to 0.5 mm in the arable layer of site Stavěšice

	Spearman correlation	
Variable	Humus	10 - 0.5 mm
Humus	1,000000	0,483333
10 - 0.5 mm	0,483333	1,000000

Table 14 Correlation humus content with the size of aggregates from 10 to 0.5 mm in the arable layer of site Svatobořice–Mistřín

	Spearman correlation	
Variable	Humus	10 - 0.5 mm
Humus	1,000000	0,483333
10 - 0.5 mm	0,483333	1,000000

Conclusion

From the attached results is possible to assess the condition of soil structure Chernozem on loess hillsides in selected localities of South Moravia is not very favorable. The statistical evaluation uses Tukey's test found a statistically significant difference between the humus content in the arable layer top, middle and bottom of the slope of the observation plots. There is a spatial variation of humus content - reduce the erosion of the slope.

The stability of soil structure in the arable layer was highest at site Domanín u Bzence in the top position, which reaches 89%. The lowest coefficient stability is of site Svatobořice-Mistřín, where it reaches only 15% in the middle of the slope. But otherwise the stability rates ranged below 40 %, which is by Fulajtár [2] unsatisfactory condition of. Arable layer is the study sites can be characterized as unstable simultaneously with high susceptibility to soil erosion.

Positive correlation relationship macroaggregates and humus content, we managed to prove only one case of site Domanín u Bzence.

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Combined effect of intercrop cultivation and method of fertilization on mineral nitrogen leaching: Lysimetric experiment

ANTONIN KINTL, JAKUB ELBL, LUKAS PLOSEK, JAROSLAV ZAHORA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

antonin.kintl@mendelu.cz

Abstract: Under the term of mixed culture or intercrop, we understand the cultivation of two different crops at same field simultaneously, in particular mixture of leguminous and non-leguminous crops. The need to cultivate these crops for improving soil fertility and sustainability is often neglected, but positive influence of legumes in crop rotation is widely recognized. Legumes have always been important crops, which have represented a source of food for humans, feed for animals and atmospheric nitrogen for soil. At present the importance of legumes is ignored, despite the fact that the cultivation of these plants brings a lot of benefits.
The aim of this study is to describe the influence of intercrop cultivation and method of fertilization on leaching of mineral nitrogen from arable soil. This effect was tested by lysimetric experiment. Six variants (C1 a C2, A1 – A4) of the experiment were prepared, each one was prepared in three repetitions. These variants were divided into two groups. First group consists of two variants: C1 (Winter Wheat - Sole crops, control without addition of fertilizers) and C2 (Winter Wheat - Sole crops, 140 kg of N per hectare and year were applied here). Second group consists of four variants A1 – A4, mixed culture of Winter Wheat (*Triticum aestivum*) and White Clover (*Trifolium repens*) were cultivated here. These variants were fertilized by mineral and organic fertilizers.
Significant differences (ANOVA; $P<0.05$) in leaching of mineral nitrogen were found between variants with intercrop (White Clover; A1-A4) and variants without (C1-C2). The significant lowest values were always found in variant A2 with intercrop. Based on these results we conclude that cultivation of intercrop/mixed culture represents new opportunity to mitigate the negative influences of extensive agriculture – leaching of mineral nitrogen.

Key-Words: winter wheat, white clover, intercrops, leaching of mineral nitrogen

Introduction

Under the term of mixed culture or intercropping we understand the cultivation of two different crops at same field simultaneously, in particular mixture of leguminous and non-leguminous crops. The need to cultivate these crops for improving soil fertility and sustainability is often neglected, but positive influence of legumes in crop rotation is widely recognized [1][2],[3]. Mixing species in cropping systems may lead to a range of benefits that are expressed on various space and time scales from a short-term increase in crop yield and quality to longer-term agroecosystem sustainability [4]. For farming systems to remain productive, it will be necessary to replenish the reserves of nutrients which are removed or lost from the soil. In the case of nitrogen (N), inputs into agricultural systems may be derived from atmospheric N_2 via biological N_2 fixation [5]. Biological nitrogen fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity [6].

Crop yield depends on ability to extract sufficient amount of nutrients (especially Nitrogen) and water from soil. Uptake of nutrients and water is dependent on the availability of nutrients in rhizosphere. Nitrogen is a key element for all living organisms because it is an essential component of proteins and nucleic acids. Although the element nitrogen is extremely abundant, making up 78 % of the Earth's atmosphere, it exists mainly as unreactive di-nitrogen (N_2). By contrast, to be useable by most plants and animals, reactive nitrogen forms are needed. These include

oxidized and reduced nitrogen compounds, such as nitric acid, ammonia, nitrates, ammonium and organic nitrogen compounds, each of which is normally scarce in the natural environment. The most important form of reactive nitrogen in the soil is the mineral nitrogen which is formed by nitrate and ammonium nitrogen [7]. Leaching of nitrate-N (NO_3^--N) from intensive agro-systems is a main environmental problem in many countries [8]. The use of legumes grown in rotations or intercropping is now regarded as an alternative and sustainable way of introducing N into lower input into agro-systems [9].

The nitrates are most dangerous in comparison with ammonium nitrogen (NH_4^+-N) because they are very mobile in soil. They have a negative charge and soil sorption complex has minimal affinity for negatively charged particles. Leaching of nitrate-N from arable soil is a major threat to the quality of drinking water from underground reservoirs [10].

Grain leguminous can cover cereal nitrogen demand from biological fixation of atmospheric N_2 [11][12] and therefore, they compete less for soil N_{min} in intercropping with cereals [13]. The success of intercrop farming systems depends initially on effective nitrogen fixation, and more importantly, on subsequent transfer of nitrogen to the non-legume [14]. Lupwayi & Rice [15] states that legume-based crop rotations are more sustainable crop management systems. Moreover Jarvis et al. [16] reported: "legumes are able to accumulate substantial quantities of nitrogen, and the soil's population of microbes has an enormous capacity to cycle this N in the right conditions".

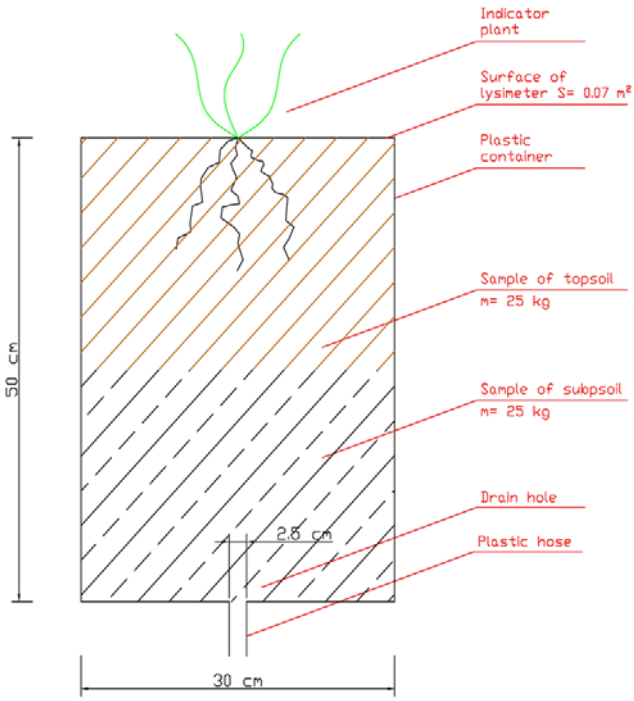
Therefore, we focused on impact of mixed culture cultivation on leaching of mineral nitrogen (N_{min}); this hypothesis was tested: cultivation of Winter Pea together with Winter Wheat has positive effect on reducing the loss of N_{min} from arable soil.

Material and Methods

Design of experiment

Lysimetric experiment was used to test the above hypotheses. Eighteen lysimeters was used as experimental containers and located in the area of our interest. Lysimetric experiment and area of our interest and was described by Elbl et al [17]: The experiment was conducted in the protection zone of underground source of drinking water Březová nad Svitavou where annual climatic averages (1962-2012) are 588.47 mm of precipitation and 7.9 °C mean of annual air temperature. The lysimeters were made from PVC (polyvinyl chloride). Each lysimeter was the same size and was filled with 25 kg of subsoil and 25 kg of topsoil (arable soil). See the Fig 1.

Fig. 1 Lysimeter – experimental container [17]



Moreover Elbl et al. [17] state that: Topsoil and subsoil were collected from a field in the area. Soil samples were sieved through a sieve (grid size of 10 mm) and homogenized. Topsoil and subsoil were prepared separately. Each Lysimeter had one drain hole and plastic hose for collecting soil solution. Hose leads into the plastic bottle. All lysimeters were buried into the ground (Fig. 2). Collection of soil solution and monitoring of the lysimeters was carried out in the control shaft. Lysimeters were completed and filled in October 2012.

Fig. 2 Location of lysimeters

Six variants (C1 a C2, A1 – A4) of the experiment were prepared. Each one was prepared in three repetitions. These variants were divided into two groups. First group consists of two variant:

- C1: Winter Wheat - Sole crops (SC), control without fertilizers
- C2: Winter Wheat - Sole crops (SC), application of 140 kg of $N \cdot ha^{-1} \cdot yr^{-1}$.

Second group consists of four variants (A1 – A4), mixed culture (IC) was cultivated there – Winter Wheat (*Triticum aestivum*) in combination with White Clover (*Trifolium repens*):

- A1: 80% of recommended doses of N for Winter Wheat.
- A2: 50% of recommended doses of N and 100 % of recommended doses of C_{org} for Winter Wheat.
- A3: 50% of recommended doses of N and 50% of recommended doses of C_{org} for Winter Wheat.
- A4 (without fertilizers).

Comment for all variants: Seeds of Winter Wheat/Winter Pea were sown mixed in the rows in the same depth on the 9th of October 2012.

Information on the applied fertilizers was published in Elbl et al [17] and [18]: Nitrogen was applied as a liquid fertilizer DAM 390. DAM 390 is a solution of ammonium nitrate and urea with an average content of 30 % nitrogen (1/4 of nitrogen is in the form of ammonium, 1/4 is in the nitrate form and ½ is in the form of urea). One hundred liters of DAM 390 contain 39 kg of nitrogen. Organic carbon (C_{org}) was applied as organic fertilizer Lignohumate B (LG B). Lignohumate is a product of chemical transformation of lignosulfonate. This material is completely transformed into the final product: solution containing 90% of humic salts (1:1 ratio of humic and fulvic acids).

Determination of mineral nitrogen leaching

Concentration of mineral nitrogen was measured in soil solution which was collected from each lysimeter into plastic bottles. These bottles were placed in the control shaft. The procedure for sampling of soil solution was described by Elbl et al. [17]: The amount of the solution was monitored three times per week. If a solution was found in a bottle, it was taken for the determination. Samples were stored at 4 °C before the determination. Moreover, Elbl et al. [17] reported that: Concentration of mineral nitrogen (N_{min}) was measured using distillation-titration method by Peoples [19]. Ammonium nitrogen was determined by distillation-titration method in an alkaline solution after the addition of MgO. Nitrate nitrogen was determined in the same manner using Devard´s alloy. Concentration of $NH4_{+}^{-}N$ and NO_3^{-}-N was calculated according formula in Figure 3:

Fig. 3 The value of N_{min} was calculated as the sum of the detected ammonium and nitrate forms [17]

$$\text{mg } NH_4^+ \text{ or } NO_3^- - N = \left(\frac{\text{normality of standart HCl}}{0.03571} \right) \times 0.5 \times \text{titration}$$

Statistical analysis

Potential differences in leaching of N_{min} were analyzed by the one-way analysis of variance (ANOVA) in combination with the post-hoc Tukey´s test. All analyses were performed using Statistica 10 software.

Results and Discussion

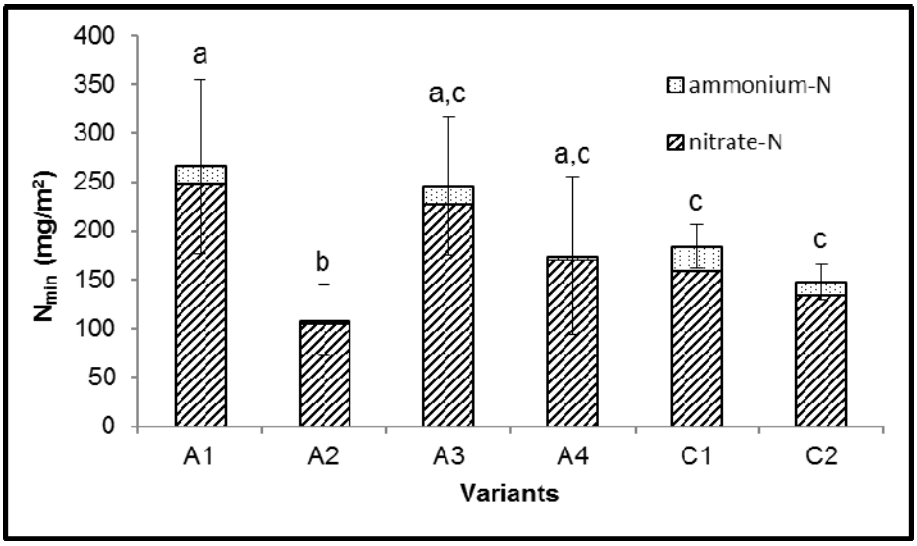
The content and quality of nitrogen in the soil is the result of soil historic development formed by natural soil forming factors and more or less by human´s activity. Therefore, every present's content of nitrogen in the soil corresponds with the present development stage of the soil and its previous commercial utilization [20].

This work presents the first results from the field/lysimetric experiment which is focused on possibilities of IC cultivation and its impact on N_{min} leaching. This experiment was established in 2012 and will continue for the next three years. The following Figures 4 – 6 present values of N_{min}

concentrations and it's both forms (ammonium-N and nitrate-N) which were measured in soil solution from January 2013 to September 2013. Moreover, Figures 5 and 6 show the loss of two most important forms of N_{min}: ammonium-N and nitrate-N.

Nitrogen is a key element for all living organisms because it is an essential component of proteins and nucleic acids. The most important form of reactive nitrogen in the soil is the mineral nitrogen which is formed by nitrate and ammonium nitrogen [7].

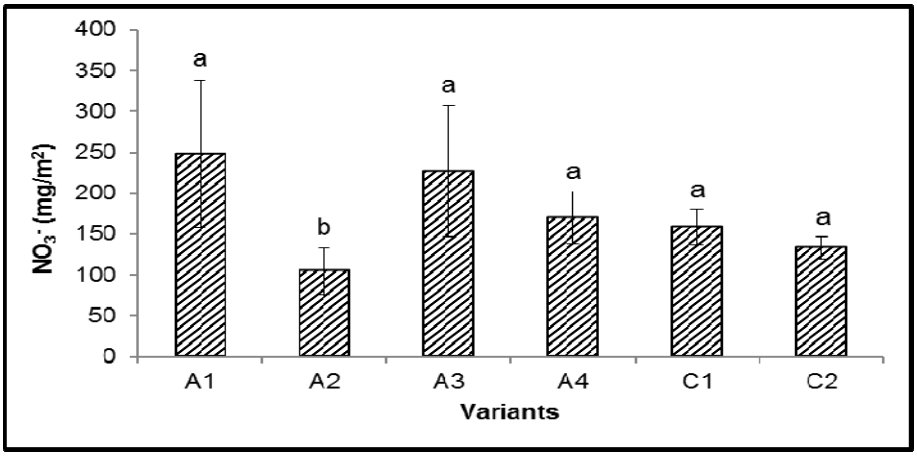
Fig. 4 Loss of N_{min} from experimental containers



Legend for Figures 5 – 7: A1 Intercrops with 80% of N; A2 Intercrops with 50% of N and 100% of LG B; A3 Intercrops with 50% of N; A4 Intercrops without addition of N or LG B; C1 Sole crops without addition of N or LG B; C2 Sole crops with 100% of N. Different small letters indicate significant differences (P<0.05) between individual variants; means values are presented n=3 with ± SD.

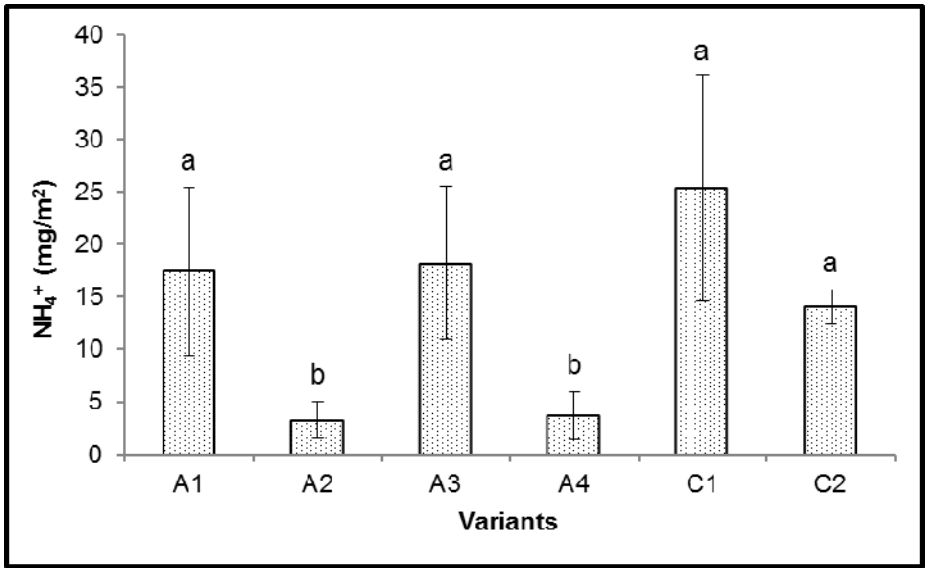
The above and following Figures (4 – 6) show significant differences between individual variant of experiment. Values of N_{min} leaching (Figures 4) indicate a positive effect of IC cultivation and reduced dose of fertilizers on loss of N_{min} from arable soil. The significant (ANOVA; $P<0.05$) lowest loss of N_{min} was found in variants A2 (108.20 mg/m^2) in comparison with variants A1; A3; A4; C1 and C2. Dubach & Russelle [21] state that legumes can transfer significant amounts of symbiotically fixed N_2 to neighbouring plants, and a putative pathway for N transfer is decomposition of fine roots and nodules. Moreover, according to Mukerji [22], legumes produce root exudates which contain a large amount of C_{org}. This fact together with application of LG B into this variant was the main reason for differences in N_{min} leaching between variant A2 and others. Impact of roots exudates from leguminous crops on soil microbial activity was confirmed by Fustec [9].

Fig. 5 Loss of N_{min} in form of nitrate-N from experimental containers



Consider values from Figures 6 and 7. The significant lowest values of ammonium-N loss were found in variants A2 (3.25 mg/m^2). We conclude that cultivation of IC and application of LG B has positive effect on soil microbial community. This community is responsible for utilization of N_{min} forms in soil. Böhme and Böhme [23] published that soil microbial biomass is fundamental for maintaining soil functions because it represents the main source of soil enzymes regulating transformation processes of elements in soils. It also controls the build-up and breaks down of organic matter, the decomposition of organic residues, and it is an early indicator of changes in soil management.

Fig. 6 Loss of N_{min} in form of ammonia-N from experimental containers



There are big differences between loss of ammonium-N and nitrate-N; the loss of nitrate-N was about 300 % higher than the loss of ammonium-N. The ammonium form of N_{min} is immobile and it may be degraded by soil microorganisms and plants. Conversely, nitrates are most dangerous in comparison with ammonium nitrogen (NH_4^+-N) because they are very mobile in soil. Elbl [10] state that nitrates are very mobile in the soil environment. Therefore, we found such great difference between these forms of nitrogen and leaching of nitrate-N from arable soil is a major threat to the quality of drinking water from underground reservoirs.

Conclusion

Our work presents first results of lysimetric experiment which was established in September 2012; therefore we must interpret these results with caution. The above results indicate that cultivation of these crops has positive influence on a decrease in leaching of mineral nitrogen. Based on these results we conclude, that cultivation of intercrop/mixed culture represents a new opportunity to mitigate the negative influences of extensive agriculture – leaching of mineral nitrogen.

Acknowledgement

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Monitoring of hydrochemical parameters, algae and cyanobacteria in newly built ponds near Hovorany village

ONDREJ KLIMA, RADOVAN KOPP
Department of Zoology, Fisheries, Hydrobiology and Apiculture
Mendel University in Brno
Zemědělská 1, 613 00 Brno
CZECH REPUBLIC

xklima7@node.mendelu.cz

Abstract: Observation of five ponds took place in the year 2014 in the cadastral area of village Hovorany. The aim was to monitor the development of these ponds in terms of hydrochemical parameters and the occurrence of algae and cyanobacteria. From each pond a sample of water was taken to analyze and measure the amounts of ammoniacal, nitrite and nitrate nitrogen, chlorides, orthophosphate, alkalinity, calcium, chlorophyll a, COD_{Cr}, total nitrogen and phosphorus. Dissolved oxygen, pH, the temperature of water, conductivity and transparency were measured *in situ*. Algae and cyanobacteria were taken using the plankton net and the identified species were divided into four groups (cyanobacteria, green algea, diatoms and others algea). According to the analyzed chemical parameters, following the method of Government Order No. 61/2003, the water was classified as "cyprinid water". In all the ponds there is a significant fluctuation in pH. The biggest pond, Sluňák, is the most stable. Cyanobacteria and algae gradually colonized all the ponds. In total, 61 species occurred (cyanobacteria 8 species and 53 species algae). The most numerous taxa were green algae (Chlorophyta), which dominated in the ponds Sluňák, Vrbatec, Mokřák and Jasaňák. The exception was the pond Olšovník, where the other algae prevail (Euglenophyta and Cryptophyta). On the basis of identified taxa algae and cyanobacteria, the ponds are eutrophic.

Key-Words: chlorophyta, pools, new habitat, chemical indicators, diatoms, season, colonization

Introduction

Cyanobacteria and algea are widespread all over the world. They occurr in the soil and water [5, 11]. They colonize extreme habitats, for example thermal springs, salt marshes, acidic water [5, 11, 14] and residual waters from uranium mining [6]. They get to new habitats by water during flooding or air as spores or cells in anabiosis [5, 12]. Chlorophyta are the ones who spread via the wind, followed by diatoms and cyanobacteria [4, 7].

In the newly built ponds the first occupants are bacteria, algae and cyanobacteria. The first manifestation of colonization is the production of biomass from anorganic matter and this way they allow the arrival of another organisms [5, 12]. Chlorophyta produce the biggest biomass [12]. They gradually create communities of plankton, benthos and neuston [5, 3, 1]. Abundance of green algae increases with the temperature of water [16]. For further development of population they create reserves of spores and resting cells in sediments. The impulse for developing spores and resting cells is light and sediment mixing [15]. Cyanobacteria and algae are indicators of water quality. The species composition of phytoplankton supplements chemical analyse of water and it gives us an image of the conditions in the ponds [5]. Low values of nitrogen and phosphorus limit the growth of biomass algae [16]. According to species of algae and cyanobacteria present, we can devide ponds into oligotrophy, mesotrophy and eutrophy [5, 8].

The aim of this study is to monitor the development of the newly built ponds near the village Hovorany in terms of hydrochemical parameters and algae and cyanobacteria occurrence.

Material and Methods

System of five ponds (figure 1) was built near the village of Hovorany on arable soil. Ponds do not have a tributary, but they are supplied by groundwater. The water depth and water surface are given in the table 1. All ponds are without fish. Macrofyta occure rarely.

In the year 2014, the ponds are being monthly observed. The results published in this article are from March to August. From each pond the sample of water, cyanobacteria and algae were taken, and hydrochemical parameters were measured.

Fig. 1 Satellite map of the ponds

Dissolved oxygen, pH and the temperature of water were measured *in situ* using a portable Hach HQ40d machine, and the conductivity of water was measured by the portable Hanna Combo HI 98129 machine. Transparency was measured *in situ* with a Secchi disk. The samples of water are taken in PE bottles with the volume of 0.5 l.

Table 1 Characteristic each ponds

Pond	Max. depth (m)	water surface (m^2)
1 Sluňák	2	4 796
2 Mokřák	2	480
3 Vrbatec	1.5	375
4 Olšovník	1	421
5 Jasaňák	0.5	355

The values of ammoniacal, nitrite and nitrate nitrogen, chlorides, orthophosphate, alkalinity (ANC) and calcium were determined using a standard method for analyzing the surface of water [9]. Chlorophyll "a" was determined according ISO 10260 [10]. For analysing COD_{Cr}, total nitrogen (Nt) and phosphorus (Pt), commercial supply sets from company WTW were used. All spectrophotometricaly analysis were carried out by a spectrofotometr PhotoLab 6600 UV-VIS.

Samples for microscopic determination of main groups of phytoplankton were taken using plankton net (average diameter of net eyes being 20 µm) into 50 ml plastic sample containers. The determination of phytoplankton was carried out in the native state using an optical microscope Olympus BX51. For the assessment of abundance of each group the seven-stage estimate scale was used [8].

The identified species were divided into four groups: cyanobacteria (Cyanobacteria), green algea (Chlorophyta), diatoms (Bacillariophyceae) and others algea (Dinophyta, Cryptophyta, Chrysophyceae, Xantophyceae, Euglenophyta). The results were processed in Microsoft Office Excel 2003.

Results and Discussion

The hydrochemical parameters of ponds are shown in the table 2. The greatest fluctuation of values was observed at pH, which moved from 8.25 to 10.00 (table 2). The alkalinity moved from 0.5 to 3.9, confirming the fluctuation of pH. High values were found organic matters, where the measured

COD_{Cr} in spring period extended to 137 $mg.l^{-1}$. By the newly built ponds there is yet no mineralized sediment, therefore a lot of organic matter occurs in the beginning. The biggest pond Sluňák was the most stable one. According to Government Order No. 61/2003, the ponds are carp water, where limits of physical and chemical parameters are determined. Value of pH should be to 9.00, all ponds exceeded this value in June and July. This occured because of the development of cyanobacteria and algea, which increase pH during photosynthesis [5, 17]. Total amount of organic matter should be to 26 $mg.l^{-1}$. All ponds except Sluňák exceeded this value many times in spring period. The low value of total nitrogen and phosphorus (table 2) show the presence of plants, which improve quality of water [18].

Table 2 Hydrochemical parameters are measured in the ponds

1 Sluňák		Average	Max	Min
Temperature of water	°C	17.27	26.00	8.30
Dissolved oxygen	%	95.38	106.80	85.70
pH		8.77	9.18	8.35
Conductivity	$mS.m^{-1}$	55.65	65.30	44.80
Nt	$mg.l^{-1}$	1.13	3.30	0.40
Pt	$mg.l^{-1}$	0.04	0.04	0.03
Chlorophyll a	$\mu g.l^{-1}$	3.70	7.40	1.48
COD_{Cr}	$mg.l^{-1}$	17.58	23.00	15.10
ANC	$mmol.l^{-1}$	2.53	3.70	1.15
2 Mokřák		**Average**	**Max**	**Min**
Temperature of water	°C	17.13	26.10	8.20
Dissolved oxygen	%	94.62	107.30	87.10
pH		8.99	9.72	8.36
Conductivity	$mS.m^{-1}$	58.87	70.20	51.40
Nt	$mg.l^{-1}$	1.02	2.70	0.40
Pt	$mg.l^{-1}$	0.04	0.04	0.03
Chlorophyll a	$\mu g.l^{-1}$	2.96	4.44	1.48
COD_{Cr}	$mg.l^{-1}$	37.73	127.00	15.50
ANC	$mmol.l^{-1}$	2.03	3.50	1.05
3 Vrbatec		**Average**	**Max**	**Min**
Temperature of water	°C	16.72	25.40	8.20
Dissolved oxygen	%	87.92	101.50	66.90
pH		8.66	9.23	8.25
Conductivity	$mS.m^{-1}$	60.83	70.50	55.10
Nt	$mg.l^{-1}$	1.28	3.60	0.30
Pt	$mg.l^{-1}$	0.05	0.06	0.04
Chlorophyll a	$\mu g.l^{-1}$	7.89	11.84	4.44
COD_{Cr}	$mg.l^{-1}$	44.45	131.00	19.80
ANC	$mmol.l^{-1}$	2.43	3.90	1.70
4 Olšovník		**Average**	**Max**	**Min**
Temperature of water	°C	16.57	25.20	8.20
Dissolved oxygen	%	85.18	100.00	57.30
pH		8.65	9.28	8.28
Conductivity	$mS.m^{-1}$	61.13	75.30	53.00
Nt	$mg.l^{-1}$	1.53	4.80	0.60
Pt	$mg.l^{-1}$	0.05	0.06	0.05
Chlorophyll a	$\mu g.l^{-1}$	4.44	7.40	1.48
COD_{Cr}	$mg.l^{-1}$	46.63	137.00	20.80
ANC	$mmol.l^{-1}$	2.15	3.90	1.35
5 Jasaňák		**Average**	**Max**	**Min**
Temperature of water	°C	16.52	24.60	8.50
Dissolved oxygen	%	93.80	141.80	67.50
pH		9.05	10.00	8.26
Conductivity	$mS.m^{-1}$	57.15	67.50	49.20
Nt	$mg.l^{-1}$	1.62	4.50	0.70
Pt	$mg.l^{-1}$	0.17	0.27	0.09
Chlorophyll a	$\mu g.l^{-1}$	14.26	51.80	1.48
COD_{Cr}	$mg.l^{-1}$	48.10	130.00	21.10
ANC	$mmol.l^{-1}$	2.32	3.45	1.20

In the ponds a total of species was found (cyanobacteria 8 species and 53 species algae). In pond Sluňák prevail golden algae (Chrysophyceae) in March, the most often occurring species being *Dinobryon divergens*. Since April most numerous were the green algae (*Zygnema sp.*, *Botryococcus braunii*, *Planktosphaeria gelatinosa*). In July, diatoms were represented the most (*Synedra acus*, *Nitzschia sigmoidea*, *Navicula sp.*). Cyanobacteria are found in large numbers only in June represented by the only species *Snowella sp.* (figure 3).

In the pond Mokřák, cyanobacteria and algae occured in March. Diatoms (*Dinobryon divergens, Malomonas sp.*) were most dominant in this month. In other samples the green algae was the most numerous (*Zygnema sp., Pandorina morum,*

Elakatothrix genevensis, Phacotus lenticularis, Spirogyra sp.). Cyanobacteria occurred more in June, namely the species *Snowella sp.* (figure 3).

In the pond Vrbatec green algae occured the most (*Staurastrum manfeldtii*, *Ankyra ancora*, *Crucigeniella apiculata*). Golden algae (*Mallomonas akrokomos*) and euglena (*Phacus longicauda*) were most numerous during March, July and August. Diatoms and cyanobacteria were rarely present (figure 3).

In the pond Olšovník euglena *(Colacium sp., Lepocinclis texta, Euglena sp.)* and Cryptophyta (*Cryptomonas sp.*) dominated the most. Green algae (*Planktosphaeria gelatinosa*, *Ankyra ancora*, *Pandorina morum*) dominated in May, July and August (figure 3).

In the pond Jasaňák, euglena (*Colacium sp.*, *Lepocinclis texta*) were dominant in spring period. Green algae (*Scenedesmus linearis*, *Oocystis lacustris*, *Botryococcus braunii*) prevailed since May. From diatoms, *Navicula sp.*, *Nitzschia sp.*, *Stephanodiscus sp.* occured. The species *Cylindrospermum mucosum* were most numerous from cyanobacteria (figure 3).

The most numerous taxa were green algae (Chlorophyta) in the ponds. The exception was the pond Olšovník, where the other algae were prevailing (Euglenophyta and Cryptophyta).

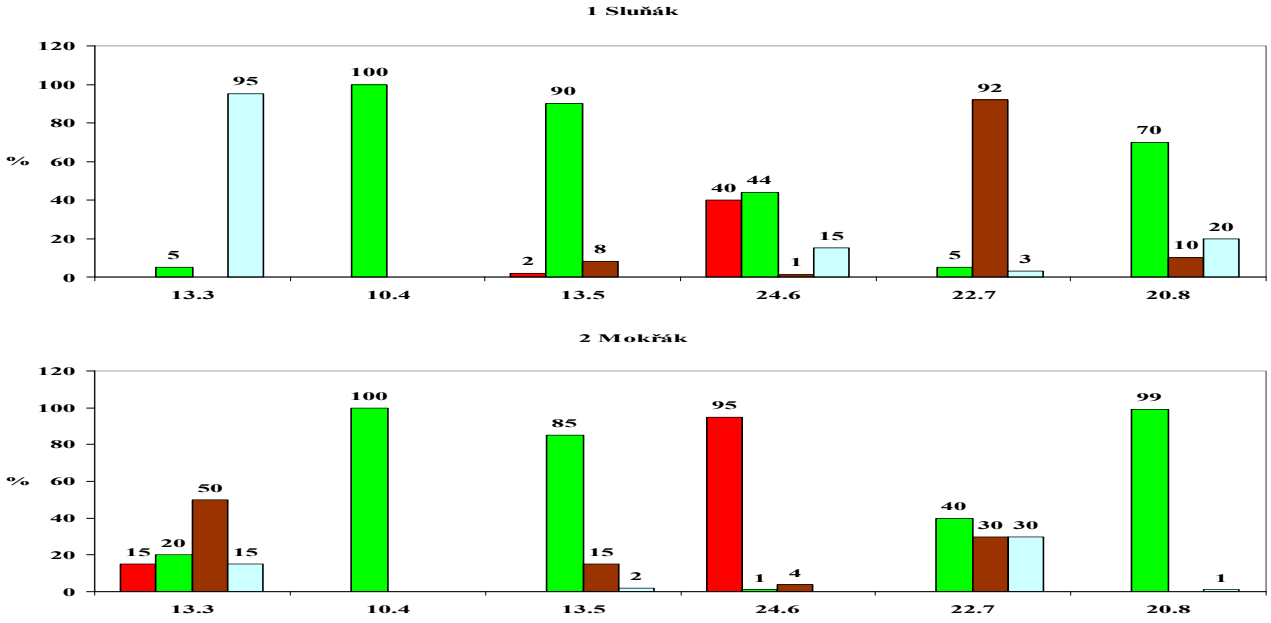
In the newly built ponds or pools the most numerous species are from the groups of Euglenophyta and Chlorophyta [3, 14]. The ability of Chlorophyta to spread by wind was confirmed [7]. Therefore they can be the first organisms in the new habitats [4, 12]. Diatoms were found both in the water column and the periphyton in accordance with Borduqui et al. [2] and Asencio [1]. The dominant occurrence of Chlorophyta indicate eutrophy in the ponds, as studies by Oikonomou et al. [13] and Asencio [1] show. Our results show that the most numerous cyanobacteria occur in the warmest period of the year. This is confirmed by Unrein et al. [17], who observed the same occurrence in nature reservoir of Laguna Grande in the South America.

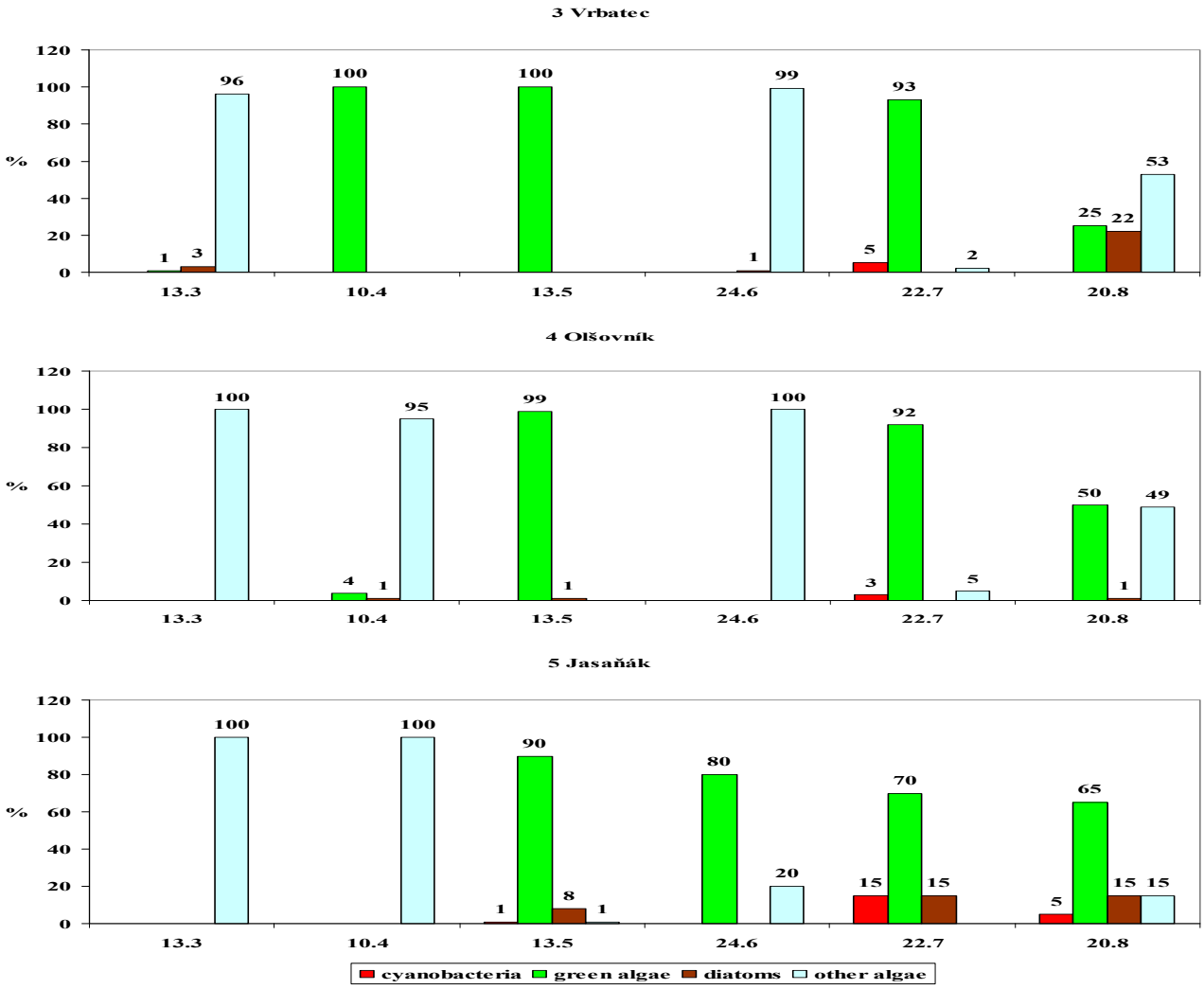
Monitoring of new habitats may help to understand the relationship between the environment and the colonizing species.

Conclusion

The ponds are being observed in the year 2014. From measured chemical parameters follows that according to Government Order No. 61/2003, the ponds are carp water. In all the ponds there is a significant fluctuation in pH. The biggest pond ,Sluňák, is the most stable, and can be suitable for the settlement of fish community. Cyanobacteria and algae gradually colonized all ponds. In total, 61 species occurred (cyanobacteria 8 species and 53 species algae). The most numerous taxa were green algae (Chlorophyta), who quickly colonized the new habitats. The exception was the pond Olšovník, where the others algae (Euglenophyta and Cryptophyta) prevail. Based on the identified taxa algae and cyanobacteria, the ponds are eutrophic.

Fig. 3 The percentage of cyanobacteria and algae in each pond





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Effect of compost amendment on arbuscular mycorrhiza in relation to bioavailability of heavy metals in contaminated soils

DANIELA KUBNA[1], JAKUB ELBL[1], LUKAS PLOSEK[1],
FLORIEN NSANGANWIMANA[2]
[1]Department of agrochemistry, soil science, microbiology and plant nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Laboratory of Soils and Environment
ISA Lille
48 boulevard Vauban, 59046 Lille Cedex
FRANCE

xkubna@node.mendelu.cz

Abstract: Concentration of heavy metals in environment has been significantly affected by human since last century. This work presents the analysis of the influence of compost amendment on arbuscular mycorrhiza and accumulation of heavy metals in *Lactuca sativa* grown in contaminated soils. To demonstrate the effect of compost the pot experiment was performed. Eight variants of soils with different concentrations of pollutants with and without compost amendment were prepared. Contaminated soils we used in our experiment come from the Nord France region Noyelles – Godault. Main pollutants were Pb, Cd and Zn. The highest decrease of heavy metals content in plants was observed by the simultaneous applications of compost to contaminated soils, from 10% to 50% in comparison with the variants without compost amendment. Moreover, we found highest level of root colonization by arbuscular mycorrhiza in compost amended variants where lowest content of heavy metals in plant tissues was observed. But these results are not significant. Based on these results, we conclude that application of organic waste compost has positive effect on immobilization of heavy metals in contaminated soils and also enhances arbuscular mycorrhiza colonization.

Key - Words: heavy metal mobility, contamination, remediation, arbuscular mycorrhiza

Introduction

Concentration of heavy metals (HM) in environment has been significantly affected by human since last century. Contamination caused by metals is mainly associated with mining, industrial activities, chemical application such as pesticides and waste production [1]. Soil pollution results dominantly from emission of fumes and smoke, which is followed by dry or wet deposition. Heavy metals remain in soil and may retard growth of plants or of soil microorganisms, may be transferred into the plant tissue and via food chain may endanger the human health [2]. In addition, many metal-polluted soils are also characterized by negative properties such as poor nutrient availability, a lack of soil structure, low organic matter (OM) content, high salinity and/or acid pH [3]. Edible plants grown in contaminated soils may accumulate elevated levels of metals that may, when consumed, increase exposures to humans. For example, crops like lettuce, spinach, carrot, radish, and zucchini have been shown to accumulate increased levels of potentially toxic metals such as Mn, Pb, Fe, Zn, Cu, etc. [4, 5]. Lettuce (*Lactuca sativa L.*) accumulates metals at relatively high internal contents because of the efficient root uptake and subsequent translocation to the shoots [6]. Lettuce is also considered a good indicator species for derivation of critical soil Cd concentrations, which generally are used in a first-tier risk assessment [7].

A conventional method of treatment of contaminated soil suffers from recognizable drawbacks and may involve some level of risk. Bioremediation is a natural process which relies on bacteria, fungi, and higher plants to

alter contaminants and environmental conditions as these organisms carry out their normal life functions and can be enhanced by adding organic amendments to soils [8]. The addition of organic amendments, such as agroindustrial wastes and composts (C_p) from different origins to contaminated soils can act on a great variety of processes, leading to improvements in physico-chemical soil properties and fertility status and even altering the heavy metal distribution in the soil [9]. Thus, high-quality C_p, rich in biologically stable and humified organic matter, non-phytotoxic and showing low concentrations of heavy metals, should be used in reclamation of polluted soil and help to reduce the mobility, the (phyto) availability and toxicity of pollutants and, at the same time, increase soil fertility in order to improve plant development [10]. Mechanisms for enhanced bioremediation of heavy metal(loid)s by organic amendments include: immobilization, reduction and rhizosphere modification. Addition of organic amendments (especially humified) to soils increases the immobilization of metal(loid)s through adsorption reactions. The organic amendment-induced retention of metal(loid)s is attributed to an increase in surface charge and the presence of metal(loid) binding compounds [11, 12].

Mycorrhizal fungi in a metal-polluted soil are the ones which provide a direct link between the soil and roots by interacting with their host plants to form a symbiotic relationship in the contaminated land [13]. Mycorrhizal fungi are commonly found in the soils of most ecosystems. Mycorrhiza can enhance the transfer of scarce nutrient elements to a plant, and facilitate the toxic metal uptake by its host. There is evidence that arbuscular mycorrhiza (AM) fungi can play a role in increasing the tolerance of some plants to toxic metal contamination by developing the metal tolerance of the fungi themselves and binding the metals to polyphosphates within the fungal hyphae implicated [14,15]. The establishment of the mycorrhizal network offers a number of basic advantages to the host plant for the acquisition of mineral nutrients: i) fungal hyphae extend beyond the area of nutrient depletion surrounding the plant's roots; ii) fungal hyphae greatly increase the surface area available for the absorption of nutrients; iii) the hyphae are able to spread into soil pores that are too small for plant roots to enter [15].

According to evidence of C_p ability to enhance heavy metal (HM) immobilization [16, 17, 18] and knowledge of AM function, in this study we want to compare the influence of C_p as AM enhancer with effect of C_p addition on accumulation of HM in *Lactuca sativa*.

Material and Methods

Characterization of samples origin and experimental design

Contaminated soils we used in our experiment come from the Nord France region Noyelles – Godault where a lead smelter called Metaleurop has been under activity for more than one hundred years. Main soil pollutants were Pb, Cd and Zn.

Samples are top soils taken at 0-25 cm deep from different distance of smelter. For each soil many point samplings were realized to cover the entire plot and to constitute large amounts (more than 50 kg). There were formed three soil samples with different level of Pb contamination: M200 (200 ppm), M500 (500 ppm), M700 (800 ppm). At laboratory, samples were air-dried, and then sieved to pass through a 10 mm mesh. Prior to use, they were stored in plastic container in a dry (not humid) chamber. From these representative samples, subsamples were prepared according to the CSN ISO 11464 standard.

Our hypotheses were tested by pot experiment (tab 1) which was carried out in grow box for 48 d in determined conditions. Day mode was set to 12 h with light intensity of 350 µmol/m·s. Day temperature was 20°C and humidity was 67%, night temperature was 18°C and humidity was 71%. Each type of soil was placed into pot in three repetitions without C_p amendment and in three repetitions with C_p amendment. Also control (non–contaminated) sample variants were set. C_p was obtained from the Central Composting Plant in Brno which is registered for agriculture use in the Czech Republic. The C_p amendment represented dose of 50 t/ha. The indicator plant lettuce was seeded next. During cultivation the pots were watered three times a week with 60 ml of demineralised water. After 48 d the pots were emptied and biomass of roots and leaves and soil were stored separately.

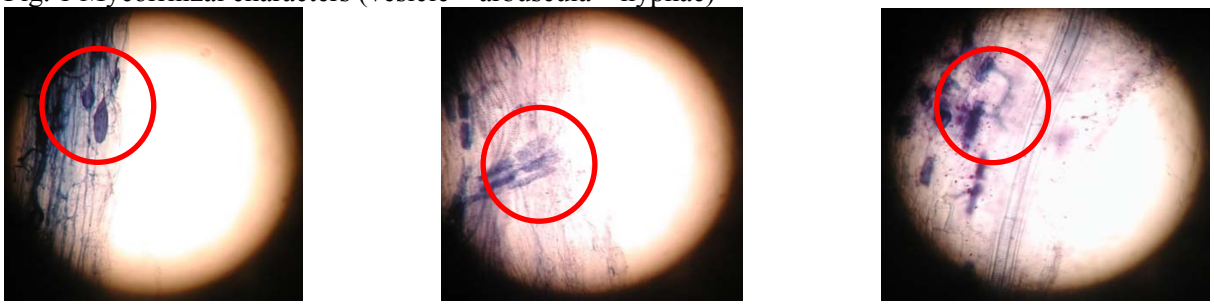
Tab. 1 Pot experiment variants

Soil sample	characteristic	repetitions	Amount of substrate in pot (soil + compost)
M2007	non-contaminated, control sample	3x	900 g
M2007 + K	M2007 with compost amendment (50 t/ha)	3x	863.7 + 36.3 g
M200	soil contaminated with approx. 200 ppm Pb	3x	900g
M200 + K	M200 plus compost amendment (50 t/ha)	3x	863.7 + 36.3 g
M500	soil contaminated with approx. 500 ppm Pb	3x	900g
M500 + K	M500 plus compost amendment (50 t/ha)	3x	863.7 + 36.3 g
M700	soil contaminated with approx. 800 ppm Pb	3x	1000g
M700 + K	M700 plus compost amendment (50 t/ha)	3x	963.7 + 36.3 g

Mycorrhizal colonization of roots

The percentage of mycorrhizal colonisation was determined in root samples, which were taken from root system of *Lactuca sativa* (from each experimental container). Root samples (3 g fresh weight) were washed in tap water and before processing stored in FAA solution (50% ethanol, acetic acid, formaldehyde). Fixed root samples were washed; cleared and stained according Koske & Gemma [19]. Stained roots were cut into 1.5 cm segments, mounted on microscope slides in glycerol gelatin and evaluate microscopically (200x MA) by Giovanetti & Mosse [20].

Fig. 1 Mycorrhizal characters (vesicle – arbuscula – hyphae)



Heavy metal concentrations in plant tissues

In the laboratory, aboveground parts of lettuce were washed in three successive baths of osmotic water. Excess water on these plant organs was blotted by a clean paper towel before cutting them into small pieces. The belowground organs were washed thoroughly with tap water to remove the soil particles.

Rhizomes were separated from roots with scissors. Both organs were rinsed in three successive RO water baths, and then cut into small pieces. All samples were oven-dried at 40 °C, and then ground and sieved to 250 µm using a knife mill (GM200) for leaves and roots, and an ultracentrifuge mill (ZM200) for stems and rhizomes. Sample digestion was realized by adding 5 ml of 70% HNO_3 (Baker Analyzed Reagent) in a tube (50 ml Digestion Cup) containing 300 mg of plant powder. The tube was covered with a watch glass and heated at 80 °C on the hot block (HOT BLOCK Environmental Express) for 1 hour under the hood box. After cooling, 5 ml of 30% H_2O_2 (Baker Analyzed Reagent) were added to the digest, and the mixture was again heated at 80 °C for 3 hours. After cooling, the volume was adjusted to 25 ml with double-distilled water and filtered (0.45 µm acetate membrane filters, Minisart). Filtrates were stored at 4 °C before Cd, Pb, and Zn determination by atomic absorption spectrophotometry (AA-6800, Shimadzu).

Quality control for chemical extraction and digestion was performed by including blanks, internal and certified (INCT-PVTL-6) reference materials. The mean recovery rates in the reference material are 97.0 % (Cd), 107.3 % (Pb), and 104.9 % (Zn). The residual moisture of the dried plant samples was measured by weighing a sample (≤ 10 g) before and after passage in an oven at 105 °C (ISO 11465) and was used to apply the moisture correction factor so as to express results on dry weight (DW) basis.

Statistical analysis

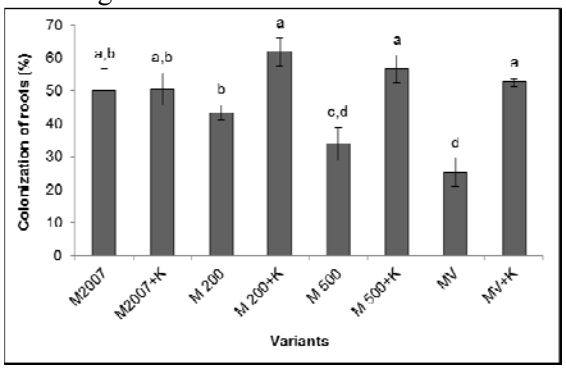
Potential differences in values of mycorrhizal colonization and content of heavy metals in plant biomass (plant biomass) were identified by ANOVA in combination with Tukey´s test ($P<0.05$). Regression was used for testing relationship between the level of mycorrhizal infection and content of heavy metals in plant biomass.

Results and Discussion

Mycorrhizal colonization of roots

Arbuscular mycorrhiza symbiosis is important mutualistic association that is formed between plant roots and soil fungi. It can be found in majority of crops. Mycorrhizal fungi are able to improve plant nutrition and growth, as well as their resistance to biotic and abiotic stress factors [21].

Fig. 2 Colonization of *Lactuca sativa* roots by AM fungi in soil



AM was observed in all variants and repetitions. The colonization varied within limits 25 – 62 %. The highest value was detected in M200+K variant, the lowest was detected in M700 in comparison with other variants. Figure 2 shows significant differences between equally contaminated variants with and without C_p amendment excluding control variant M2007. There is also shown increasing level of AM colonization in relation with increasing HM concentration. C_p amendment significantly enhanced AM colonization of lettuce roots, what is confirmed by results in Alguacil et al. [21].

Heavy metal concentration in plant tissues

The soil immediately surrounding plant roots (rhizosphere) is a modified microbiologial and chemical environment due to plant – soil – microbe interactions. The changes in soil chemistry due to soil amendment and plant growth can therefore influence the transformation, mobility and bioavailability of metals [8]. Results in Tab. 2 present lower HM uptake in C_p amended variants. The significant differences are observed between all of amended and non-amended variants for Cd as we expected due to [16, 17, 18]. There are also significant differences between variously contaminated variants and control for Cd. The same differences are observed in variants with high Pb contamination, but these are not significant excluding M700 variant. The different results were found for Zn, where C_p amendment enhanced Zn uptake, especially in highly contaminated variants M500 and M700. Zinc is essential element for plants. It is usually found in higher concentrations.

Tab. 2 Heavy metal concentrations in plant tissues, different small letters indicate a significant differences in concentration of individual heavy metals (Cd; Pb and Zn) at level 0.05 (ANOVA; $P<0.05$; post-hoc Tukey`s test) between individual variants of experiment.

Soil sample	Cd (mg/kg)	±SE	Pb (mg/kg)	±SE	Zn (mg/kg)	±SE
M2007	2.90^{a}	0.10	5.85^{a}	1.28	82.89^{a}	4.75
M2007 + K	0.76^{b}	0.09	5.22^{a}	0.63	65.13^{b}	2.99
M200	10.63^{b}	1.07	7.02^{a}	0.83	76.96^{a}	7.85
M200 + K	6.18^{c}	1.54	7.50^{a}	0.93	85.31^{a}	7.68
M500	8.96^{d}	0.54	14.32^{b}	1.91	130.66^{c}	13.32
M500 + K	6.94^{c}	0.83	12.88^{b}	1.91	107.28^{d}	9.28
M700	18.54^{e}	0.501	17.79^{c}	1.23	93.99^{a}	5.05
M700 + K	15.94^{f}	0.61	14.28^{b}	0.54	115.74^{d}	3.72

The relationship between concentration of heavy metal in plant tissues and colonization of roots by AM

The correlation between HM concentration and AM colonization for Pb and Cd was found. As the bold statistical parameters shows in tables 3 and 4, concentration of HM in plant decreased with increasing degree of AM. Reduced HM uptake, especially at higher soil metal contents, indicates a changed metal tolerance strategy in colonized plants similarly to results of Vogel-Mikuš et al. [22]. Significant correlation for Zn was not found.

Tab. 3 Regression analysis of the relationship between the level of colonization of roots by AM and concentration of cadmium in plant tissues

Parameter	Value
Multiple regression	**0.46932**
R-squared value	0.22026
Statistical power	**6.21467**
Probability	**0.02068**
Standard error	5.35133

Tab. 4 Regression analysis of the relationship between the level of colonization of roots by AM and concentration of lead in plant tissues

Parameter	Value
Multiple regression	**0.50746**
R-squared value	0.25751
Statistical power	**7.63010**
Probability	**0.01137**
Standard error	4,31191

Tab. 5 Regression analysis of the relationship between the level of colonization of roots by AM and concentration of zinc in plant tissues

Parameter	Value
Multiple regression	0.18086
R-squared value	0.03271
Statistical power	0.74397
Probability	0.39770
Standard error	23.59601

Comment for Table 3 – 5: The significant correlation between AM and concentration of heavy metals are shown in bold. The relationship between colonization of roots by AM and concentration of individual heavy metals was always compared for one variants of experiment.

Conclusion

Nowadays trends of bioremediation are heading to using compost as reclamation substrate on heavy metal contaminated areas. The aim of this experiment was evaluation of correlation of two aspect caused by compost adding. We conclude that compost amendment definitely enhances AM colonization of *Lactuca sativa* roots even in highly contaminated soil. One of the characters of this symbiosis is adapting strategy in stressful environment to protect the host plant. This strategy was confirmed for lead and cadmium, where the significant correlation between AM colonization and reduced HM uptake was observed. There appeared different behaviour of zinc, which was not evidential. The reason of different zinc behaviour could be higher affinity to forming chelates with organic compounds, which are readily available for plant. We find compost suitable as bioremediation tool, but at first the pollutant type, level of contamination and the target plant must be considered.

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The evaluation of selected indicators of water quality in observed watersheds in Znojmo region

DAGMAR MICHALCIKOVA, VERA HUBACIKOVA, PETRA OPPELTOVA,
VERONIKA PETRAKOVA, KATERINA ZAKOUTSKA
Department of Applied and Landscape Ecology, Faculty of Agronomy
Mendel University in Brno
Zemědělská 1, 613 00 Brno
CZECH REPUBLIC

xmichal2@node.mendelu.cz

Abstract: The paper is focused on the monitoring of selected water quality parameters in watersheds of Březanka and Trstěnice stream due to land use categories. Measuring point at each flow was elected according to the borders of different land use categories because of demonstrating the effect of individual category on the water quality. Since March 2014 there has been measurement monthly taking place on chosen sites and followed by laboratory analysis of water in the laboratory of the Mendel University in Brno and comparing the results with actual legislation.
Results of laboratory analyzes demonstrate the self-cleaning process of individual sections of streams, which are noticeable changes in the concentrations of monitored indicators of water quality. The results confirmed the assumption that forest land use category shows the highest degree of self-cleaning water.
One of the main results are the average values of phosphorus in Trstěnice stream 0.26 mg. l^{-1} and 0.87 mg. l^{-1} by the Březanka stream, where according to the law announcement is allowed only the value of 0.15 mg. l^{-1}. Further, the nitrate nitrogen with mean values of 17.8 mg. l^{-1} and 16.2 mg. l^{-1}, compared with ordinance stipulating 5.4 mg. l^{-1} and last but not least conductivity mainly on Březanka stream showing 177.5 $mS.m^{-1}$ instead of normal 160 $mS.m^{-1}$. The high rate of conductivity, according to CSN, points to the grade indicating a quality of polluted, heavily and very heavily polluted water, which points to run-off from agricultural lands. In place of the sharp decline do not take place the large areas of fields. Levels of phosphorus and nitrogen showed higher values for the both flows, mainly in agriculturally used land ends and urban areas, which were subsequently reduced after passing through the forest vegetation by several orders. The main problem is usage of phosphate and nitrogen in agricultural land fertilizers and household washing and cleaning detergents containing polyphosphates, which WWTP cannot completely remove. The great influence of the forest is in removing these substances, namely vegetation uses a lot of nutrients (nitrogen and phosphorus). All measured values indicate the emergence of eutrophication and degradation of natural habitats.

Key-Words: Březanka and Trstěnice stream, land use, self-cleaning process, water quality, monitoring, laboratory analysis

Introduction

Water is high substantial factor for formation stronger landscape stability [1].

Trstěnice even Březanka stream is located in municipality district of the town Zlín in South Moravia Region. Both sites are 2 km far from the town Zlín and 45 km far from Brno city. The area belongs to the Black Sea drainage, the Danube basin, sub-basin of Thaya.

The Březanka stream (originaly Mackovice stream) springs in the Mackovice cadastral; there are two ponds on the stream. The Březanka stream near the village of Hrusovany upon Jeviškovka empties into the Jevišovka river. Březanka´s catchment area is 5.49 km^2 [2].

Trstěnice stream sprint in the cadastral of the village Trstěnice, subsequently flows into the Skalička stream that discounts with Jevišovka river. The catchment area is 6.05 km^2 [3]. There are two small water ponds.

It has established a total of ten sampling profiles for water sampling, which, according to set parameters further processed and evaluated.

Nitrogen and phosphorus are the main elements causing eutrophication and determining its scope. The

increase in phosphorus and nitrogen in the monitored areas is mainly due to the human factor.

Eutrophication processes cause excessive occurrence of certain species of cyanobacteria and algae along with the associated health risks.

Detergents in wastewater and surface runoff from farm soil and fertilization are considered the main sources of pollution [1].

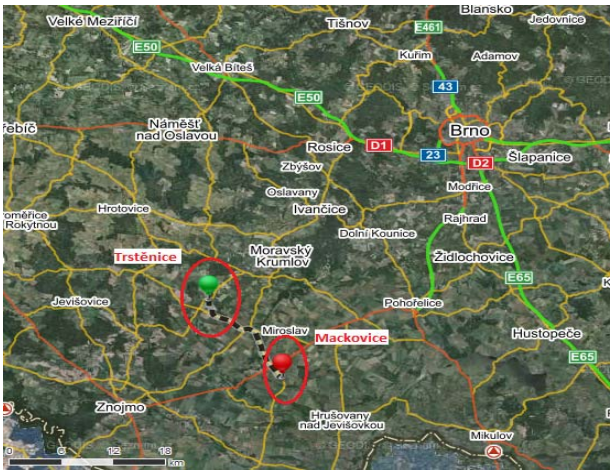
The concentration of nitrate is the basic indicator of farming on the surrounding land [4]. Nitrates are concentrated in surface waters. It´s sources are mainly fertilization, mineralization of plants residues and atmospheric water. High leak of nitrates form agriculture processes (fertilizers) is decreases with the help of legislative support so-called Nitrates Directive [5].

COD means the amount of oxygen consumed for the oxidation of organic substances, which is an indicator of total organic pollution of surface waters, according to which it can judge the importance of self-cleaning process in a flow. The value of COD is therefore a measure of the total content of organic substances in water [4].

Phosphorus gets into water from fertilizers, detergents, leaching soils etc. It is a limiting factor in the waters and their processes, because its value is compared to others constant or continuously increasing. Phosphorus in waters occurs mainly in the form of phosphates [6]. Phosphorus compounds are important for the cycling of matter in nature and are key to the eutrophication of surface waters [4].

Conductivity (concentration of electrolytes in the water) depends on the concentration of ions, their charge, mobility and temperature [4]. This is the concentration of ion solutes and total water mineralization.

Fig. 1 definition of the area – represented by the red circle (www.mapy.cz, edited by the author)



There are several options to reduce these indicators in the water, one of them are self-cleaning flow processes. These processes are dependent on the type and use of the landscape. According to laboratory analysis of water quality and the use of land use categories best practices for sound land use in terms of water pollution will be evaluated.

Materials and Methods

Sites

Sampling is carried out from April 2014 and as part of the thesis will continue until March 2015, so it is a yearly measurement of water quality at selected sections of two streams. Each of these streams is monitored in five sites.

The first profile on Březanka stream is its spring located directly on agricultural land, which was this year especially in the dry summer months. The second one is located on the border of the farming land with the edge of small local woods. The third one is located flow down the water pond; the fourth on the end of the village, under the outfall of waste water treatment plant and the fifth is under the next water pond in a small wood above the confluence with the amelioration system.

The first site on the Trstěnice stream is not directly the spring of the stream, because it is permanently dry this year. An unnamed tributary was therefore designed as a site, which passes around the farm, intended for breeding pigs. The second site is situated in the wood below two ponds and right on the confluence with the Trstěnice stream. The third one is on the end of boundaries of the village, where there is the outfall of waste pipe. The fourth site in located on the border of farmland and wood. The last site is in the wood above the confluence with the Skalička stream.

Sampling in the field

Samples are collected monthly, at about the same time, into plastic bottles, which are subsequently kept refrigerated and processed within 24 hours in the laboratory. Additionally on the sites the water temperature, dissolved oxygen, pH and electrolytic conductivity are measured by portable multimeter HQd (HACH LANGE Company)

Laboratory Processing of Samples

Samples are processed in the laboratory of the Department of Applied and Landscape Ecology, Faculty of Agronomy, Mendel University (DALE). All samples are processed according to standardized methods pursuant to the Hach Lange methodology on the spectrophotometer DR 4000. Subsequently the

results are graphically presented, evaluated and compared with the actual law.

Fig. 2 picture of the selected profiles (www.mapy.cz, edited by the author)

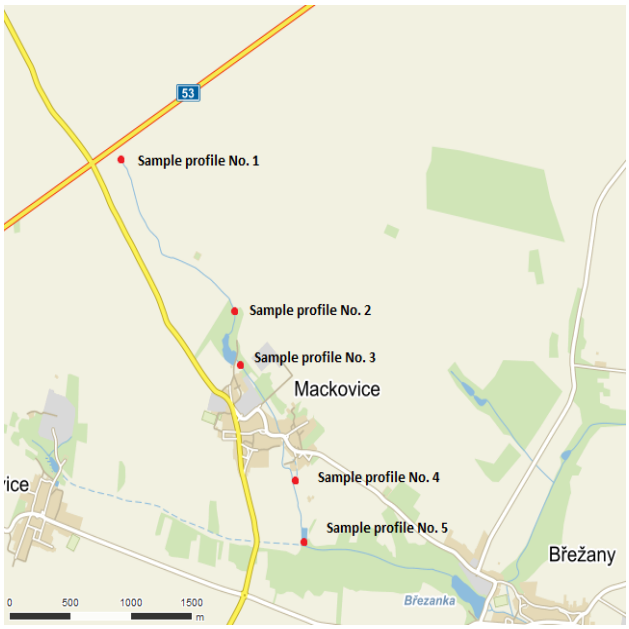


Fig. 3 picture of the selected profiles (www.mapy.cz, edited by the author)

Results and Discussion

Comparison of the Results

The results from sampling and measurement on both streams present self-cleaning processes of individual stream parts and there are evident changes in concentrations of observed indicators of water quality. These results were compared with the environmental quality standard for bodies of surface water, according to Government Regulation No. 61/2003 and CSN 75 7221.

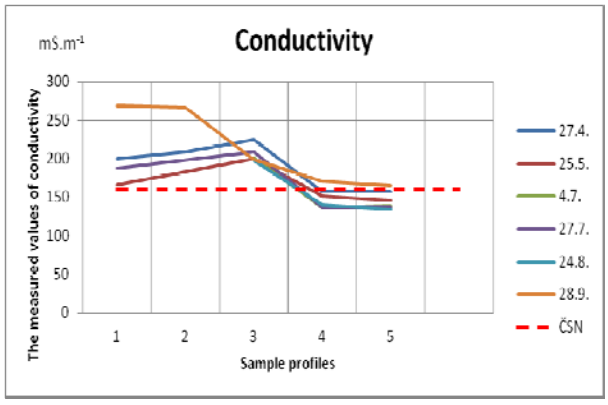
Březanka Stream´s Resultes

Levels of phosphorus, nitrate nitrogen, COD and conductivity measurements exceed the legal standards according to the resulting analysis. After evaluation of the sampling sites and their surroundings using land use, the fastest growth of these values is visible on site of the urban village under the water tank (sampling profile No. 3). Positive results of self-cleaning process are noticeable especially after the passage of the forest (sites No. 2 and 5).

The increase of phosphorus concentration was gradual and the values were the highest at sites No. 3 and 4. It is therefore primarily a problem of application of phosphate fertilizers on agricultural land use and household washing and cleaning agents containing polyphosphates [7], which subsequently WWTP will not be able to completely remove. Lower values for the profile No. 5 confirmed the great influence of the forest in removing this material, vegetation uses a lot of nutrients (nitrogen and phosphorus).

Nitrate nitrogen was highest in site No. 4, so it is a problem of increased minerals. Wastewater is the significant factor. Sites within farming area show increased incidence of nitrates which probably come from nitrogenous fertilizers. Even the first site shows higher concentration of nitrates, reduction of nitrogen emissions from livestock would be an appropriate countermeasure [5].

Fig. 4 – Values of conductivity in Březanka stream and the Environmental standard according to CSN (author)



The lowest value is on the third site (downstream the pond), where the reduction nitrates into nitrites is done (denitrification), and on the fifth site which is affected by the wood ecosystem with lot of fytoplankton in the surface water, which drains the nutrients from water and use them for body construction.

The COD was commonly the highest on the third site under the water tank. The water tank and its surrounding is contaminated with organic polluters, e.g. leaching from soil, leaves and waste water.

The conductivity was measured in the field and its high rate, according to CSN, points to the third, fourth and fifth grade indicating the quality of polluted heavily and very heavily polluted water [8]. Conductivity increases between the first and the third profile, which points to runoff from agricultural land.

Trstěnice Stream's Results

According to the results, values of phosphorus, nitrates nitrate, COD and dissolved oxygen were higher than the standard.

Curve of phosphorus refers to the highest representation in the consumption profile No. 3, (in the village), which points to the fact that the municipality does not have a WWTP. The fourth site (beginning of forest) has also elevated levels, so at the end of the agricultural land where contamination may be similar to the case of the Břežanka stream and the site No. 1, which is located in a field behind the building for a pig factory farm. On all other (forest) sites there are mostly in decline or stagnation of this element.

High concentrations of nitrate nitrogen were observed already in the first site; the effects on such results have surely economic object and nitrogen fertilizer applied to the land. Among the first and the second site, there was the self-cleaning process. On the rest of monitored location there was a slight increase of nitrate nitrogen which is probably caused by wastewater from municipalities and leaches nitrogen compounds from fields.

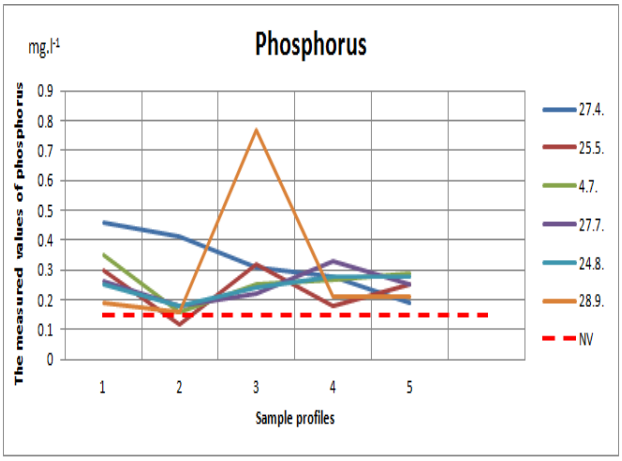
COD had a huge increase in profile No. 3 (below the village) and profile No. 4 (fields), this result points again to the waste from agriculture and extracts of these soils. Other sites´ values of COD were below the standard according to the notice, but is also worth mentioning site No. 2 (in the forest), which had also increasing the value that was mostly due to natural pollution (leachate from decaying wood or plant products and animals) [4].

The level of conductivity is largely located in the third group of surface water quality, according to CSN, which means the water has been polluted [8]. The high degree of conductivity indicates a high concentration of dissolved salts (minerals) that are present in such a large extent, mainly due to nutrient runoff from cultivated fields. It was also proved the rule that a change in temperature causes a change of conductivity. PH is indeed normal, but most of the time with the profile no. 1 found elevated levels that gradually drop to profile No. 4, which has the lowest value. PH on these sites shows the existence of lush aquatic vegetation on runoff of soil from deciduous forests and, last but not least, the possible evasion or violation of procedures or slurry storage and its application to the farmland [9].

If the pH is increased due to the photosynthetic assimilation of green plants, which leads to depletion of free carbon dioxide[4], so reduce the pH can be achieved by cuting-out lush local vegetation, thereby limit photosynthesis [7].

After evaluation of the sampling sites and their surroundings using land use, the fastest growth of these values is visible on site of the urban village (sampling site No. 3) and on the first site. Positive results of self-cleaning process are noticeable especially after the passage of the forest (sites No. 2 and 5).

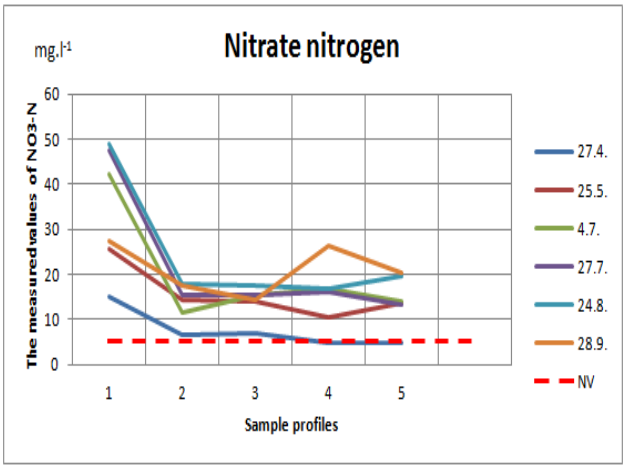
Fig. 5 – The amount of phosphorus in the selected profile Trstěnice and the level of premissible phosphorus amount (NEQ) (author)



Overall recommendations, as the context requires, are based on the application of phosphate limitation and nitrogen fertilizer on agricultural land use and household detergents containing polyphosphates. Construction of wastewater treatment plant in the village Trstěnice and in the village Mackovice greater control of phosphorus discharged is another useful measure, as well as increased checks on possible leaks or failure to observe the storage of slurry or its application to the farmland.

To support all three phases of processes (physical, chemical and biological) is necessary for self-cleaning process [1]. Creating infiltration strips around streams near the fields would limit the quantity of substances released into surface waters through run-off [10]. The construction of wetlands, planting of vegetation for greater oxygen supply, leading stream boulders or support aquatic fauna also will support self-cleaning processes.

Fig. 6 – The amount of nitrate in the selected profile of Trstěnice and the level of permissible nitrate amount (NEQ) (author)



Conclusion

The confirmation that different land use has different effects on the water quality is the main output. The selected points on the borders of individual land use categories point to the fact water quality changes in a negative sense, especially on farmed land, urban areas of municipalities and local wastewater treatment plant, where the levels of phosphorus, nitrate nitrogen, COD and conductivity generally increased. Conversely, positive changes in the concentrations of the indicators confirmed the assumption that the use of forest land for the process of self-purification of water is the best option. Appropriate measures for self-purification capacity in the most polluted sampling sites were recommended. Sampling, analysis and processing laboratory analysis and studying of recommended measures will continue.

Acknowledgement

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Microbial transformation of nitrogen in soil after the biochar addition

IRINA MIKAJLO[1], JAROSLAV ZAHORA[1], JAKUB ELBL[1], MARINA KRIVCOVA[2]
[1]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Department of Genetics, Physiology of Plants and Microbiology
Faculty of Biology
Uzhgorod National University
Voloshyna 32, 88000 Uzhgorod
UKRAINE

xmikaylo@mendelu.cz

Abstract: It is apparent from the several studies that soil amended with biochar (pyrolysed plant-derived material in absence of oxygen) can influence the abundance, diversity and activity of soil organisms. The objective of the present work is to characterize the impact of soil treatment with various amounts of mineral nitrogen fertilizers on specialized group of free-living N_2-fixing bacteria (diazotrophic) bacteria as a potential indicator of the effect of biochar amendment on the soil nitrogen transformation in the planned experiments. The high sensitivity of diazotrophic bacteria from the genus *Azotobacter* to the increasing doses of nitrate fertilizers has been found out in our studies. In terms of model experiment it has been showed that the number of *Azotobacter* was reduced under the influence of nitrogen fertilizers. Moreover, the higher concentration of mineral nitrogen, the more distinct the decreasing trend in the percentage of nitrogen-fixing bacteria has been observed. It was found that soya bean cultivation has led to the increase of the amount of nitrate-nitrogen in the soil, which explains the significant reduction in the percentage of *Azotobacter* genus bacteria. Based on the high value of correlation coefficients between the inversely correlated studied values is to be recommended to use these as an indicator of the effect of above mentioned biochar amendment.

Key-Words: biochar, soil, genus *Azotobacter*, nitrogen fertilizers

Introduction

Biochar, a material obtained from the carbonization of biomass, has many uses as a soil improver and also as a method of carbon sequestration. Therefore, biochar addition has been explored in the last years as a way to mitigate global warming. Different studies have found that biochar can improve both the nutrient- and water-holding properties of soil, and its popularity in recent years also owes to its ability to reduce greenhouse gases by storing carbon in soil, in some cases for many centuries [1,2].

However, responses of plant – soil - microbial complexes to biochar addition in particular in relation to changes in microbial activities changing the form and the availability of soil nitrogen are seldom documented. Nitrogen (N) is an essential nutrient, and research to date shows that biochar has the ability to manipulate the rates of N cycling in soil systems by influencing nitrification rates and adsorption of ammonia and increasing NH_4^+ storage by enhancing cation exchange capacity in soils and it may cause the reduction of gaseous N losses such as N_2O and nitrate leaching [3]. A greater abundance of microorganisms after biochar additions to soil has been relatively well described [4]. But still, it is not satisfactory explained, how biochar application into the soil influences free-living diazotrophs, the transformation mechanisms and fate of N. Finding an answer to some of these questions will be the aim of our future studies.

Though, modern technologies of crops cultivation tend to ensure complete recovery of nitrogen spends [5]. Due to the usage of such fertilizers as mineral and fertilizers of organic origin plants receive a sufficient amount of nutrients. However, fertilizers can not only enhance physiological processes, but also inhibit microbiological processes, including biological nitrogen fixation [6]. It is known, that in the

structure of microbial coenosis, *Azotobacter* acts as a sensitive indicator of soil condition changes under the influence of anthropogenic factors and as an indicator of the amount of soil nutrients [7, 8, 9].

The main goal of our previous studies in Ukraine was to determine a quantitative composition of free-living diazotrophs in soils after the application of the nitrate fertilizers and to find possibilities of studying the ways of using *Azotobacter* as a microbial indicator to assess soil state.

Material and Methods

In laboratory conditions, the amount of nitrogen-fixing bacteria in soil samples enriched with nitrate fertilizers has been determined before and after germination of soya bean (*Glycine max* Moench.).

The studies were conducted during 2012 -2013 years in a sod-podzolic soil type. Different fertilizer levels of calcium nitrate (Ca $(NO_3)_2$) and ammonium nitrate (NH_4NO_3) have been added to experimental soil samples. Calcium nitrate in the amount of 30 g, 60 g, 90 g per 100 cm^2 and ammonium nitrate in the amount of 15 g, 30 g, 45 g per 100 cm^2 have been applied into special containers with soil and then soya beans were seeded there. Soil without fertilizers was taken as a control sample. *Azotobacter* genus representatives were determined on nitrogen-free Ashby medium by the method of application and the percentage soil lumps fouling [10]. The concentration of nitrates in soil has been also estimated using ion-selective electrode method with an ion analyzer AI-123 before and after soya bean cultivation [11] and the correlation analysis between the percentage of *Azotobacter* genus representatives and the concentration of nitrate ions in differently fertilized soils before and after soya bean cultivation has been carried out.

Results and Discussion

According to the research results it has been found that the percentage of free-living nitrogen-fixing bacteria has gradually decreased with increasing doses of nitrate fertilizers, with a more clear tendency of *Azotobacter* percentage decrease noted after 60 days of observation (Table 1). Before soya bean planting, the control sample showed a high number of *Azotobacter* – 98.4 ± 1.65%.The greatest inhibition of diazotrophic bacteria has been observed during the application of calcium nitrate in the highest concentration of 90 g/cm^2, at which their number was on the 30th day of the experiment 1.1 times lower (88.4 ± 1.65%), compared to the control sample (98.4±1.65%). Similarly on the 60th day of the experiment was their number 1.3 times lower (76.6 ± 0.3%), compared to the control sample (96.65±3.35%). During the application of ammonium nitrate in the concentration of 45 g/cm^2 has been recorded the following inhibition of nitrogen-fixing microorganisms – it dropped in 1.2 times (81.7 ± 1.65%) on the 30th day of the experiment and in 1.24 times (78.3 ± 1.5%) on the 60th day of the experiment (Table 1).

Table 1 Percentage of the *Azotobacter* genus representatives during nitrate fertilizers application (x from each variant; n = 3; ± σ)

Fertilizer concentration	Nitrogen-fixing microorganisms	
	30th day of experiment	60th day of experiment
g/cm^2	%	
	Calcium nitrate	
0 (control)	98.4±1.65	96.65±3.35
30	95±1.70	83.4±6.65
60	96.7±3.35	81.7±1.65
90	88.4±1.65	76.6±0.30
	Ammonium nitrate	
0 (control)	98.4±1.65	96.65±3.35
15	93.3±0.50	81.7±1.65
30	93.3±0.50	83.3±1.00
45	81.7±1.65	78.3±1.50

Nitrate fertilizers influence on *Azotobacter* throughout soya bean cultivation has been characterized by further suppression of the microorganisms number (Table 2). Thus, during the application of calcium nitrate in the concentration of 90 g/cm^2 nitrogen-fixing bacteria content decreased in 1.4 times (63.3 ± 3.3%) on the 30th day of the experiment compared to the control sample (91.6 ± 1.65%) and in 3.8 times (21.6 ± 1.65%) on the 60th day of the experiment,

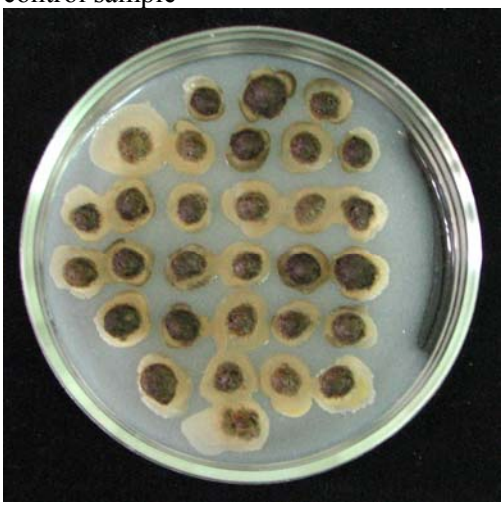
compared to the control sample (81,65±1,65%). Ammonium nitrate application throughout soya bean cultivation has also led to the nitrogen-fixing microorganisms inhibition in 1.7 times (53.3 ± 3.3%) on the 30th day of the experiment and in 2.1 times (38.3 ± 2.7%) on the 60th day of the experiment compared to the control samples (Table 2, see Fig.1).

Table 2 Percentage of the *Azotobacter* genus representatives during nitrate fertilizers application and cultivation of soya bean (x from each variant; n = 3; ± σ)

Fertilizer concentration	Nitrogen-fixing microorganisms	
	30th day of experiment	60th day of experiment
g/cm^2	%	
	Calcium nitrate	
0 (control)	91.6±1.65	81.65±1.65
30	85±1.65	70±6.65
60	76.6±1.70	21.6±1.65
90	63.3±3.30	21.6±1.65
	Ammonium nitrate	
0 (control)	91.6±1.65	81.65±1.65
15	70±0.30	70±3.35
30	66.6±3.35	41.6±8.35
45	53.3±3.30	38.3±2.70

Fig. 1 Representation of the ammonium nitrate fertilizer influence on the *Azotobacter* genus representatives throughout cultivation of soya bean (60th day of experiment)

A) Untreated with nitrate fertilizers control sample



B) Treated with ammonium nitrate fertilizer in concentration of 45 g/cm^2 sample

It has been showed in the works [12, 13, 14] that a long-term usage of mineral fertilizers in high doses inhibits germination of nitrogen-fixing microorganisms. Experimental results testify on whether or not the fertilization with increasing doses of nitrogen, such as ammonium nitrate and calcium nitrate, the percentage of the *Azotobacter* genus representatives reduces. Throughout soya bean cultivation a further nitrogen-fixing bacteria inhibition has been recorded. Using an ion-selective electrode method for the nitrates determination in the soil we have also discovered, that soya bean cultivation leds to an increase of mineral nitrogen amount in the soil, which explains the significant percentage reduction of the investigated bacteria genus (Table 3).

Table 3 Nitrate concentration changes before and after soya bean cultivation in the fertilized soil (x from each variant; n = 3; ± σ)

Fertilizer concentration	Nitrates concentration	
	Before soya bean cultivation	After soya bean cultivation
g/cm^2	mg/kg	
	Calcium nitrate	
0 (control)	151±8	210±15
30	203.4±6	428±7
60	668.1±32	758.2±26
90	712.8±28	1465.4±82
	Ammonium nitrate	
0 (control)	151±8	210±15
15	373.4±18,5	773.5±14,5
30	538.8±28	811.2±19
45	1105±40	1021±8

Our data are consistent with the data of other authors who have observed that the cultivation of legumes in monoculture and legume-cereal stock mixtures is accompanied by the *Azotobacter* number decrease comparing to growing cereals, which in turn is linked with a competition between symbiotic and associative nitrogen-fixing microorganisms, that are specifically supported by the plants in their own rhizosphere suppressing the development of free-living nitrogen-fixing bacteria [15, 16].

Correlation analysis between the percentage of *Azotobacter* in treated soil and the concentration of nitrate ions has been conducted. High and reliable correlation coefficients between the studied values, which have inversely proportional correlational relationships has been defined (Table 4).

Table 4 Correlation between the percentage of *Azotobacter* genus representatives and the concentration of nitrate ions in the fertilized soil before and after soya bean cultivation

Correlation coefficient			
30th day of experiment	60th day of experiment	30th day of experiment	60th day of experiment
Before soya bean cultivation		After soya bean cultivation	
	Calcium nitrate		
-0.62	-0.79	-0.99	-0.85
	Ammonium nitrate		
-0.99	-0.79	-0.99	-0.85

No data currently exists on the effect of biochar on free-living N fixers, however it is possible that these organisms would benefit from a reduced partial pressure of oxygen in the small pores of biochar (since oxygen destroys enzymes required for the biological fixation of N). For free-living diazotrophs, the fine pores of biochar create a habitat where reduced O_2 tensions are likely [17].

According to Thies and Rillig [18], if iron and Mn are sufficiently available for free-living N fixers, they could be favoured on and in biochar particles. If Fe and Mo are available in sufficient supply, the fixation of atmospheric N_2 will increase an organism's competitiveness in the biochar environment and, thus, their proportional representation within the biochar and soil community [17, 18].

Adding biochar to soil also appeared to stimulate the N_2-fixing activity of free-living diazotrophs. Ogawa [19] proposed that these bacteria might be poorer competitors whose survival in soil may be enhanced by their ability to colonize the biochar pores. Most biochars are very low in inorganic N content, giving diazotrophs a competitive advantage for surface colonization [17, 19].

Rondon et al. [20] in their studies found that the proportion of N derived symbiotically increased and the proportion of N derived from the soil decreased as more biochar was applied.

Conclusion

It has been established, that *Azotobacter* is a sensitive indicator to nitrogen fertilizers load, therefore a research conduction aimed at the study of

quantitative composition changes of these microorganisms in fertilized soils of different types in model experiment conditions is of great importance.

Our research conducted in Ukraine has showed that the number of free-living nitrogen-fixing bacteria has been reduced under the influence of nitrogen fertilizers. According to the laboratory experiment research results concerning the nitrate fertilizers application into the soil has revealed that the percentage of nitrogen-fixing microorganisms gradually decreases with the increasing doses of applied nitrate fertilizers, and as a result the number of nitrogen-fixing bacteria decreased on average in 1.1 - 3.8 times compared to a control sample.

In addition, high inverse correlation coefficients up to -0.99 on the 30th day of experiment between the percentage of *Azotobacter* and the concentration of nitrate ions in the studied soil have been obtained.

Specific changes in the quantitative composition of nitrogen-fixing microorganisms of soil, treated by nitrate addition, allows us to confirm the high sensitivity of this kind of microorganisms to anthropogenic impact and to recommend them as an ecological indicators of the possible changes in anthropogenically transformed soil, that support the possibility of using *Azotobacter* for bioindication in our further studies with a biochar application.

Acknowledgement

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A Trend of the Five-Year Development Temperatures computed from Average Monthly Temperatures in the Territory of the Czech Republic

RENATA OSICKOVA, STANISLAV BARTON, VOJTECH KUMBAR
Department of Technology and Automobile Transport
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xosicko7@node.mendelu.cz

Abstract: In this paper, its authors process and analyze values of average monthly temperatures recorded in 34 meteorological stations since January 2003 till December 2013 that are uniformly distributed in the territory of the Czech Republic. At first statistical relevance of each term of the used regression function is evaluated using the sum of squared residuals per degree of freedom. A second function containing only significant terms is tested on the edge of statistical reliability equal to 99%, 95% and 90% with respect to its individual terms. The Fisher-Snedecor function was used to determine really important terms of the evaluated function. The Final function enabled computation coefficients of 7 regression functions explaining recorded data in 7 time steps of 5 year intervals. These functions were used to determine border positions splitting areas of the Czech Republic into zones of local warming and cooling. Recorded data is plotted graphically and shows that this border oscillates over the whole territory of the Czech Republic.

Key-Words: global warming, mathematical modelling, regression function, linear correlation, space and time coincidence, temperature trends

Introduction

Problems of global warming represent a widely discussed theme that is the focus of interest of the major part of world population, for example [2, 9]. Many authors publish papers that accentuate the fact that the process of global warming is real and quite inevitable while some others write that this is a disputable phenomenon and that the global warming is a mere fiction, [3, 5].

In this paper we present a mathematical study of the development of diurnal temperatures in the territory of the Czech Republic within the period of the recent decade from January 2003 till December 2013. Using a Maple application, see [6], based on the method of least squares, we have developed a regression function $T(t,x,y,h)$, which explains the dependence of temperature on time, geographical position and height above the sea. To determine the significance of the regression function members that have been tested with a confidence interval of 90%, 95% and 99% have been used in the Fischer-Snedecor function (4). The resulting functions allow us to calculate the coefficients of the regression function, which is used at the end and are necessary for determining the motion boundary warming and cooling in the area of the Czech Republic. Calculations were made for seven five-year cycles, and gradually shifted by one year.

Material and Methods

Data concerning average monthly temperatures as recorded within the period of last ten years in 22 selected meteorological stations are normally available on the Internet, [10]. As far as further 12 stations are concerned, similarly data can be obtained from graphs that are available at the web page, [11].

The Czech Hydrometeorological Institute collects data about daily temperatures, as measured and recorded in a much higher number of meteorological stations already for a long time period. These data, however, can be obtained only on the base of payments and for that reason they are not available for wider public.

Nevertheless, data recorded in available 34 meteorological stations cover the territory of the Czech Republic adequately and in a satisfactory manner. The minimum airline distance between two stations is 12 km while the maximum does not exceed 54.7 km. Distances were recomputed from GPS coordinates onto *XY* coordinates with respect to [8, 12]. Data presented in this paper informs about an exact geographical location of the station, about its altitude and also about average monthly air

temperatures. Temporary data is expressed as yearly fractions and the time t = 0 corresponds with the 1st January 2003. In case that partial data about the temperature is excluded or missing, the temperature is rewritten by -99 °C. Stations with incomplete data are highlighted in red, stations in Group 1, or in blue, Group 2. Data from Group 2 is reconstructed from graphs.

$$F(t,x,y,h) = c_1 x\cos(kt) + c_2 x\sin(kt) + c_3 x + c_4 xt + c_5 xt^2 + c_6 y\cos(kt) + c_7 y\sin(kt) + c_8 y + c_9 yt + c_{10} yt^2 + c_{11} h\cos(kt) + c_{12} h\sin(kt) + c_{13} h + c_{14} ht + c_{16} ht^2 + c_{17}\cos(kt) + c_{18}\sin(kt) + c_{19} t + c_{20} t^2, \quad (1)$$

The first step is to determine the statistical significance of each member of the regression function (1), where $k = 2\pi$, using the sum of squared residues on one degree of freedom – SQR_1 with a precision of 15 significant digits, (2).

$$SQR_1 = \frac{\sum_{i=1}^{N}\left(F(t_i,x_i,y_i,h_i) - T_i\right)^2}{N-p}, \quad (2)$$

where N = number of measurements, t = time, $[x,y,h]$ = spatial coordinates, p = number of function parameters F, F = seeking regression function, T_i = measured temperature. It turned out, not all members of the function (1) leads to a reduction in the sum squared residuals at one degree of freedom. Therefore, the resulting function (3) contains only members that meet this condition. In addition, each member in the list was sorted according to their importance.

$$F_3(t,x,y,h) = 10.9319 - 10.5003\cos(kt) - 2.4811\sin(kt) - 0.0059h + 0.0012h\cos(kt) - 0.0004yt - 0.0022x - 0.0010x\cos(kt) - 0.0013x\sin(kt) - 0.0004h\sin(kt) - 0.0000ht^2 + 0.0015y\cos(kt) + 0.0001ht - 0.0022y + 0.0000xt^2, \quad (3)$$

The accuracy of calculation was then verified with a precision of 36 digits in force according to another algorithm, see [4]. All computations were done in the programme Maple, [6]. Both calculations differed only within the selected numerical precision, which means that their relative difference was of the order of 10^{13}%. The coefficient of linear correlation for each station for the entire period 2003-2013 ranges from 0.961 to 0.975, average correlation coefficient of linear spatial temperature distribution in the Czech Republic is 0.931. This means that the regression function can be considered as satisfactory.

Fischer-Snedecor function, see [13, 7], *F(z),* (4), is used for testing and determining the significance of members of the regression function with more coefficients compared to simpler regression function. Level of the uncertainty is α.

$$\int_0^q F(z)\,dz = 1-\alpha, \text{ where } F(z) = \frac{\left(\frac{\kappa}{n}\right)^{\left(\frac{\kappa}{2}\right)} z^{\left(\frac{\kappa}{2}-1\right)}}{B\left(\frac{\kappa}{2},\frac{n}{2}\right)\left(1+\frac{z\,\kappa}{n}\right)^{\left(\frac{\kappa}{2}+\frac{n}{2}\right)}}, \quad \begin{matrix}\kappa = \text{difference of count of parameters of functions}\\ n = \text{count of measurements.}\end{matrix} \quad (4)$$

This feature, with accuracy *1-*α, tells us how to change the statistical significance of the function, if we add more function members. More complex functions is statistically significant if it satisfies the condition (5). Selection of the most suitable model is performed on the basis of a test which is based on the inequality (5), see tables XVIII 4a-4c in [1].

$$\frac{\frac{S_R(1)-S_R(2)}{p_2-p_1}}{\frac{S_R(2)}{n-p_2}} \geq q \quad \text{where} \quad \begin{matrix}S_R(1) = \text{is the residual sum of squares of a simple model}\\ S_R(2) = \text{is the residual sum of squares of a complex model}\\ p_1 = \text{number of coefficien ts of a simple model}\\ p_2 = \text{number of coefficien ts of a complex model}\end{matrix}, \quad \kappa = p_2 - p_1. \quad (5)$$

Results and Discussion

Determining individual members of regression function

For each member of the regression function (1) containing 20 members is calculated the sum of squared residuals per 1 degree of freedom, further shorten as **SQR1,** see [14]. Selected is the value where **SQR1** is the lowest. This selected member is searched from the remaining 19 members, another member such that **SQR1** for two members was the lowest. It is being continued for each subsequent member by this manner until they are exhausted all the members or **SQR1** begins to rise. In this way, we find that members *2xt, y,* sin*(kt), yt*^{2}*, t, t*^{2} are meaningless. Graph showing **SQR1** as a function of the number of members is shown below, (see Fig. 1). From this graph it is clear that with the increasing number of sum of squared residuals on 1 degree of freedom to the 15th member decreases. Graph showing **SQR1** for two consecutive functions that differ from each other about member is for 5th to 15th member shown below, (see Fig. 2).

Fig. 1 **SQR1** as a function of count of operands of F3

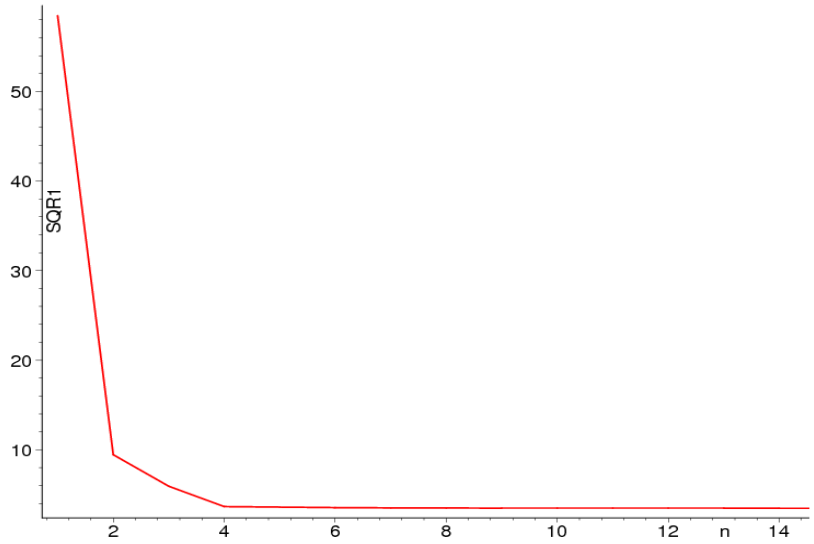
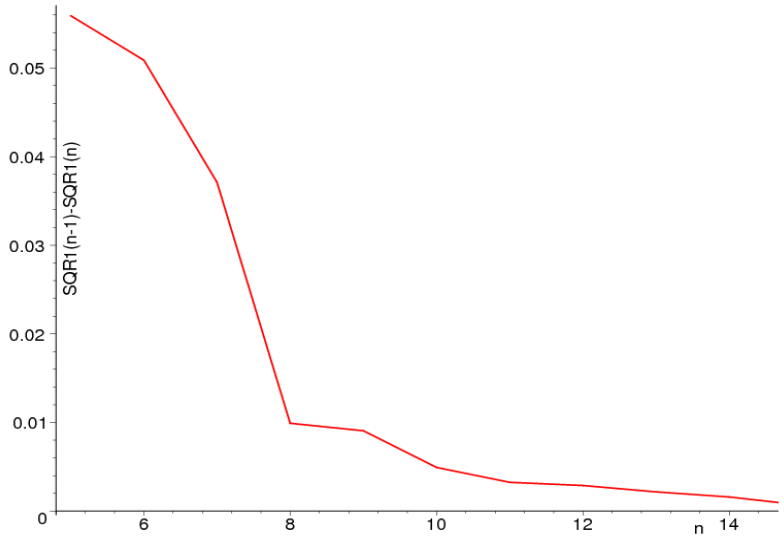
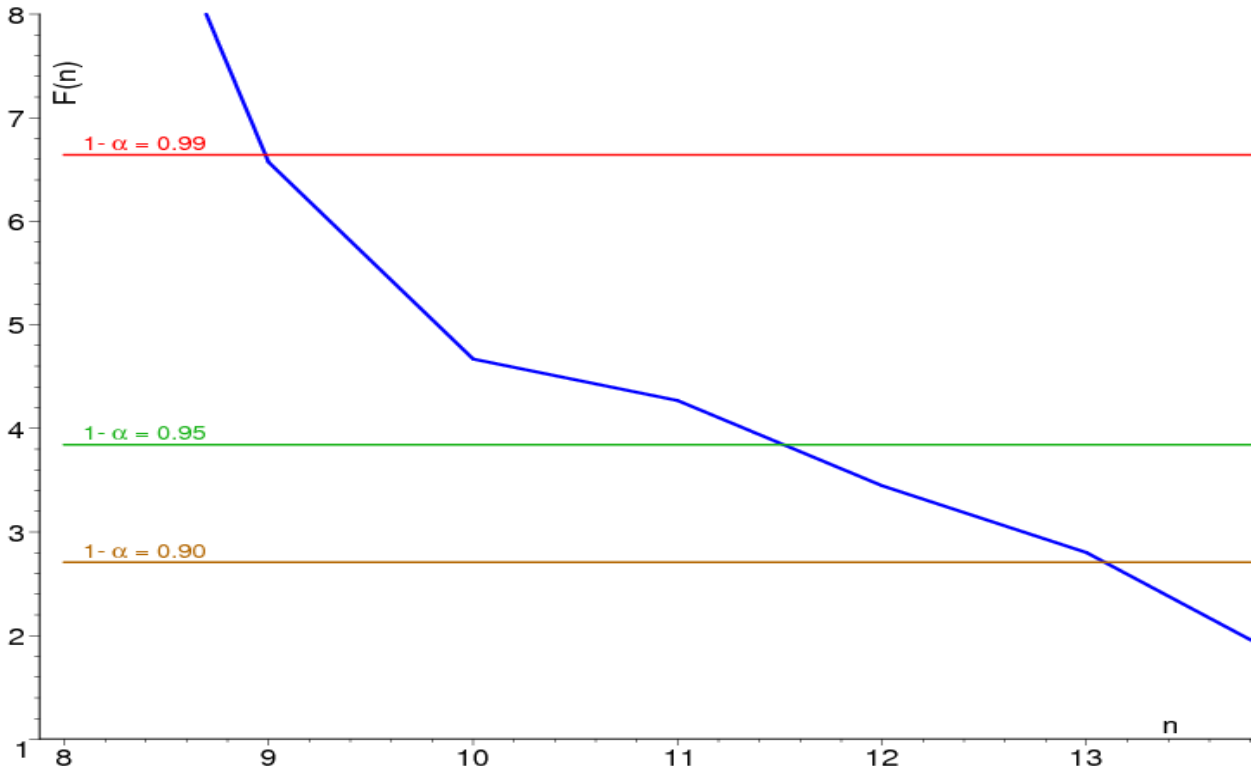


Fig. 2 **SQR1(n) - SQR1(n-1)** as a function of count of operands of F3



Test to determine whether a more complex model (multivariable) better than the simpler model is done with respect to the equation (5). The more complex function is omitted if $\boldsymbol{F} > \boldsymbol{F}_{1-\alpha}(p2-p1,\ n-p2)$.The count of operands of functions that correspond to the reliability of α = 90%, α = 95% and α = 99%, (see Fig. 3).

Fig. 3 Value of the q, (5), as a function of the count of operands of the function F3



Periodic components sin*(kt)* and cos*(kt)* are removed of these functions appropriate to the individual reliability. Then a derivation function by time is done, see (6).

$$\begin{aligned} f_{99} &= 10.93199 - 0.00586\,h - 0.00038\,y\,t - 0.00222\,x \\ f_{95} &= 10.93199 - 0.00586\,h - 0.00038\,y\,t - 0.00222\,x - 0.00001\,h\,t^2 \\ f_{90} &= 10.93199 - 0.00586\,h - 0.00038\,y\,t - 0.00222\,x - 0.00001\,h\,t^2 + 0.00014\,h\,t\,. \end{aligned} \tag{6}$$

These derivatives are set equal to zero, see (7). Furthermore, the coordinates *y* are calculated and the exact position of 3 boundary for a 5-year intervals are found.

$$\begin{aligned} ft_{99} &= -0.00038\,y \\ ft_{95} &= -0.00038\,y - 0.00003\,h\,t \\ ft_{90} &= -0.00038\,y - 0.00003\,h\,t + 0.00014\,h\,. \end{aligned} \tag{7}$$

The functions corresponding reliability α = 99% passes through the center of gravity of the Czech Republic. This fact shows the graph in Fig 4 – positions on the map of the Czech Republic and Fig. 5 showing average *y* position of the boundaries in 7 five years intervals.

From the calculations above it is clear that members of the 9 explain 99% of the data, the members of the 13 explain 95% of the data and the members of the 14 explain 90% of the data. The boundary, 95% and 99% reliability are plotted on the graph, (see Fig. 4).

In our case, we work with 95% reliability, for which the corresponding function contains 14 members. This reliability is designed for more complex models. The boundaries, corresponding to 99%, is stable and passes through the center of gravity of the Czech Republic. The functions corresponding 99% reliability, contains 9 members and it is independent on time.

Fig. 4 Average cooling/warming border position of 5 year intervals in the Czech Republic for 1-α = 95%

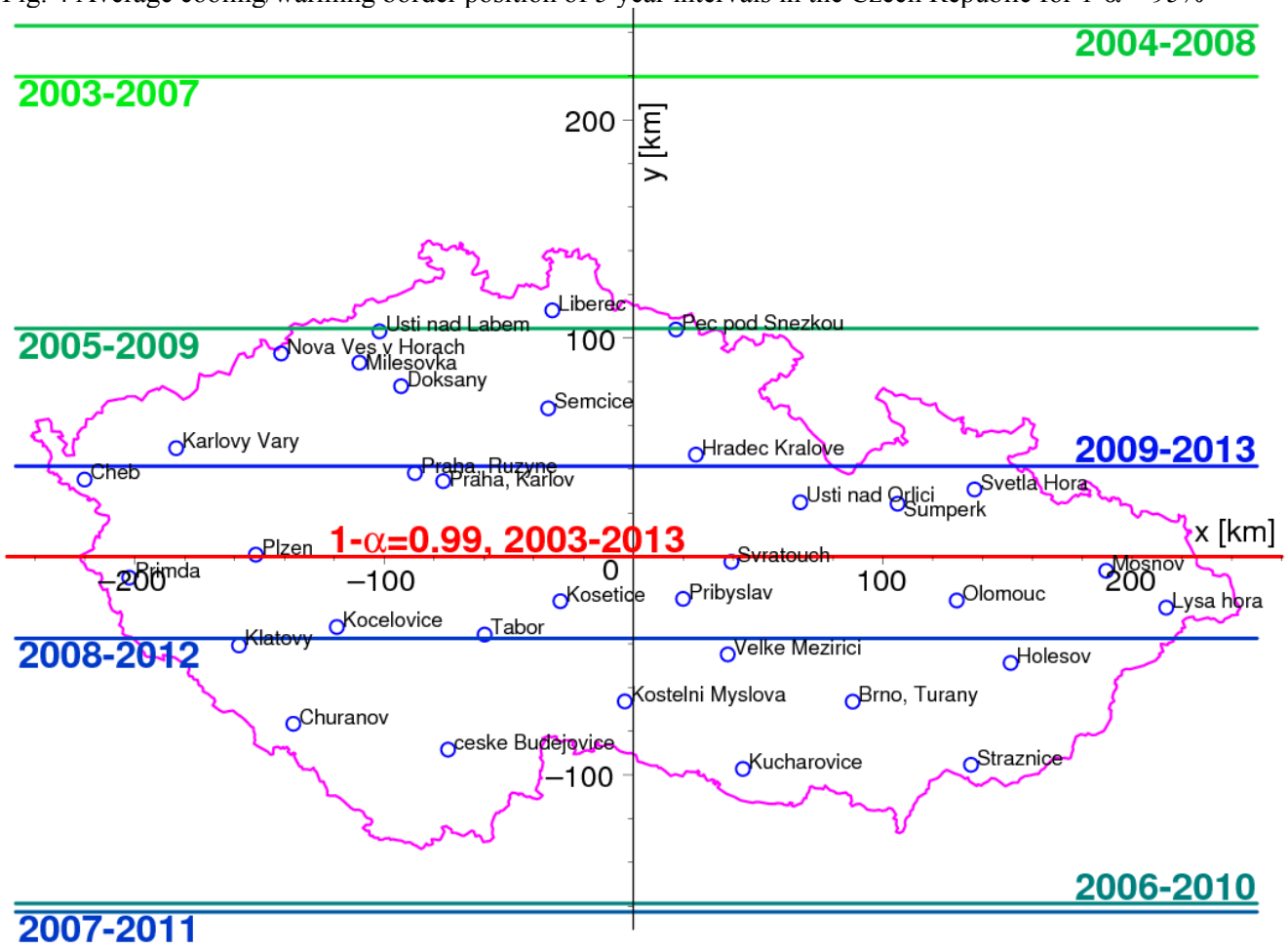
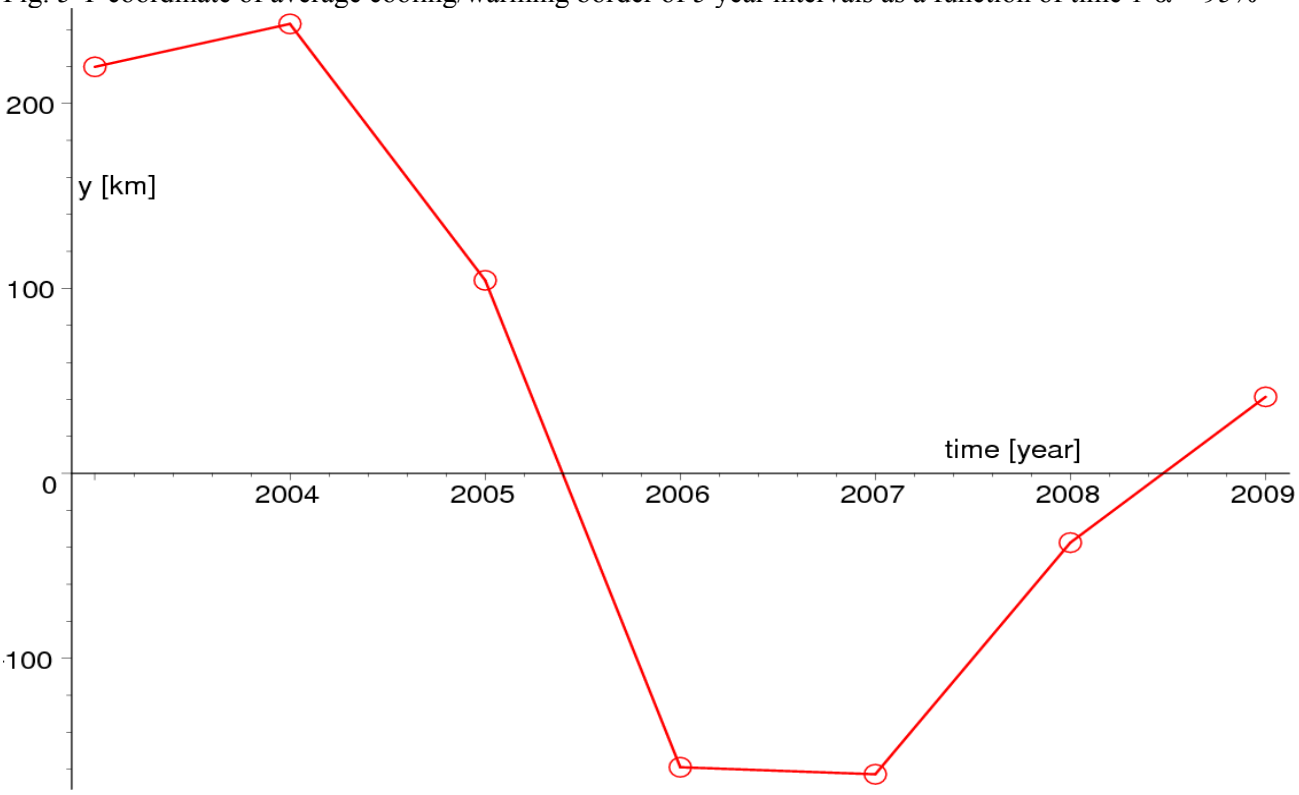


Fig. 5 Y coordinate of average cooling/warming border of 5 year intervals as a function of time 1-α = 95%



Conclusion

In area of the Czech Republic there is a border between areas where warming and cooling are occurring. If we use a simpler function, then a position of this boundary has coordinates y = 0 (the border passes through the center of gravity of the Czech Republic) and is not dependent on time. This feature is able to explain 99% of the measured data.

From the properties of the more complex function, it follows that the position of the boundary is moved over the whole area of the Czech Republic, (see Fig. 4 and Fig. 5).

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The Effect Evaluation of Selected Land Use Category on the Change of Water Quality

VERONIKA PETRAKOVA, VERA HUBACIKOVA, PETRA OPPELTOVA,
KATERINA ZAKOUTSKA
Department of Applied and Landscape Ecology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xpetrak1@node.mendelu.cz

Abstract: Thanks to a current human effect on the landscape, the surface water quality depends on the methods of landscape management. The purity and quality of surface water results in both the characteristics of the water itself and the surrounding environment and also the biotic and abiotic conditions that this environment creates. The investigation was under way from March until November 2014 and it took place on chosen river flows on the border profiles between the individual land use categories. Small river flows with the watershed size to 10 km^2 were chosen in order to investigate how the water quality parameters (pH value, temperature, COD, conductivity, O_2, NO_3^- N, PO_4^{-3} and P_{total}) change when measured in selected sampling sites .The analysis of water quality indicators was realized in the laboratory of Mendel University. According to the comparison to each other the effect of land use categories on the water quality will be evaluated. Following land use categiroes were assessed: Forests, Permanent Grassland, Arable land, Scattered greenery, Built-up area, surface Water, Gardens, Roads and Orchards. Only four of six listed water quality parametres (pH value, temperature, NO_3^- N and PO_4^{-3}) are assessed in this paper. The analysis resulted in the deterioration of pH value, temperature and PO_4^{-3} in the third sampling profile – Built – up area. The highest nitrate nitrogen concentration was determined at the first sampling profile that is the source of the stream.

Key-Words: land use, self – purification, water quality, waste water, pollutants enrichment

Introduction

The Czech Republic is located in the territory of free main European watersheds. And thus the purity and quality of surface waters, which origin and flow through Czech and Moravian territory, is strictly controlled, because such flows represent the a life-giving source of water both in the Czech Republic and certain ecosystems bound directly or indirectly on water with specific supply to water quality. The land use belongs to the factors affecting the quality of surface water. These are the following categories: Forests, Permanent Grassland, Arable land, Scattered greenery, Built-up area, surface Water, Gardens, Roads and Orchards [2]. The land Use represents one of many factors, which can affect the surface water quality in the abroad, where the rivers subsequently flow. An increasing human effect on the natural resources may gradually lead to their depreciation, which can result in changes of their biological, physical or chemical characteristics. Such changes may result in damage or the disappearance of certain ecosystems which are directly or indirectly linked to the water resource. Partial results of an effect of concrete land use categories on the water quality change which were measured in six months (April – September), are summarized in this paper.

Material and Methods

The rivers stream Pernička was selected in order to evaluate the impact of individual land use categories on the water quality change. The Pernička origins above the Počítky village which is situated in the southern part of National Reserve Žďárské vrchy, which belongs to both the protected area and protected area of natural water accumulation called Žďárské vrchy. The exact location of Počítky village and the Pernička stream is shown in the Fig. 5. The selected catchment area ranged from 5 – 11 km^2 .The origin and whole upper part of Pernička stream is situated in the area with livestock breeding and pasture. The village Pernička is not equipped by the waste water treatment plant. Five sampling sites were selected on the river flow resulting in 3 220 m length, just on the borderline of individual land use categories. The order of land use categories which Pernička stream flows through is following: Permanent grassland – Built – up area – Arable land

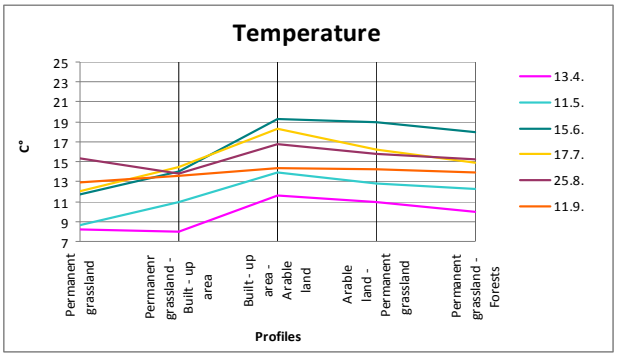
– Permanent grassland – Forests. Measurement and sampling are realized once a month from March 2014 to November 2014. The characteristics of water quality were analysed directly in situ using portable multimeter HQ30d by Hach-Lange in order to measure pH value, water conductivity, oxygen content and temperature. From the point of water quality change view the significant characteristics ($P_{total.}$, PO_4, COD, NO_3^- N, etc.) were determined in laboratory. Hubačíková (2014) deals with the similar theme [1]. According to measured values the impact of individual land use and the change of water quality were assessed on individual sites of observed stream. For now it is not possible to evaluate the impact of individual land use categories. This paper is focused only on the evaluation of certain indicators of water quality for the chosen stream – pH, temperature and NO_3^- N, PO_4^{3-}.

Results

- Temperature - t

The development of the temperature value is equal for all six measurements. During the flow through Permanent grassland the temperature was being reduced. Afterwards during the flow through Built – up area the temperature increased and then in decreased when flowing through Arable land and Permanent grassland. There is a small water pond above the third sampling site. Water accumulated in that pond is being warmed up. And thus the Built – up area significantly affects the water stream temperature.

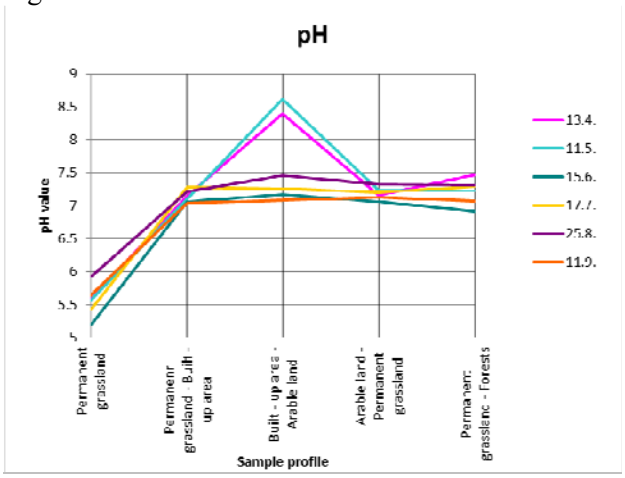
Fig. 1 Temperature value



- pH value

First sampling site (the flow origin with the livestock pasture) resulted in similar value for all the measurements resulting pH value under 6.0 concretely between 5.2 – 5.93. According the pH scale it corresponds to moderate acid to neutral pH value. In the second sampling site (Permanent grassland – Built – up area) the pH value increased to neutral value ranging from 7.04 – 7.22. According to the measurement in April and May the value increased again up to the value approximately 8.5 (8.4 – 8.62) in the third sampling site (Built – up area – Arable land). From July to September the value did not significantly changed in the third sampling site. It ranged between 7.0 – 7.5 corresponding to neutral pH value. The same results were observed for the fourth and the fifth sampling sites. No significant deflection from neutral pH value was measured during whole sampling period. According to Meybeck nad Helmer (1992) in the global scale acidification is considered to cause occasional or regional deteritoration of water quality [7].

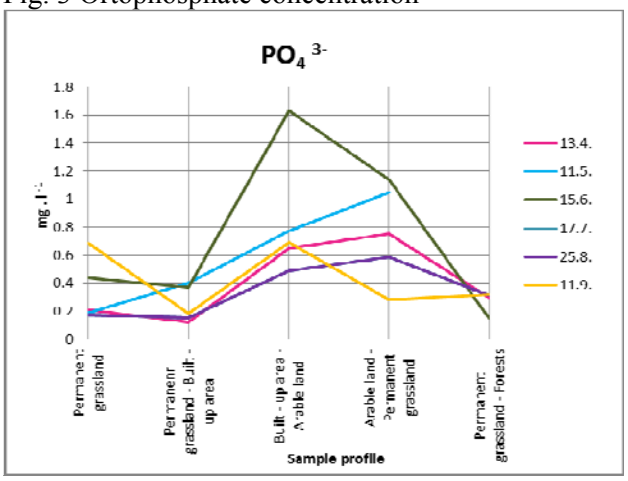
Fig. 2 Ph value



- Orthophosphates - PO_4^{-3}

The presence and concentration of orthophosphate were determined in the laboratory within 24 hours after sampling. In the second sampling site (Permanent grassland –Built – up area), where the livestock is grazed, five measurements resulted in decrease of orthophosphate concentration according to the values measured in the origin site. The increase of orthophosphate concentration was observed in the third sampling site for all samples. In the third site the highest value was measured in the middle of June, when the orthophosphate concentration reached 1.6 mg * l^{-1} which is equal to a double concentration value of other five measurements. In the fourth sampling site, the concentration slightly increased in three cases and it decreased in two cases. In the fourth sampling site (Arable land – Permanent grassland), the concentration slightly decreased again in all samples. There are no results for orthophosphate concentration for fifth sampling site in May due to the extensive growth of plants in the riverbed.

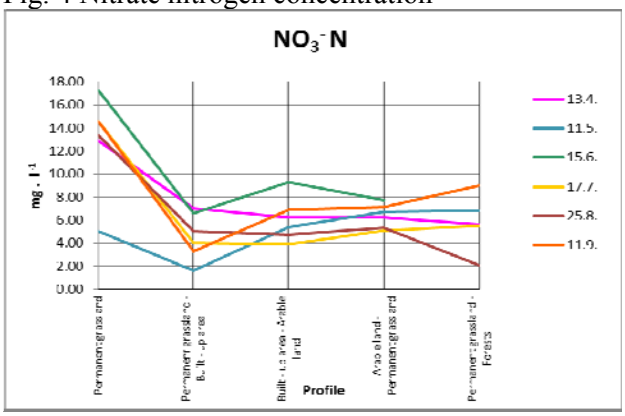
Fig. 3 Ortophosphate concentration



- Nitrate nitrogen - NO_3^-N

The presence and concentration of nitrate nitrogen were determined in the laboratory within 24 hours after sampling. The highest values (12.9 – 17.3 mg $*l^{-1}$) were measured in five samples in first sampling site. The analysis proved obvious decrease of NO_3^-N concentration in all samples collected in the second sampling site (Permanent grassland – Built – up area). Three samples resulted in decrease and three samples resulted in increase of nitrate nitrogen concentration in the third sampling site. There are no results for nitrate nitrogen concentration for fifth sampling site in May due to the extensive growth of plants in the riverbed. According to Meybeck nad Helmer (1992) in the global scale Nitrate as a pollutant is considered to cause occasional or regional deteritoration of water quality [7].

Fig. 4 Nitrate nitrogen concentration



Discussion

The measured values obtained during a six months measurement at five sampling profiles were averaged and these values were compared with values given in Regulation No. 61/2003 Coll., Government Regulation on indicators and values of acceptable pollution of surface water and wastewaters, details of the permit to discharge wastewater into surface water and sewerage systems and sensitive areas, Annex. 3 - Indicators expressing the status of water in the watercourse, environmental quality standards and requirements for water use. (Tab.1).[5] Nitrate nitrogen value exceeded the prescribed value in all five sampling profiles. The pH value exceeded the prescribed value only in the third sampling profile. The PO_4^{3-} value and temperature did not exceeded prescribed value during whole sampling period.

The measured values were then compared with Czech national standard No. 75 7221 Water Quality - Classification of surface water quality [4]. According to this standard the results obtained during at least one year can be evaluated. Additionally, only the samples with following analyzed parametres: saprobic index of macroinvertebrates, biochemical oxygen demand, chemical oxygen demand by dichromate, nitrate nitrogen, ammonia nitrogen and total phosphorus, can be classified. The values presented in this paper were obtained during a period of six months. Only 3 parameters of the mandatory parameters needed for the classification of the surface water quality were analyzed (nitrate nitrogen, total phosphorus and chemical oxygen demand). Therefore, the following comparison is only approximate. Preliminary results showed that a significant impact of different land use on water quality changes have Built up area and Permanent grassland, with cattle grazing. The classification of surface water quality is according to the Czech National Standard No. 75 7221[4] is given in Tab. 2. According to Stoate and Boatman et al. (2001) the greatest impact of the water quality is associated with simplified, high input arable systems where the nitrates and some other pesticides entre groundwater follofing leaching from arable land [6].

Conclusion

The results presented in this paper are only a partial output of the presented project dealing with the categorization of stream sections according to the land use. The previously measured results show that municipalities have significantly greater negative impact on the water quality than agriculturally cultivated land. The Pernička village households are not equipped by the waste water treatment plant and thus all the waste water pollution is treated in individual septic tanks. There is a probability of leach of such waste water into the Pernička stream. The measured data confirmed the assumption that Built – up area and Permanent grassland with

livestock grazing have got an significant effect on the water quality change.

Acknowledgement
The paper was created with support of Internal Grant Agency MENDELU project "Categorization of selected streams based on their degree of self-purification capacity with respect to landuse".

Fig. 5 The Pernička stream flowing through Počítky village (geoportal.gov.cz)

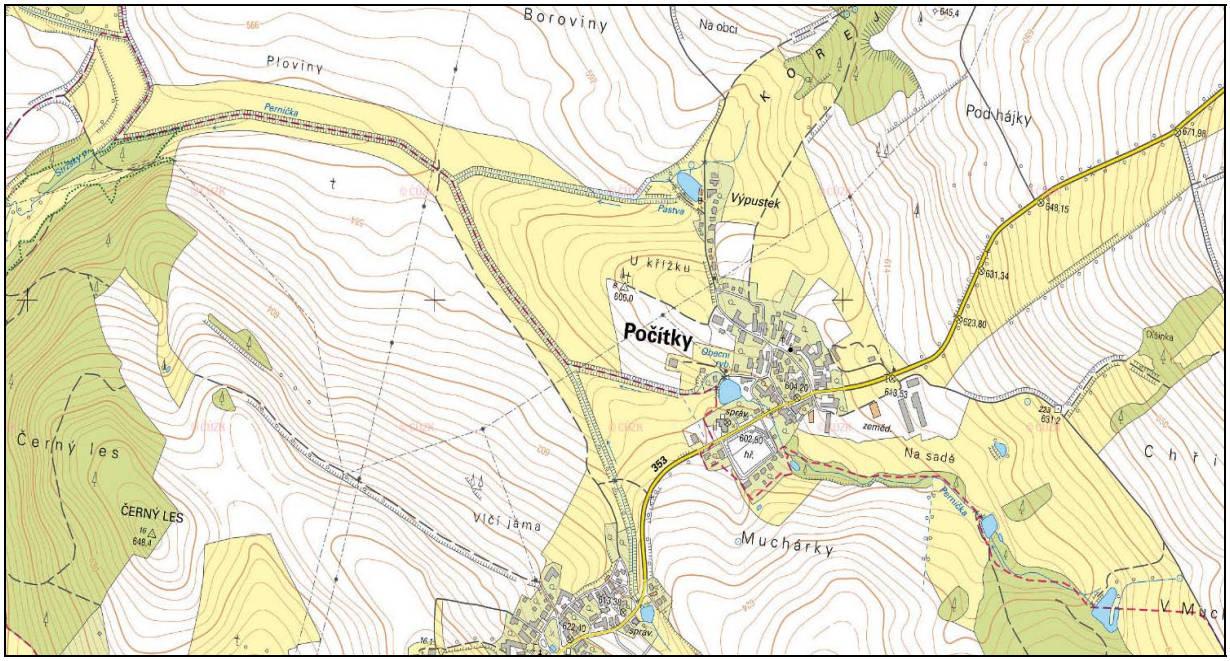


Table 1 Comparison of standardized values (Government regulation No. 61/2003) and averaged measured values

	Standartized value	Permanent grassland	Permanent grassland - Built - up area	Built - up area - Arable land	Arable land - Permanent grassland	Permanent grassland - Forests
NO^{3-} N	5.4	15.56	5.5	7.28	7.62	5.8
PO_4^{3-}		0.3756	0.277	0.9442	0.881	0.2794
pH	6.0 – 9.0	6.672	8.572	9.198	8.624	8.66
t	29	13.76	14.96	18.86	17.8	16.86

Table 2 Comparison of averaged analyzed characteristics with Czech national standard value (ČSN 75 7221)

	Permanent grassland		Permanent grassland - Built - up area		Built - up area - Arable land		Arable land - Permanent grassland		Permanent grassland - Forests	
	sample	Czech national standard	sample	Czech national standard	sample	Czech national standard	sample	Czech national standard	sample	Czech national standard
NO^{3-} N	1.56	5	5.5	2	7.28	3	7.62	3	5.8	2
P_{total}	7.07	5	11.78	5	7.05	5	6.79	5	6.14	5
COD	6.06	1	41.01	3	6.05	1	5.82	1	5.27	1

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[3] Government regulation No. 23/2011 Coll. about indicators and values of permitted pollution of surface waters and waste waters, essentials permit to discharge wastewater into surface waters and sewers and sensitive areas

[4] Czech Technical standard No. 75 7221 Water Quality - Classification of surface water quality

[5] Regulation No. 61/2003 Coll., Government Regulation on indicators and values of acceptable pollution of surface water and wastewaters, details of the permit to discharge wastewater into surface water and sewerage systems and sensitive areas, Annex. 3 Indicators expressing the status of water in the watercourse, environmental quality standards and requirements for water use

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Comparison of mineral nitrogen leaching in lysimetric and laboratory experiment with different types of fertilization

LUKAS PLOSEK, JAROSLAV ZAHORA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

lukas.plosek@mendelu.cz

Abstract: Compost can influence soil fertility and plant health. At the same time compost can play an important role in the nitrogen cycle and it can influence leaching of mineral nitrogen from soil to underground water.
This paper deals with the influence of compost addition and mineral nitrogen fertilizer on leaching of mineral nitrogen in the laboratory pot experiment and field lysimetric experiment - twenty one lysimeters were filed with topsoil and subsoil collected in the area of protection zone of underground source of drinking water - Březová nad Svitavou.
There are large differences between arable soils with addition of compost and arable soils without, as it was expected. Compost has a positive impact on the soil environment.

Key-Words: compost, mineral nitrogen leaching, carbon, fertilization

Introduction

Application of compost in agriculture is very desirable worldwide. In the Czech Republic, compost is the most often used to improve soil structure and increase the content of soil organic matter.

The advantages of the use of organic wastes such as compost as fertilizers are evident. Their use would reduce the consumption of commercial fertilizers which need in their production high cost and energy [11]. Compost amendment improves physical, chemical and biological properties of soils, in particular by increasing available nutrients mainly in the organic soil fractions [4, 5] and there is evidence that application of organic amendments to soils could also reduce soil mineral N [13]. Many studies have reported that addition of a high C, low N organic amendment to soil can stimulate microbes to take up the available N from their environment for their own growth, in a process known as immobilisation [1, 6, 10]. Also, the application of compost increases the plant cover and stimulates soil microbial growth and activity [11, 12]. On the other hand, if the compost is applied in high doses it can negatively influence desirable groups of microorganisms, reduce yield of crops, increase leaching of nutrients [4].

Leaching of mineral nitrogen (consisting of NH_4^+-N and NO_3^--N) from arable land is a major threat to the quality of drinking water from underground reservoirs in the Czech Republic [6]. In the present paper, effect of compost addition on leaching of mineral nitrogen from arable land was investigated and evaluated in the laboratory pot experiment and in the field lysimetric experiment.

Material and Methods

Laboratory pot experiment

Experiment was performed in experimental containers with circular floor plan (Figure 1).
Containers were filled with 550 g of soil with added fertilizers according to the designed variants. Six seed of *Lactuca sativa* L. (salad) were planted in each container. After one week, we left one germinated seed in each container. During whole experiment, plants were kept in a climate chamber at 22 °C with a day length of 16 h and a light intensity of 300 $\mu mol \cdot m^{-1} \cdot s^{-1}$. Every day, salad was irrigated with 20 ml of distilled water.

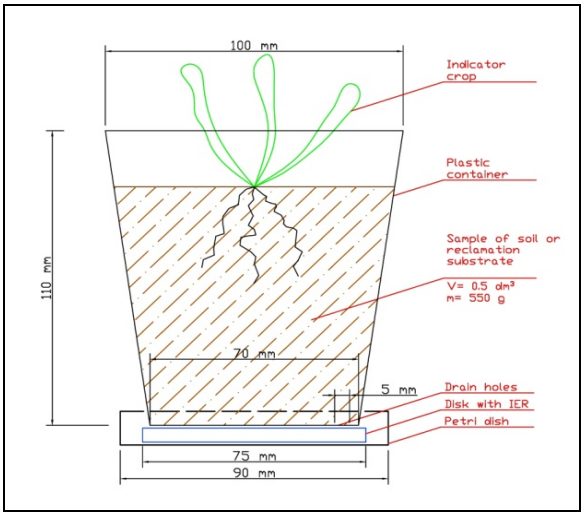
Variants of the experiment:

- C - soil as a control variant without addition of compost or mineral fertilizer;
- ČD 50 - variant with addition of compost Černý drak (28 g) from Central composting plant in Brno. In conversion, this dose represents 50 $Mg \cdot ha^{-1}$;
- CMC 50 - variant with addition of compost CMC (28 g) from composting plant in Náměšť nad

Oslavou. In conversion, this dose represents 50 $Mg \cdot ha^{-1}$;

- ČD 100 - variant with addition of compost Černý drak (56 g) from Central composting plant in Brno. In conversion, this dose represents 100 $Mg \cdot ha^{-1}$;
- CMC 100 - variant with addition of compost CMC (56 g) from composting plant in Náměšť nad Oslavou. In conversion, this dose represents 100 $Mg \cdot ha^{-1}$;
- NPK - variant with addition of inorganic fertilizer NPK (0,6 g) from Lovochemie a.s. In conversion, this dose represents 60 kg $N \cdot ha^{-1}$. NPK is common mineral fertilizers containing N, P, K and S in the ratio 10:10:10:13. The NPK fertilizers were dissolved in 200 ml of distilled water (for each variant with NPK). NPK fertilizers irrigation was applied twice a week;
- ČD 50 + NPK - variant with combination of compost addition and inorganic fertilization.
- CMC 50 + NPK - variant with combination of compost addition and inorganic fertilization.

Fig. 1 Design of the laboratory pot experiment [6]



Measurement of the leakage of mineral nitrogen

For measurement leakage of mineral forms of nitrogen (NH_4^+-N and NO_3^--N) was used modified methods of [2, 8]

Mineral nitrogen (NH_4^+-N and NO_3^--N) leached from the soil was captured by special discs with mixed IER (Ion Exchange Resin), which were located under each experimental container (see Figure 1). The discs were made from plastic (PVC) tubes. Each disc was 75 mm in diameter and 5 mm thick. From both sides of each disc, nylon mesh was glued (grid size of 0.1 mm). Mixed IER (CER– Cation Exchange Resin and AER– Anion Exchange Resin in ratio 1:1) were then placed into the inner space of annular flat cover [8].

For the quantification of N_{min} trapped by the resin (CER and AER), the IER were allowed to dry at room temperature. Captured N_{min} was extracted from resin using 100 ml of 1.7 M NaCl. Released N_{min} was determined by distillation and titration method [9]. The results obtained from the Ion Exchange Discs were expressed in mg/dm^3 of N_{min}, NH_4^+-N and NO_3^--N of soil.

Lysimetric experiment

Twenty one lysimeters have been used as experimental containers and located in the area. The experiment was conducted in the protection zone of underground source of drinking water Březová nad Svitavou, where annual climatic averages (1962-2012) are 588.47 mm of precipitation and 7.9 °C mean of annual air temperature [6]. The lysimeters were made from PVC (polyvinyl chloride). Each lysimeter was the same size and was filled with 25 kg of subsoil and 25 kg of topsoil (arable soil). See Figure 2.

Topsoil and subsoil were collected from a field in the area. Soil samples were sieved through a sieve (grid size of 10 mm) and homogenized. Topsoil and subsoil were prepared separately. Each lysimeter had one drain hole and PVC hose for collecting soil solution. Hose leads into the plastic bottle. All lysimeters were buried into the ground. Collection of soil solution and monitoring of the lysimeters was carried out in the control shaft. Lysimeters were completed and filled in October 2012. Winter wheat (*Triticum aestivum*) was used as a model plant to determine the effect of fertilizers and on plant production. The model crop was planted into each lysimeter in October 2012.

Seven variants (C1, C2 and K1 - K5) were prepared: C1 (control - without fertilization), C2 (control - with 100 % of recommended doses of N), K1 (100 % of recommended doses of compost), K2 (100 % of compost, 25 % of N), K3 (100 % compost, 50 % of N), K4 (100 % of compost, 100 % of N), K5 (200 % of compost).

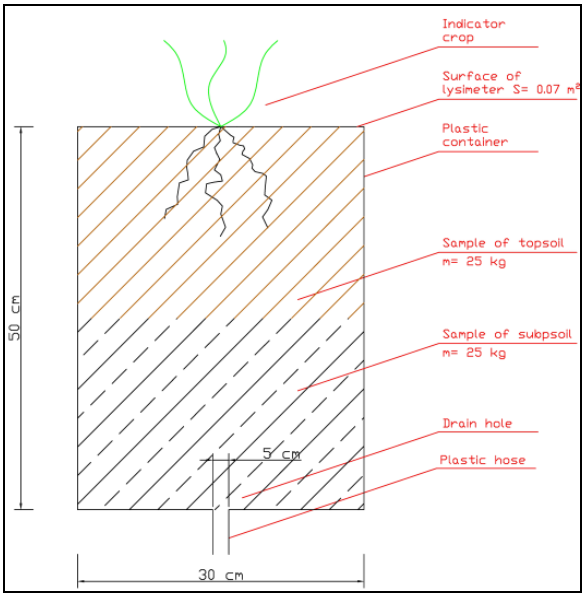
Information on the applied fertilizers: Compost was applied in recommended doses of 50 Mg ha^{-1} from Central composting plant in Brno (Compost Černý drak). Nitrogen was applied as a liquid fertilizer DAM 390 converted in dose of 140 kg ha^{-1} of N.

Determination of mineral nitrogen

Soil solution was collected into plastic bottles, which were placed in the control shaft. The amount of the solution was monitored three times per week. If a solution was found in a bottle, it was taken for

the determination. Samples were stored at 4°C before the determination.

Fig. 2 Detail of the lysimetric experiment [10]



Concentration of mineral nitrogen (N_{min}) was measured using distillation-titration method by [9]. Ammonium nitrogen was determined by distillation-titration method in an alkaline solution after the addition of MgO. Nitrate nitrogen was determined in the same manner using Devard´s alloy. Concentration of NH_4^+-N and NO_3^--N was calculated:

$$mg\,NH_4^+ \text{ or } NO_3^- - N = \left(\frac{\text{normality of standard HCl}}{\text{aliquot}}\right) \times 0.6 \times \text{titration} \quad [3]$$

The value of N_{min} was calculated as the sum of the detected ammonium and nitrate forms.
Determination of N_{min} was performed after each sampling of the soil solution and in each sample. The results obtained from the analyses of soil solution were expressed in mg of N_{min} per m^2 (mg/m^2).

Results and Discussion

Mineral nitrogen leaching in laboratory pot experiment

Leaching of mineral nitrogen was determined as the capture of ammonium and nitrate forms on the Ion Exchange Resin (mg N_{min} $dm^{-3}$of soil). Expression of results was explained in the preceding section.
Table 1 presents differences between variants with different fertilization. The highest leakage of mineral nitrogen was detected in variant NPK - only mineral fertilization (489,60 mg N_{min} m^{-2}). On the other hand the lowest with higher dose of compost - ČD 100 (15,18 mg N_{min} m^{-2}).

Table 1 Leaching of mineral nitrogen - pot experiment. Different letters indicate significant differences (ANOVA, $P < 0.05$)

	mg N-NH4 m^{-2}	± SE	mg N-NO3 m^{-2}	± SE	mg N_{min} m^{-2}	± SE
C	18,06^{a}	5,05	143,99^{a}	51,01	162,05^{a}	56,06
ČD 50	6,16^{b}	1,15	33,04^{bd}	12,10	39,20^{b}	13,25
CMC 50	4,48^{b}	1,55	38,33^{bd}	19,79	42,81^{b}	21,34
ČD 100	2,34^{c}	0,42	12,84^{c}	2,17	15,18^{c}	2,59
CMC 100	3,26^{bc}	0,73	20,72^{b}	2,52	23,98^{b}	3,25
NPK	441,97^{d}	47,16	47,63^{d}	19,91	489,60^{d}	67,08
ČD+NPK	64,67^{e}	34,19	14,69^{bce}	15,39	79,36^{ab}	49,58
CMC+NPK	109,28^{e}	51,25	7,23^{e}	1,34	116,51^{ab}	52,59

These results indicate positive influence of C_{org} (organic carbon) addition on leaching of N_{min} from arable soils. All variants with addition of C_{org} showed lower detection of nitrogen than variants without.

Mineral nitrogen leaching in Lysimetric experiment

Table 2 present distribution of leakage of N_{min} to the part ammonium nitrogen and nitrate nitrogen. Leaching of ammonium nitrogen was not significant among treatments.

On the other hand high addition of compost (variant K5) had a positive effect on concentration of nitrate nitrogen in soil solution. 200 % dose of compost in variant K5 significantly decreased leaching of N_{min} compared with all variants (Table 2).

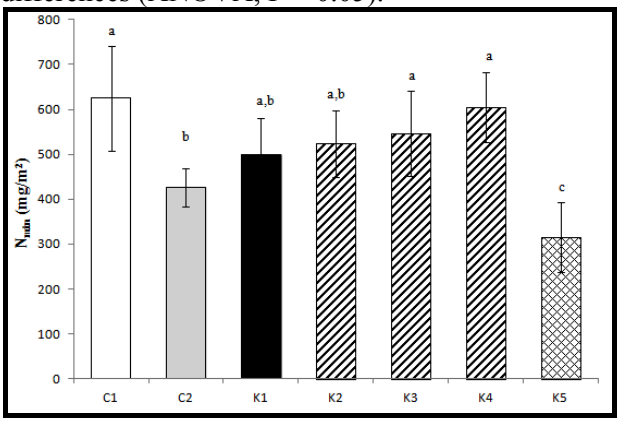
Figure 3 shows leaching of N_{min} from soil. The highest concentration of N_{min} was detected in variant

C1 - with addition 100 % of recommended dose of nitrogen (964.58 mg m^{-2}) and the lowest concentration of N_{min} was detected in variant K5 - with addition of 200% of recommended dose of compost (315.51 mg m^{-2}).

Table 2 Concentration of ammonium and nitrate nitrogen in soil solution

	mg NH_4^+-N m^{-2}	±SD	mg NO_3^--N m^{-2}	±SD
C1	56,13	31,92	568,46	85,78
C2	21,10	8,31	405,90	34,11
K1	17,68	2,34	482,10	78,05
K2	14,69	2,86	508,89	72,03
K3	35,18	18,70	510,47	75,93
K4	28,75	12,60	576,09	65,23
K5	27,63	6,64	287,88	70,69

Fig. 3 Detection of mineral nitrogen in soil solution. Different letters indicate significant differences (ANOVA, $P < 0.05$).



Positive effect of compost addition on leaching of N_{min} was confirmed by various scientific studies [6, 7, 10, 12], which confirm that C_{org} is a source of energy for soil microorganisms and its application in form of compost has a positive effect on microbial activities in soil.

Conclusion

Based on these results, we can conclude that the addition of compost have a positive effect on microbial activity and decrease in leaching of mineral nitrogen from the soil. For these comparisons, we may draw the following conclusions. There are large differences between arable soils with addition of compost and arable soils without, as it was expected. Compost has a positive impact on the soil environment. This positive effect is manifested in all variants of fertilization.

Acknowledgement

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The influence of intensive farming practices on biodiversity of selected species of invertebrates

JANA PODHORNA, MARIE BORKOVCOVA
Department of Zoology, Fisheries, Hydrobiology and Apiculture
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jana.podhorna@mendelu.cz

Abstract: Biodiversity of selected species of invertebrates focused on necrophagous species was monitored in the South Moravia region (Czech Republic) using pitfall traps. Traps were placed in total in 6 locations divided into 2 types A and B. Three localities marked as A are wine regions with almost no disturbance of the landscape, while the other three sites marked as B are in the intensive agricultural farming area. Monitoring was conducted from May to August 2014. In total were laid 216 traps and for the processing were used 110 traps. Each trap was evaluated for the species spectrum of trapped animals, and counted the total number of individuals of each species. The results were processed using biodiversity indexes. In the localities A were captured in total 31 species and 3024 specimens. In the localities B were 18 species and 3708 individuals. Index of species diversity in the localities A was 1.8, in the localities B 0.9. Simpson's Diversity Index evaluation in the localities A was 0.63 and 0.46 in the B localities. Dominance indexes evaluation shows undisturbed character in habitats marked A, with typical occurrence of subordinate species, on the contrary, in areas B was significantly higher occurrence of eudominant species. These observations are consistent with the results of the other authors that intensive anthropogenic activity - intensive agriculture farming - can also affect species diversity at necrophagous species.

Key-Words: necrophagous insect, carabids, vineyard, intensive agricultural farming, South Moravia region

Introduction

Arable (agricultural) landscapes, grassland farming systems, as well as forests, uplands and freshwater habitats are often the point of many research studies not only in Central and Northern Europe, whilst there are minority of those have been carried out in agro-systems, such as vineyards, despite their economic importance for farmers [1]. Vineyards must however be prevented to avoid future biotic losses and these measures should be supported by all stakeholders while at the same time conserving the natural complement of biodiversity [2].

The global decline in biodiversity needs to identify the drivers responsible for conflicts between human activities and the conservation of European biodiversity. There is an effort to promote the management of these conflicts [3]. In grassland strips, and only marginally in vineyards, we found that the positive effect of organic farming was more pronounced for perennial than annual species [1].

As North 2007 states we represent habitat quality as a continuously varying surface over the two-dimensional landscape and the quality affects either fecundity or probability of propagule production. Control of the properties of the landscape is conducted by two parameters, which we call the patch size (the characteristic length scale in quality variation), and the level of heterogeneity (the characteristic quality difference between poor quality and high quality areas) [4].

Generally heterogeneity has positive effect on population density, and hence it is beneficial to improve best quality habitat at the expense of worst quality habitat. Large-scale disturbance events simultaneously affect contiguous blocks of sites. Increasing spatial autocorrelation in the disturbances has a harmful effect to population density so its decreasing equilibrium [5]. The presence of spatially structured fixed habitat heterogeneity, increasing local spatial autocorrelation in habitat generally has a beneficial effect on such populations which increase the equilibrium of population density [5]. The overall suitability of an environment is characterized by the principal eigenvalue of the corresponding linearized equation on the spatial arrangement of regions [6].

Favourable and unfavourable habitat on boundary conditions is analysed.

Material and Methods

Characterization of localities

The experiments were carried out in six localities (Fig. 1) in the South Moravian region in about 40 km range southward from Brno as following Brno-Venkov, Znojmo and Breclav districts with different farming practices. Locations were divided into 2 types A and B. Localities A are characteristic for the almost no disturbance of the landscape. B localities are placed in the intensive agricultural farming area. Three localities marked as A constitute vineyards and orchards. These regions have typical hilly landscape features and on the sunny hillside are grown grapevine, apricots and peaches. Forest steppe and steppe associations are present. Experimental localities were placed nearby Moravsky Krumlov, Nosislav and Kobyli.

Moravsky Krumlov is situated 225 MASL. The average annual rainfall is 500-550 mm. The average annual temperature is 9° C. Abiotic conditions, their number and variations (humidity, local chemistry of the soil, orientation, slope) in this area has developed a number of association of different nature. The whole territory is very rich of extraordinary species. Area has a warm, dry climate with shorter sun shines and mild winters.

An average altitude of Nosislav village is 192 m. The average annual temperature is 9° C. The average rainfall amount is 551 mm per year. Slopes location, warm climate and mild winters support traditional crops growing as grapevine, apricots and peaches.

Kobyli is situated 205 MASL. The average annual rainfall is 515 mm. The average annual temperature is 9° C. Favourable climatic conditions allow the existence of protected steppe association. Fields, orchards and vineyards are predominant extensive agricultural crops. In recent decades there was also a lot of terracing slopes.

The next three localities marked as B are cultivated in an intensive crop farming way focused on maize, sunflower, cereals and vegetables production. Agriculture management greatly affects the appearance of the landscape. Due to the warm climate harvest takes place twice a year. Localities were as follows: Lodenice, Nesvacilka and Zajeci.

Sample collecting

The study was focused preferably to necrophagous species with the use of pitfall traps. Pitfall traps were formed from the one litre size bottle. On the bottom was poured layer of soil up to height of about 3 cm. On this layer was placed bait – raw fish meat weighing 70 g. This amount was empirically verified before the beginning of the experiment. Bottles with bait were always buried in three in a row, on every site were made 3 rows at a distance of 1 km. Traps were covered with stone or brick, thus invertebrates could fly in but not to get out of the bottle. After 7 days, the traps were removed and processed at the Institute of Zoology Fisheries, Hydrobiology and Apiculture.

Fig. 1 Observed localities in the South Moravian region

Results and Discussion

Monitoring was conducted from May to August 2014. In total were laid 216 traps and for the processing were used 110 traps. Each trap was evaluated for the species spectrum of trapped animals, and counted the total number of individuals of each species. The results were processed using biodiversity indexes. In the localities A were captured in total 31 species and 3024 specimens. In the localities B were 18 species and 3708 individuals (tab. 1). Index of species diversity in the localities A was 1.8, in the localities B 0.9. Simpson's Diversity Index evaluation in the localities A was 0.63 and 0.46 in the B localities. Dominance indexes evaluation shows undisturbed

character in habitat marked A, with typical occurrence of subordinate species, on the contrary, in areas B was significantly higher occurrence of eudominant species. These observations are consistent with the results of the other authors that intensive anthropogenic activity can also affect species diversity at necrophagous species [7].

Table 1 Number of species in both localities

A localities	taxonomy	species	specimens in total	B localities	taxonomy	species	specimens in total
necrophagous		10	162	necrophagous		7	216
carabids	Carabidae	8	378	carabids	Carabidae	4	486
rove beetles	Staphylinidae	4	162	rove beetles	Staphylinidae	3	189
true flies	Diptera	4	2268	true flies	Diptera	2	2754
others		5	54	others		2	63
in total		31	3024	in total		18	3708

Conclusion

In 6 localities in southern Moravia region were collected samples of insect from pitfall traps. In the areas A were 3024 individuals captured in total 31 species. In the localities B were 18 species and 3708 individuals gathered. Simpson's Diversity Index evaluation in the localities A was 0.63 and 0.46 in the B localities. Dominance indexes evaluation shows undisturbed character in habitats marked A, with typical occurrence of subordinate species (Carabidae), on the contrary, in areas B was significantly higher occurrence of eudominant species as genus *Calliphora* and *Lucillia* from the order Diptera. Location A index of species dominance evaluate as less anthropogenically influenced. The differences in between locality A were not in very large range in comparison with the location B.

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Effect of burning on epigeal beetle (Coleoptera) diversity

JIRI SCHILLER, VLADIMIR HULA, ZDENEK LASTUVKA
Department of Zoology, Fisheries, Hydrobiology and Apiculture
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xschille@node.mendelu.cz

Abstract: In presented work we have focused on biodiversity of epigeic beetles of the hill Výhon at Židlochovice in the areas that had been burned. This location is one of the northernmost outpost (historical and contemporary) of Pannonian biogeographical region, with many species of invertebrates. Although, we are missing a species list of this remarkable locality is missing. The whole Výhon hill was historically a system of small fields, grassland patches, vineyards and orchards, and it is now a Natural Park with an area of 1700 ha, occupying the entire massif Výhon. Current conditions include mostly arable land, growths of non-native trees (*Robinia pseudoacia, Ailanthus altissimus*) and vineyards. The whole hill was terraced for the cultivation of wine, fruit trees and generally arable during end of the last century. We used pitfall traps, always in line of three traps of the hillside terraces. Determination currently underway, but so detected very important species of our steppe fauna were frequently detected, e.g.: *Sisyphus schaefferi, Brachinus crepitans* and *Brachinus explodens*. All of these species are important from the rareness point of view. Majority of them are protected by Czech law (as threatened) and some of them are listed under the Red List of Invertebrates of the Czech Republic. Species of the genus *Brachinus* amount the largest proportion of the material. Populations of collected species are large enough, to deserve targeted management.

Key-Words: biodiversity, invertebrates**,** burning, epigeic beetles

Introduction

There are large differences of opinion regarding the harmful effects of burning vegetation. The degree of harmfulness of burning forests and its impact on biodiversity is mainly due to seasonality. The damage to plants and animals is smaller in winter and early spring , because the plants are in dormant state and animals are hidden. The greatest damage has grass burning in the growing season and autumn. Burning vegetation can have a big impact on animals that move slowly (eg. Molluscs) or insects in sensitive developmental stages (eggs, larvae, pupae). Controlled burning is used in some areas for maintenance xerophilous heaths and meadows. The countryside of south Moravia is characteristic due agricultural intensification followed by loosing of open xerothermic and light forest. Almost all these xeric habitats were terraced for agricultural production (for wine and fruit trees mainly) in the second half of 20th century. At that time it was really negative and sizable transformation of ecosystem and landscape character. It is indisputable, that not only invertebrate fauna changed with these changes. Nowadays the value of the Výhon Hill is in the character of this landscape, because of a lot of smaller mosaic patches, which guarantee landscape heterogeneity. This heterogeneity is also product of human activities and land use in landscape. Though, landscape character was not disturbed essentially. Výhon Hill has historical, geological and paleontological importance. This locality is one of the northernmost refuges for many invertebrate species of Pannonian biogeographical area. It was the reason of Výhon hill declaration as Nature Park. The Výhon Hill was terraced from south, east and west. Landscape mosaic consist of vineyards, intensive and extensive fruit orchards, small fields and gardens, we can find also steppe habitats there. Occurrence of the invasive *Robinia pseudoacacia* is problematic, which spreads very quickly [1]. My study is focused on epigeic beetles of area mentioned above. Some important invertebrate species were found here, for example Hemiptera [4] or Carabidae [6].

Material and Methods

Experimental localities

Research was conducted on two localities of the

Výhon Hill in faunistic square 6965 [5]. Experimental localities and their basic characteristic:

Fromoušek – locality is characterized by meadow, (fig. 1)

Sad – old plantation with a long apple planting tradition (fig. 2)

Collecting and important species interpretation

Pitfall traps were used as collecting method. Pitfall traps are suitable for epigeic invertebrate fauna collecting, their main advantage is that pitfall traps do not work with a mistake of collector [7]. Pitfall traps presented by [8] were used, covered with wood cover. There was used as a fixative fluid 4% formaldehyde solution. Two lines were installed (each line with 3 pitfall traps) at each locality during growing season (from Aprit to September). Biological material was collected each month. Collected material was conservated in 70% ethanol, then particularly groups of invertebrates were separated. Epigeic Coleoptera were precisely determined.

Results and Discussion

Currently, the collected material is partly determinated. First results show important steppe species of our fauna such as *Sisyphus schaefferi* (Linnaeus, 1758). *S. schaefferi* is under low protection (Decree 395/1992Sb. according to 176/2006Sb.) and it is also listed in Red list of invertebrates in the Czech Republic [2] as vulnerable species. It is necessary to mention also *Brachinus crepitans* (Linnaeus, 1758) and *Brachinus explodens* Duftschmidt, 1812, which are both listed under low mentioned above.

Tab.1

Locality	Sad	
	12.5.2014	15.6. 2014
Abax parallelepipedus	2	2
Amara similata		1
Anchomenus dorsalis	8	14
Bembidion properans	3	
Callistus lunatus	1	
Carabus ulrichi	5	2
Drypta dentata	1	1
Harpalus rubripes	2	
Leistus ferrugineus	2	3
Notiophilus aquaticus	1	1
Ophonus azureus	2	1
Poecilus cupreus	1	
Pseudoophonus rufipes	7	10

Tab. 2

Locality	Fromoušek	
	12.5.2014	15.6. 2014
Amara apricaria	1	1
Amara bifrons	2	1
Amara similata		2
Anchomenus dorsalis	12	5
Bembidion properans	1	
Brachinus explodens	10	1
Callistus lunatus	4	8
Carabus ulrichi	2	2
Harpalus rubripes	1	
Harpalus signaticornis	6	
Leistus ferrugineus	1	
Microlestes maurus	3	
Ophonus azureus	6	1
Poecilus cupreus	5	
Pseudoophonus griseus	7	3
Pseudoophonus rufipes	4	10

Here are 3 examples: interesting species of ground beetles:

Carabus ulrichi

Robust convex species. Upper surface usually shiny, coppery, frequently with green hue, underside and appendages black. Central an southeast Europe, from lowlands to foothills in meadows, fields, shrubby formations and open forests. In ČR and SR 2 subspecies [3].

Notiophilus aquaticus

Dark brown-coppery, appendages black, 1st palpomere and 3 basal antennomeres red-brown. Elytra blue or black. Circumboreal. In ČR and SR common in moderately dry to humid habitats: woodland, heaths, lowlands to alpine zone of mountains [3].

Callistus lunatus

Head and underside of body black, head with slight bluish lustre elytral surface yellow, each elytronwith 3 black spots: small, round humeral spot, middle spot extending from elytral margin to interval 2, and preapical spot in form of common transverse band in front of yellow apex of elytra, connected to middle spot at lateral margin, pronotun black. West Palaeartic species, reaching Middle Asia, in ČR and SR sporadic to rare, especially in dry, unshaded habitats: pastures, steppe, forest edges, lowlands to foothills, mostly in hills [3].

Fig.1 Situation of landscape mosaic of the surroundings of locality Fromoušek (experimental plot is marks by red colour)

Fig.2 Situation of landscape mosaic of the surroundings of locality Sad (experimental plot is marks by red colour)

Conclusion

In the past, some places on the hill Výhon regularly fired. At present, when the burning is prohibited, many valuable sites overgrown seeding wood and steamed large biomass of grasses. Various diversity of species on the sites shows a large influence of the surrounding areas and the size of burned areas. It turns out that burning small areas has no great influence on the diversity of species, because the damaged area is quickly colonized from unaffected areas. Further data will be available after end of the season 2014.

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The difference the grain size distribution forehead vineyard terraces of South Moravia with respect to ground cover vegetation cover

JANA SIMECKOVA, ANNA HAMMEROVA, BEATA HRABOVSKA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xsimecko@node.mendelu.cz

Abstract: Soil is an intricate system. Different characteristics are used to his. One of them belong grain size distribution. The soil texture affects many other physical, physicochemical and biological properties.The paper deals with grain size distribution of forehead terraces with respect to ground cover vegetation cover. We have distinguished the place – bare, grass and shrubs. For comparison was used pipetting method and of determining soil texture by Novak (grouped according to particles smaller than 0.01 mm).
The laboratory results show that most of the monitoring locations and places are soil class sand loamy. The results were statistically processed. These results suggest that not only the grain size distribution of individual places vary but also vary also grain size distribution one location to different places (bare, grass, shrubs). The places bare and shrubs are in terms of the grain size distribution statistically proven different.

Key-Words: soil class, terrace

Introduction

One of the physical properties of the soil is the soil texture. The texture is formed by mechanical particles of mineral origin. [1] Elementary particles are grouped according size into grain size fractions. Quantitative representation of individual fractions in soil is referred to as soil texture. Quantitative representation fractions are given in weight percent. [2]

The soil is divided into two fractions: skeleton and fine earth. In the soil is monitored primarily fine earth because is a principal component of soil. Particle size affects many other physical and physical-chemical properties. We can talk about porosity, air and maximum water capacity, soil permeability to water and air, adhesion, plasticity, soil sorption, soil temperature, and others. According soil grain size determines the possible use of the land or the method of processing and reclamation of land. From this standpoint belongs soil granularity to the basic characteristics of the soil. [2] The soil texture affects the biological properties of the soil also. The size of microbial activity depends on the grain size distribution. [3]
Impact the texture to the other processes in the soil is also dealing some scientific work. An example can be the contribution that dealt with aspects influencing the yield of sugarcane. There was an association between the occurrence amount eldana larvae and soil texture. The authors explain the clay content. If the soil contains little clay, the plant can suffer from water stress and it affects the presence of N in the stem of sugarcane. Higher incidence of N in aboveground plant parts makes it easier to attack eldana larvae. [4]

It has been detected differences in the intensity of the assault soybean cyst nematode [5] or *Renynchulus reniformis* [6].

Soil texture can also affect the release into the atmosphere of certain substances applied to the land for the protection of plants against pests. Certain textures are able to retain substances in the soil longer, which allows extending the time for contact with the pests. [7]

The authors Sağlam and Dengiz [8] dealt the difference of soil organic matter, water stable aggregates, hydraulic conductivity, bulk density of different soil classes and different land use. There were statistically significant differences between soil type and soil organic matter content, hydraulic conductivity and water stable aggregates. However, different with volume weight was not found. The authors based their results argue that soils with higher content clay particles are better able to treat the water and make better use of nutrients.

Material and Methods

This article analyzes the grain size distribution of terraces. It is at a location Morkuvky, Mutenice, Nemcicky, Velka Slunecna by Dolni Dunajovice. All sites are located on the vineyard terraces.

Terraces generally originated mainly in the last century. It was combined with the state agricultural policy of the former regime. There was a demand for the used of all locations that are suitable thanks the climate. Thus began to build terraces on slopes with too large longitudinal slope. The total area of the terraces in the Czech Republic is 3 899.76 hectares. It is located in the district of Brno-venkov, Breclav, Hodonin, Melnik, Uherské Hradiste, Vyskov and Znojmo. Of the total area of terraces is 1 390.79 hectares used for the cultivation of the vine. [9]

Terrace consists of two parts - the production area and forehead terrace, which has just cope longitudinal slope. Forehead terraces are now developing succession; they are left without human intervention. It creates conditions for the growth of rare plants. Because the forehead terraces are without human intervention, there is a good opportunity for the life of various animal species. We assume that the soil texture could affect on what species the sites will be found. In this paper, we focus only on the analysis of particle-size distribution of each of these locations.

At each location were identified three sites with respect to ground cover vegetation cover. At each location had to find place bare - with ground cover up to 40 %, grass - with ground cover above 40 % without greater representation of trees or shrubs, shrubs - sufficient coverage consisting of shrubs or trees.

Loose samples for grain size distribution analysis were collected from the surface layer approximately the middle of the slope, where it was placed pitfall traps for insects.

Loose samples were dried under applicable methodology. Grain size distribution analysis was performed using the pipette method. The method consists in that it is based on values of the specific weight of each sample is determined sedimentation time, which is different for each particle density determined tabulating. Creates a water suspension and identified by sedimentation times specific weights for each fraction is performed pipetting. Pipetted amount in a weighing bottle is deferred on the hotplate, where there is evaporation of excess water. After evaporation, the sample is inserted into an oven set at 105°C. After drying, the sample weight is weighed weighing dish. [10]

Using this method we gain weight value of the fraction and using Excel we gain weight percentages of each fraction in the sample. Based on these values, we are able to determine the soil class.

In this work, we used to label size fraction classification by Kopecky and to determine the class of soil samples used Novak classification that soil types classified by content loam particles (<0.01 mm).

This article also contains the statistical data processing. We focused on comparing the content loam particles. Each other was compared all the bare places, place grass or shrubs too. In addition it was compared the contents loam particles at various places each location.

In statistics, ANOVA was used when the data were normally distributed. In the event, that data was not normally distributed used the Kruskal-Wallis one-way analysis of variance. For tests, in which appeared statistically significant difference was performed subsequent analysis using the appropriate post-hoc tests for determining the values between which the difference is statistically significant.

Results and Discussion

As stated above, the grain size distribution is of the basic characteristics of the soil. If we know the grain size distribution of the soil, we can estimate other soil properties. The representation of the different grain size fraction directly influences other soil properties.

For locations described in this article were found differences in soil class, not only between locations but also between different places of one location. Overview of soil class by location and place is in Table 1.

Table 1 Overview of soil class by location and place

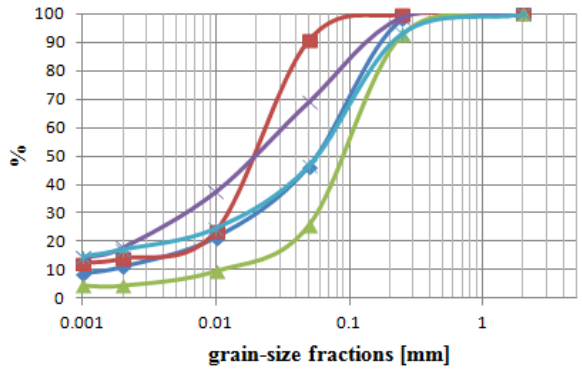
LOCATION/PLACE	BARE	GRASS	SHRUBS
MORKUVKY	sand loamy	sand loamy	sand loamy
MUTENICE	sand loamy	sand loamy	clay loam
NEMCICKY	sand	loamy sand	sand loamy
TESANY	loam	clay loam	clay loam
VELKA SLUNECNA	sand loamy	sand loamy	loam

The predominant soil class is sandy loam soil class and the total of 8 places. This soil class contains loam particles (particles smaller than 0.01 mm)

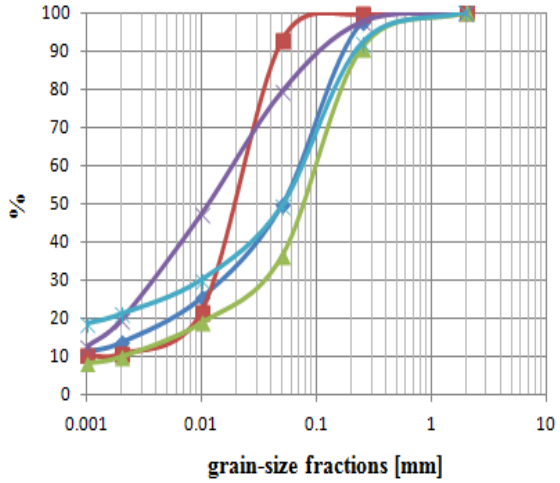
between 20-30 %. This soil class has compliant porosity, permeability and air capacity. [2]
Second soil class was clay loam. It was on three monitored places. Content loam particles are 45-60 %. These soils have a high sorption and retention capacity. [3]

Fig. 1 Particle-size distribution curve for locations

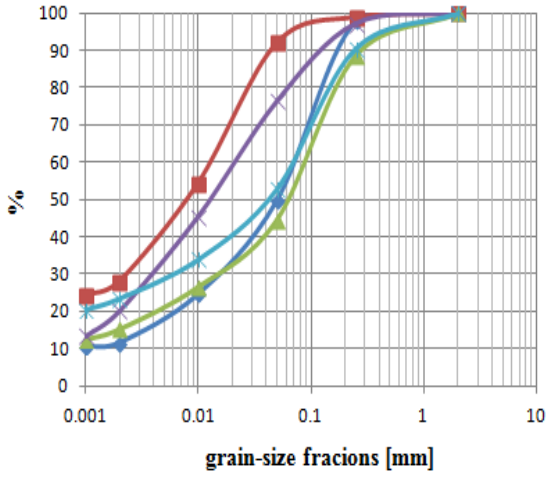
a) bare



b) grass



c) shrubs



Legend:

Soil class loam was at the two places only. They contain 30-45 % loam particles. And the one place is sandy soil class and one is loamy sand. Sandy soil type contains 0-10 % loam particles and loamy 10-20 %.

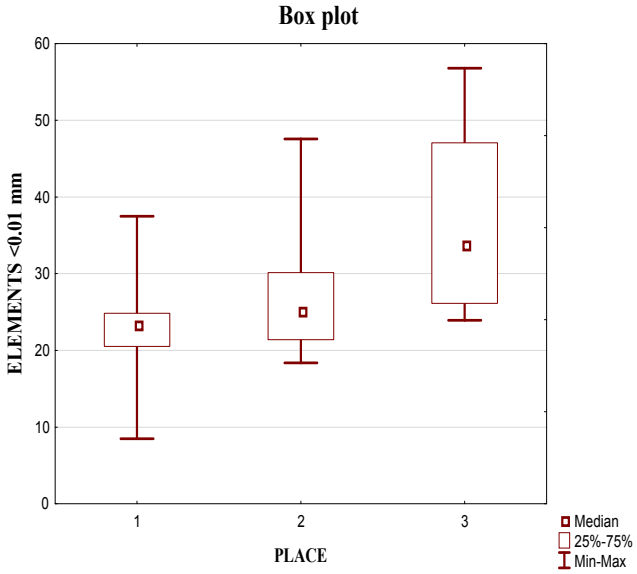
The statistics shows that the locations are different in various places in terms of soil class. For the places bare is statistically significant difference for all places, exceptions are locations Mutenice with Velka Slunecna. In place of grass was statistically significant difference at all locations. In place of shrubs was no statistically significant difference to demonstrate only between the locations Morkuvky - Nemcicky.

Table 2 Kruskal-Walis one-way analysis of varaiance all locations between places

	Kruskal-Wallis one way analysis of variance: p = .0029		
	1	2	3
<0.01 mm	R:15.733	R:21.367	R:31.990
1		0.720427	**0.00225**
2	0.720427		0.0842
3	**0.00225**	0.0842	

Legend: 1 – bare; 2 – grass; 3 – shrubs

Fig. 2 Box plot all locations between places



Legend: 1 – bare; 2 – grass; 3 – shrubs

In addition to the differences between the locations by places were also found statistically significant differences between places in different locations. A statistically significant difference between all places has been found in locations Nechvalin and Velka Slunecna. For locations Nikolcice and Morkuvky was statistically significantly difference bare place from grass and shrubs. U Mutenice again statistically significantly difference place bare of bushes and grass.

For statistical comparison of all places divided by points with a statistically significant difference was found only between the place bare and shrubs.

Conclusion

The laboratory results show that the grain size distribution of the vineyard terraces is different. The predominant soil class of the monitored locations is soil class sandy loam.

With statistics showed that content loam particles which are used to determine the soil class, significantly different from each other. This difference was found between both locations categorized by the places, but is also found in the statistics of individual places at one location or all locations statistical comparison between them. From this we can derive that there is a possible influence of soil class on the present animal species in the case that some species prefers a certain soil composition.

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Soil properties of terraces South Moravia intended of vegetation cover

JANA SIMECKOVA, BEATA HRABOVSKA, ANNA HAMMEROVA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xsimecko@node.mendelu.cz

Abstract: The soil is part of the environment. Soil is a very complex system, which may be in its soil properties highly variable. It is caused by how the development soil was. In addition to development, which is long-term criterion may affect on soil vegetation cover. Among the vegetation and the soil is interaction.
The paper describes the properties of the soil forehead of terraces on the basis of determining the composition of the vegetation cover and the subsequent study of ecological conditions that require relevant plant species. Soil properties for locations investigated in this work can be summarized: soils should be medium, or light, there is likely gritting, pH alkaline to neutral values. All localities by vegetation have a sufficient supply of nutrients.

Key-Words: soil, vineyard, permanent grassland, forehead of terraces

Introduction

Acreage Czech Republic is made up almost half of the agricultural soil fond. According to law no. 344/1992 Sb., valid according amended, the agricultural soil fond divided by the arable land, pastures, meadows, gardens, orchards, hop fields and vineyards. [1]

Due to the development of agriculture caused by the political leadership of our country in the second half of the last century was the fact that in some places began to create terraces. It was due to the fact that he was a demand for the use of all places that have suitable climatic conditions for agricultural production. The terraces were built in places with large cross slope.

In our area, the terrace is an area of 3 899.76 ha. Their main use is for growing grapes (1 390.79 ha) and arable land (1 122.29 ha). The terraces are typical for districts in Moravia Brno-venkov, Vyskov, Hodonin, Uherske Hradiste, Znojmo, and in Bohemia Melnik. [2]

The terraces consist of the production area and the slope, which offsets the longitudinal slope. We call them foreheads. These parts are currently without mechanization interference, allowing the natural development of vegetation present, respectively to climax stadium.

Soil is the basis for the life of many plant species on our earth. The soil allows plants to extract water, nutrients, etc. needed for their life. [3]

The total number of plant species is not precisely known, but there is a very great diversity. The different soil properties at different locations are one reason for this diversity. Each plant species has specific requirements for the soil environment and they are limiting the occurrence of the species observed on the location. [3]

It is shown that soil properties affect the occurrence of plant species. For example, it was found influence of soil properties on vegetation composition of semi-natural grassland. [4]
It was watched the development of sand dunes in ten steps. There was a relatively high correspondence between vegetation and soil factors. The most influence soil properties were marked content of organic C, total N, pH, slope, very fine sand and soil water. [5]

Another paper focused on the diversity of vegetation of subtropical rainforest in Nanjenshan Reserve. The authors argue that the topographic variables and vegetation types are need to explain the different soil properties. As the actors that influence were identified: pH, total N, the content of exchangeable elements Al, K, Ca and Mg. [6]
There is a computer model that is capable after entering the input data to determine the composition of vegetation cover. As one of the input data required soil texture (it is one of the physical properties of the soil) [7]

We can say that if we know the composition of the vegetation monitored location, we are able to study under coenology requirements of the plant species to determine soil properties.

Material and Methods

The paper describes the soil properties of selected localities of southern Moravia. Locations were selected according to several criteria. The first was that the location is located on the terrace. The second requirement was that at one location occurred vegetative growth with different abundances. It required coverage to 40% - barren land, over 40% but no greater representation of shrubs - place with grass, and sufficient covering of shrubs or trees - so called shrubs.

In this paper, we focus only on the five selected locations. This is Mikulov, Nechvalin, Nemcicky Nikolcice, and Satov. Terraces at these locations are Terraces at these locations are used as a vineyard, only Nemcicky is permanent grassland. Localization of monitored locations showed Fig. 1.

Fig. 1 Localization of locations monitored in the paper (the map was created in the program BaseCamp TOPO Czech 2012) [8]

Legend: 1 – Mikulov 2 – Nechvalin 3 – Nemcicky 4 – Nikolcice 5 – Satov
vineyard *permanent grassland*

All monitored locations fall under the wine growing region of Morava. This area includes 96% acreage of all vineyards occurring in the Czech Republic. The wine-growing region of Morava in terms climate we can characterize as follows: average temperature 9.42°C, average annual rainfall 510 mm, and average hours of sunshine 2 244 hours per year. [9]

Orientation of slope monitored locations is to the south (Satov), respectively southwest (Mikulov, Nemcicky, Nechvalin, Nikolcice). The down slope of the slopes all locations are between 30 - 43 %. Length of slopes at various sites is different. Mikulov was chosen forehead with a length 11.5 m, Nechvalin 5.5 m, Nemcicky 18.8 m, Nikolčice 23 m and Satov 13 m.

Phytosociological survey was carried out 2 to 9 June 2014, when it was recorded all plant species that are found in certain localities. On the basis thereof is formed summarization plant species - in Table 1.

Results and Discussion

By studying the plant species occurring on the monitored locations were evaluated for various soil properties.

The composition of the vegetation at the location Mikulov confirms that the location was created by anthropogenic activities. Many of the present plant species were found predominantly in anthropic affected areas. In addition, there occurring plant species require repeated wounding of the soil surface. Soil class should be loam with the possibility of grittiness, mineral rich. The chemistry of the soil should range from weakly alkaline to neutral. The soil should be dry. Increased soil moisture should appear at the foot of the slope. This part in places grass and shrubs occurs, which requires freshly moist soil. *Crataegus laevigata* is in this part in places grass and shrubs. The plants require freshly moist soil. [10, 11, 12, 13]

For location Nechvalin was plant cover composition the same for all the places (bare, grass, shrubs). All plant species occurring in this location require soil rich in nutrients and well supplied with nitrogen. Soil class should be loam. The soil profile should be deeper and especially mellow. The chemistry of the soil should be about neutral pH. As for the occurrence of water in the soil profile the soil should be weakly moist until freshly. It is assumed a higher groundwater table or higher rainfall. The second option is possible because the location is located adjacent to the Zdanicky forest. [10, 11, 12, 13, 14, 15, 16]

The soil at the location Nemcicky should be light, rather loamy sand, sandy with options stoniness. The soil profile should be mellow and drying. In terms of soil supply of nutrients is more complicated. Part vegetation requires nutrient-poor places, while other species of nutrient-rich sites they want. These plant species are at the top or bottom edge of the slope. It is likely the supply of nutrients from higher lying areas. The chemistry of the soil should be neutral. Composition of the vegetative cover suggests that it is a secondary place.

Some present plant species are typical of the different developmental time succession. [10, 11, 12, 13, 14, 16, 17, 18, 19, 20]

Location Nikolcice has different soil properties at the place of bare and grass shrubs compared the place shrubs. The places bare and grass can be characterized as a place with a weak acid base.

In the shrubs are plant species (*Hippophae rhamnoides* and *Poa Pragensia*), which require moist places. In addition, they prefer sandy and gravelly soil; soil pH should be neutral to alkaline. In all places but growing species requiring ground sufficiently supplied with nutrients. [10, 11, 12, 13, 16, 21, 22]

Satov in terms of soil properties of the monitored places varied, at least indicates vegetation cover. For places bare entirely predominant vegetation *Salvia nemorosa*. This herbal species requires wounding, landslide place. Soil class should be loamy clay. The soil should be rich in minerals and humus. The chemistry of the soil should be alkaline to neutral. [10, 11, 12, 13, 14, 21, 23]

In place of grass were species require a weak acidic environment. Soil should be moist. It is likely that there is a campaign washout substance from the points lying higher on a slope, because at the bottom of the slope there are more species requiring a supply of soil nutrients. In contrast, in the upper part are the species at nutrients unpretentious. Place labeled as shrubs should have higher soil moisture. Furthermore, there are species require deep soil substrate and loam. Chemistry should be about neutral pH.

Conclusion

Soil characteristics are quite variable. Their values will affect many other factors. One such factor is the vegetation cover.

Fig. 2 Location Satov, place bare (photo by Hammerova)

For locations investigated in this work can be summarized as follows soil properties. There are especially soils medium, or light. There is likely gritting. pH of the soil should be alkaline to neutral values. All localities by vegetation have a sufficient supply of nutrients.

In addition, the location Nemcicky place bare was found protected species *Linaria genistifolia*. This species is included in the protection of C3 (endangered taxa of vascular plants). At the same locality but place grass and shrub were recorded individuals *Asgragalus danicus*. This species is also in the protection category C3. [24]

Fig. 3 Location Nikolcice, place grass (photo by Hrabovska)

Fig. 4 Location Nemcicky, place shrubs (photo by Simeckova)

Table 1 Summarization plant species of locations (the authors created this table based on field survey) [25]

LOCATION	DESIGNATION	GRASS - Poaceae	HERBS		Shrubs/Trees
			Legumes	Other	
MIKULOV	bare		Melilotus officinalis	Salvia nemorasa	Prunus mahaleb subsp. simonakii, Rosa canina
	grass	Arhenatherum elativ		Verbascum nigrum	Crataegus laevigata, Rosa canina
	shrubs	Arhenatherum elativ		Origanum vulgare	Crataegus laevigata, Rosa canina
NECHVALIN	bare	Arhenatherum elativ		Artemisia vulgaris, Euphorbia cyparissias, Gallium mollugo, Plantago lanceolata	Cornus sanguinea, Rosa canina
	grass			Artemisia vulgaris, Euphorbia cyparissias, Gallium mollugo, Plantago lanceolata	Cornus sanguinea
	shrubs	Arhenatherum elativ		Artemisia vulgaris, Euphorbia cyparissias, Gallium mollugo, Plantago lanceolata	Cornus sanguinea, Rosa canina, Viburnum opulus
NEMCICKY	bare	Bromus japonicus, Melica transsilvanica		Artemisia campestris, Carduus acanthoides, Euphorbia esula, Hypericum preforatum, Linaria genistifolia, Tripleruspermum inodorum	Rosa canina, Vitis vinifera subsp. vinifera
	grass	Bromus japonicus, Melica transsilvanica		Artemisia campestris, Astragalus danicus, Carduus canthoides, Cerinthe minor	Prunus spinosa, Robinia pseudocacia, Rosa canina
	shrubs	Bromus japonicus, Melica transsilvanica	Melilotus officinalis	Artemisia campestris, Astragalus danicus	Betula pendula, Prunus spinosa, Rosa canina, Vitis vinifera subsp. vinifera
NIKOLCICE	bare	Arhenatherum elativ, Dactylis glomerata			Rosa canina
	grass		Trifolium reperis	Euphorbia cyparissias, Origanum vulgare, Reseda odorata	Rosa canina
	shrubs	Poa pratensis			Hippophae rhamnoides
SATOV	bare			Salvia nemorosa	Rosa canina
	grass	Anthoxanthum odoratum		Asparagus officinalis , Cihcorium intybus, Salvia nemorosa	Rubus caesius
	shrubs	Calamagrotis epigejos, Dactylis glomerata		Achillea milleforium	Rosa canina, Prunus cerasifera

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The effects of biochar on soil respiration in rhizosphere and non-rhizosphere soil

OLGA URBANKOVA, JAKUB ELBL, JAROSLAV ZAHORA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

olga.urbankova@mendelu.cz

Abstract: This paper presents the result from laboratory experiment (under controlled conditions), which is focused to influence of the burned plant biomass (biochar) to soil respiration in rhizosphere and soil without roots. The twelve Mitscherlich's containers were used and were filled with subsoil and topsoil from the area of Březová nad Svitavou. The experiment was divided into three variants: C presented the control variants without addition of biochar, V2 presented variants with 20 t of biochar per ha and V5 presented addition of 50 t of biochar per ha. It was used to nylon membrane for separating rhizosphere soil and non-rhizosphere soil.
Soil respiration was determined by the soda-lime method, when CO_2 is captured on the granules of soda-lime during anaerobic incubation. The results confirm the hypothesis that addition of biochar has positive effect on soil microbial activity and biomass production. The highest influence of biochar to soil respiration was measured in variant V5, the highest influence of biochar to biomass production was measured in variant V2.

Key-Words: biochar, soil microbial activity, rhizosphere soil, bulk soil, soda-lime, biomass

Introduction

Soil contains a huge amount of carbon. But soil carbon contained in soil organic matter is lost gradually due to poor land management. However, the quantity of organic matter and understanding the control on the dynamics of soil carbon is important because of the central role soil carbon plays in ecosystem sustainability, nutrient availability and the production of global greenhouse gases. The activity of the soil biota controls, in part, the release of soil carbon to atmosphere. Relatively little is known regarding how changes in microbial community composition or metabolic capacity may alter the types or amounts of soil carbon consumed and respired [1]. One of the possibilities how to increase the amount of soil carbon is application of biochar [2, 3, 4]. Biochar is a fine-grained material nature of charcoal, which is made by a process called pyrolysis. Pyrolysis is a thermal decomposition of the organic material at relatively low temperatures (300°-700°C) in the air deficiency conditions. Biochar is different from the coal, because for it's applied to the soil for purpose of changing soil properties including soil fertility and ecosystem change through the carbon sequestration. From the chemical point of view, biochar is carbon-rich product, which is more resistant to microbial degradation than other forms of carbon produces from biomass in the soil environment [5].

The authors [4, 6, 7, 8] reported a different knowledge about the effect of biochar to soil properties and soil microbial activity depending on a climatic conditions, soil type, type of vegetation and also on the properties of biochar. The chemical and physical properties of biochar can be different depending on the material from which biochar is made and the amount of the temperature during pyrolysis. For example, charcoals rich in volatile organic compounds or salts can be toxic for roots and soil microorganisms [9].

The work deals with a soil respiration - one of the indicators of soil microbial activity. It's expected that biochar added to the soil isn't inert but can provide a certain amount of labile carbon, which is readily available to microorganisms. This causes the soil respiration is stimulated in the early days of incubation by [10, 11, 12]. However, some sources [3, 7] indicates that application of biochar has no effect to soil respiration.

The aim of this work is determine how the various additions of biochar influence to 2 type of soil:

a) the soil stimulating by the growth of roots (rhizosphere soil)

b) the soil without roots (non-rhizosphere soil)

It's known that the soil near the root system has different properties than soil without roots (bulk soil). A series of soil processes is induced either by the plant's rhizosphere activities directly or by the plant's microflora. The roots aren't only a reservoir of nutrients which are transported to the shoot by the flow mass and diffusion but also the roots accept ions or water, which leads to a reduction or accumulation ions or water near roots. Roots also secreted H^+ or $(HCO_3)^-$ and CO_2, which causes changes in pH, the roots also accept or release O_2, which may cause changes in the redox potential. Furthermore roots secrete the low molecular root exudates rich in carbon substances and can mobilize the nutrients directly or indirectly supplying energy for the activity of microorganisms, which are in close proximity to the roots [13], [14]. Conversely, the microbial community in the bulk soil is thought to be carbon limited.

Material and Methods

Soil samples

The pot experiment was established in greenhouse on the 5th April 2014. The Mitscherlich's container with capacity 6 liters was used for this experiment. Each container was the same size and it was filled with 1l of subsoil and 5,5l of topsoil. The soil originated from locality Březová nad Svitavou.

The experiment consisted of 3variants: control variant without addition of biochar, V2: variant with calculated addition of 20 t of biochar per ha and V5: variant with 50 t biochar per ha. The variants had always 3 repetitions. Biochar was supplied by EKOGRILL®, the material was beech wood (Table 1). Biochar was sieved through a sieve (grid size 4mm) and homogenized. It was used a nylon membrane for separating the rhizosphere soil and soil without roots. The nylon membranes were in the shape of stocking and their diameter mesh were 0,4 µm. This type of mesh is impassable for roots and root hair but bacterias and fungal hyphae can penetrate it. The plant *Zea mays* was indicator plant.

Soil respiration

It was measured soil respiration weekly from 13th April to 31st July by the soda-lime method [14]. The principle of this method is absorbing of CO_2 which is exhaled through the soil microorganisms to the soda-lime granules. CO_2 is the ultimate product of the mineralization of organic substances in the soil. Therefore, the amount and the dynamics of CO_2 released from soil are considered as a significant measure of the intensity of mineralization processes in the soil and global activities of soil organisms. The weight gain of the soda-lime in incubated samples is directly proportional to the amount of CO_2 which is released by microbial activity of microorganisms in the samples. This weigh must be added further quantity of water which enters to the reaction between CO_2 and the absorbent. This water is evaporated during drying of the absorbent after incubation. Therefore it's necessary to multiply the resulting value by a coefficient of 1.43 [15].

The incubation lasted for 24 hours for each sample. There were measured the increase in the values of soda-lime between the individual measurements in the samples.

Table 1 Properties of biochar:

biochar material	beech wood
C	87,7%
H	2,6%
N	0,3%
S	< 0,1%
O	2,3%
H_2O	2,8%
ash	2,5%
pH (H_2O)	7,9%

Determination of dry matter biomass

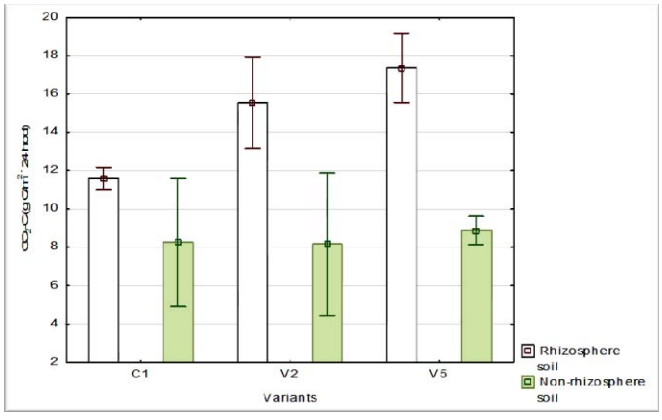
The individual samples of *Zea mays* were removed from the containers after the termination of experiment. The roots were washed by water. Aboveground and belowground biomass was dried in to constant temperature and it was weighed.

Results and Discussion

Soil respiration

The amount of CO_2 absorbed in soda-lime was determined at weekly intervals. The total amount of CO_2 absorbed by granules of soda-lime is shown at Figure 1.

Fig. 1 Cumulative production of CO_2 – basal soil respiration (mean values ± standard error, n = 3)

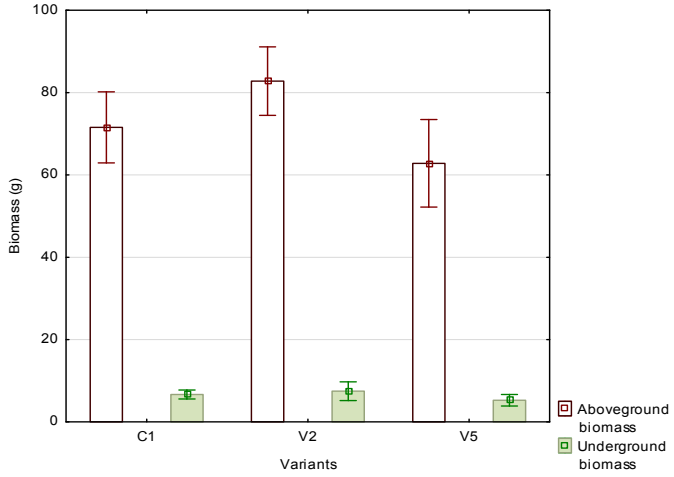


The graph shows statistically differences of soil respiration in rhizosphere and non-rhizosphere soil (between individual variants at the level 0.05; ANOVA; P<0.05). It corresponds to [13, 14, 16], when secretion of exudates by roots (rhizodeposition) allows 5–100 times more organisms per unit volume to be supported in the rhizosphere than in nearby bulk soil. As well, significant differences were observed at rhizospher soil at V5, when addition of biochar increased the microbial activity [10, 11, 12]. The influence of biochar on soil respiration in samples of non-rhizosphere wasn't statistically significant.

Biomass

Production of aboveground and belowground biomass is one of the factors which determined the influence of biochar on plant growth. Production values of biomass are shown at Figure 2, the values are expressed in g of dry weight. The uppercase letters indicate a significant differences in aboveground biomass and the small letters indicate significant differences in underground biomass (between individual variants at the level 0,05; ANOVA, p<0,05).

Fig. 2 Production of plant biomass (mean values ± standard error, n = 3)



The graph shows the highest aboveground biomass production in V2, while the lowest production in variants V5. This results correspond with [3, 4, 9, 10, 11, 12, 17], that addition of biochar have positive effect to plant grow, but high rate of biochar inhibit plant's roots or microbial biomass in soil. These results were observed at aboveground biomass and belowground biomass too.

Conclusion

This contribution presents the results of laboratory experiment to determine the effect of biochar on soil microbial activity. The measures values indicate the influence of biochar addition to soil respiration in rhizosphere soil and biomass production. The influence of biochar addition in non-rhizosphere soil has not been demonstrated.

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Botanical pesticides and their human health safety on the example of *Citrus sinensis* essential oil and *Oulema melanopus* under laboratory conditions

LENKA ZARUBOVA[1], LENKA KOURIMSKA[1], PAVEL NOVY[1], MILOSLAV ZOUHAR[2], ONDREJ DOUDA[3], JIRI SKUHROVEC[4]
[1]Department of Quality of Agricultural Products
[2]Department of Plant Protection, Faculty of Agrobiology
Faculty of Agrobiology, Food and Natural Resources
Czech University of Life Sciences Prague
Kamycka 129 165 21 Prague 6
CZECH REPUBLIC
[3] Department of Entomology
[4]Department of Plant Ecology and Weed Science
Crop Research Institut,
Drnovska 507/73 161 06 Prague 6
CZECH REPUBLIC

zaruboval@af.czu.cz

Abstract: Bio-pesticides derived from plants have recently received increasing interest as an alternative to conventional chemicals suitable for integrated pest management and organic farming. In this study, *Citrus sinensis* (Osbeck) essential oil was tested for its potential for wheat protection against *Oulema melanopus* (L.). The chemical composition of the essential oil was analysed by gas chromatography-mass spectrometry (GC-MS). Twenty-five compounds were identified in the oil among which d-limonene was predominant component (89.49%), followed by myrcene, α-pinene, linalool, sabinene, decanal. and other minor components. Direct contact toxicity assay showed the effectiveness of the essential oil against *O. melanopus* larvae causing the mortality of 85% during 48 hours. The persistence study revealed high dissipation rate of the oil from treated plants whereas concentrations lower than 0.01 g/kg were detected by GC-MS 5 min after the application of the oil, suggesting its environmental and food safety. These results, together with previous literature reports reviewed in this study, indicate the great potential of the *C. sinensis* essential oil for future use in crop protection against insect pests.

Keywords: crop protection; bio-pesticides; d–limonene; larvicidal; organic agriculture; *Triticum aestivum*

Introduction

Synthetic pesticides are widely used for decades in agriculture for plant pest and disease control and their overuse has caused serious health and environmental hazards in the past. Although the new EU and USA legislation led to a dramatic decrease in approved products and to significant safety improvement the application of synthetic pesticides is still considered as a potential threat to human health, food safety and the environment [6]. In this respect botanicals pose little risk. Due to their natural origin they are an ideal eco-friendly alternative to chemical control [32] suitable for implementation into the integrated crop protection systems [30], particularly in organic farming where the use of synthetic chemicals is banned [15].

Considerably large group of potential biopesticides is represented by essential oils known to exert a wide spectrum of biological effects including various pests controlling and repellent properties [15]. Some of the oils exhibit certain degree of phytotoxicity, usually inhibiting seed germination, root and seedling growth, which indicates their possible use for weed management. On the other hand many other oils with none or very low phytotoxicity

[32] suggesting their potential for direct application against pests and diseases.

Probably the most conspicuous pests in green grain crops are small cereal leaf beetles (CLB) *Oulema melanopus* L. and *O. galleciana* Heyden (Coleoptera: Chrysomelidae). Both are found in many species of cultural and weed grasses whereas *Triticum aestivum* L. is their preferable host plant. They can cause economically important damages especially on wheat and barley (*Hordeum vulgare* L.). Beetles arrive in late April and early May. After feeding on grasses they are moving in the fields of grain, where the beetles damaged the leaves. After mating, the female deposits from mid-May its bright yellow oval eggs individually or in short rows on top of the highest ranking leaves. Extraordinarily warm and dry spring and summer weather encourages reproduction of the pest whereas each larva destroys 2.5 to 3.5 cm^2 leaf area, which in wheat accounts for about 10 % of the flag leaf. The damages caused by CLB adults are not economically important but pesticides are commonly applied against CLB larvae [4]. Conventional pesticides play a very important role in the plant protection, but their negative impact on the environment and human health leads to a re-evaluation of plant protection systems. One of the reasons is the incidence of resistant pest populations to widely used active substances as in case of the beetle *O. oryzae* Kuwayama, an important pest of rice, that has developed fipronil resistance in Japan [23]. In this regard, it is advisable to look for new procedures that will reduce the emergence of this phenomenon. Extending the range of applicable plant protection methods is also an essential prerequisite for the application of integrated pest management principles. Several alternatives for *O. melanopus* control have been described previously such as the use of enthomopathogenic nematodes [19], selection of appropriate varieties according to natural resistance parameters [26] or the application of resistance inducers [7].

So far there are just few essential oils allowed for plant protection in the EU: citronella, clove, spear mint, tea tree [9] and most recently orange oil derived from *Citrus sinensis* (L.) Osbeck, the first insecticidal essential oil approved [10] authorized in France for the control of whiteflies on pumpkins and tomatoes [26].

Essential oil from *C. sinensis* was found as an effective pesticide on different plant and storage products pests. Its effect has previously been described against *Sternechus subsignatus* Fabricius and *Rhyssomatus subtilis* Fiedler [32], *Tribolium castaneum* Herbst, *Sitophilus granarius* L. and *Callosobruchus maculatus* Fabricius [21] *Bemisia tabaci* Gennadius [30] and *Tetranychus urticae* Koch [4] Moreover, *C. sinensis* has been reported to exert activity against common house fly [18, 25] and several mosquito species [38, 27, 3, 12, 22].

The main components of *C. sinensis* essential oil reported in literature are d-limonene, aplha-pinene, myrcene and linalool [34, 33, 2, 18, 40] d-limonene (Fig. 1), as the most abundant component of the oil, is considered to be responsible for the majority of the anti-insect activity. As well as *C. sinensis* essential oil, d-limonene has also been reported to be effective against various crop pests [5,13,14,16,17,37,40] stored product pests [98,11,20] and some other insects [36,12,1].

Although high concentrations of d-limonene can be phytotoxic to sugar beet, cabbage, carrot and strawberry seedlings [13] however, it should be otherwise relatively harmless to the environment because it is biodegradable and highly volatile and thus probably low-persistent compound. d-limonene is relatively safe also for humans since it is used as food additive whereas its ADI was established up to 1.5 mg/kg bw [39].

The aim of this work was to monitor the effect of essential oil from *C. sinensis* on *O. melanopus* in correlation with its evaporation from leaves of wheat (var. Bohemia) which enable us to obtain the information about the food and feed safety of plants treated with these bio-pesticides and focused on level of safety application. In addition to residuals analysis of *C. sinensis* and its decrease during the time the effect of essential oil on larvae and adults *O. melanopus* was also tested.

Material and Methods

Chemicals

C. sinensis essential oil, Limonene and Tween 20% were purchased from Sigma-Aldrich (Prague, CZ). Hexane (Merck, Prague, CZ) was used as extraction solvent.

Contact toxicity assay

Specimens of *O. melanopus* used for experiments were obtained from field cultures of wheat from the locality Semice (N 50.157709, W 14.871727). The *C. sinensis* essential oil was diluted in water to the concentration of 1% with 0.5% Tween 20 and subsequently used for the experiments. Specimens of *O. melanopus* larvae and adults were placed individually into Petri dishes (90 mm) and 1 µl of diluted plant essence was pipetted topically on insect body. Mortality of larvae and adults was evaluated visually in the time intervals of 1, 24, and 48 hours in four independent experiments whereas 10 replicates were included within the evaluation. Larvae and adults treated with 0.5% solution of Tween 20 were included as a negative control groups.

Persistence study

Plants of wheat (var. Bohemia) were cultivated under controlled conditions till the essential oil application. The mixture of 1% *C. sinensis* essential oil and 0.5% Tween 20% in H_2O was prepared and the leaves of each plant were sprayed until runoff to achieve maximum coverage of the leaves. The leave samples were then taken in the time intervals of 1, 5, 10, 20, 30 and 60 min. 0.5 g of leaves was frozen by liquid nitrogen, powdered and extracted to 0.5 mL of hexane for 5 min shaking. Samples were subsequently centrifuged; the supernatants were transferred to glass vials and stored in a freezer till the chemical analysis.

GC-MS analysis

Samples were analysed by GC/MS using Agilent 7890A GC coupled to Agilent 5975C single-quadrupole mass detector with a HP-5MS column (30 m × 0.25 mm, 0.25 µm film) from Agilent (Santa Clara, CA, USA). The sample volume of 1 µL was injected in splitless mode, the injector temperature was 250 °C and the electron ionization energy set at 70eV. The oven temperature started at 60 °C for 3 min. and was programmed to 250 °C at a rate of 3 °C/min, and then kept constant for 10 min. The flow rate was 1 mL/min. and helium was used as carrier gas. The analysis of the essential oil composition was carried out in full scan mode. In case of residue detection the concentration of limonene, the prevailing constituent of the oil (89.49%), was examined in the selected ion-monitoring mode (m/z 68, 93, 136). The identification of constituents was based on the comparison of their mass spectra and relative retention indices with the National Institute of Standards and Technology Library (NIST, USA).

Results

Essential oil composition

The volatile components of fruit peel essential oil of *C. sinensis* (obtained by cold-press extraction and analyzed by GC-MS) are listed in Table 1. A total of 25 different compounds with 99.19% of total areas were isolated and identified using spectroscopic (mass spectra) criteria. d-limonene was the most prevailing component of the oil (89.49%) followed by myrcene (3.55%), *a*-pinene (1.26%), linalool (1.04%), sabinene (0.62%) and decanal (0.62%).

Table 1 Chemical composition of *Citrus sinensis* essential oil

Peak	Compounds	Area (%)	RT[a] (min)
1	*a*-pinene	1.26	6.713
2	sabinene	0.62	8.079
3	myrcene	3.55	8.738
4	octanal	0.49	9.186
5	*d*-3-carene	0.23	9.461
6	d-limonene	89.49	10.455
7	1-octanol	0.08	12.062
8	linalool	1.04	13.343
9	nonanal	0.09	13.453
10	*cis*-limonene oxide	0.11	14.705
11	*trans*-limonene oxide	0.12	14.908
12	citronellal	0.33	15.631
13	a-terpineol	0.15	17.394
14	decanal	0.62	17.990
15	citronellol	0.07	19.077
16	neral	0.08	19.568
17	carvone	0.31	19.682
18	citral	0.18	20.900
19	perillal	0.06	21.018
20	anethole	0.28	21.593
21	copaene	0.07	25.395
22	dodecanal	0.11	26.841
23	germacrene D	0.09	27.619
24	valencene	0.11	30.249
25	cadinene	0.08	31.471

RT: retention time

Persistence of Citrus sinensis essential oil on wheat leaves

The concentrations of *C. sinensis* essential oil were examined in time intervals (Table 2) after the application of its 1% (v/v) suspension in

water whereas d-limonene, as the most abundant compound (89.49%), was used for the residue detection. The GC-MS analysis revealed decreasing concentrations of the oil in time whereas the concentration of 0.01038 µL/g was detected 5 min after application (Fig. 2) which is below the minimum residue limit (0.01 mg/kg) established for substances that are not included in any of the annexes in EU regulations.

Table 2 Decreasing essential oil concentration on wheat leaves in time

Time (min)	Concentration (µL/g)
1	0.01803
5	0.01038
10	0.00979
20	0.00474
30	0.00292
60	0.00287

The effect of essential oil on Oulema melanopus

In our toxicity assay, the *C. sinensis* essential oil showed to be highly effective against *O. melanopus* larvae but no insecticidal effect was observed on the adults during 48 hours (Table 3).

Table 3 Mortality of *Oulema melanopus* larvae and adults after apical application of *Citrus sinensis* essential oil

Exposition time	Mortality (%)[a]	
	larvae	adults
1	12.5 ± 4.3	0
24	42.5 ± 8.3	0
48	85.0 ± 5.0	0

a mortality in % ± standard deviation of 4 repetitions

The larvae mortality of 10% was observed even after only one hour of exposure. The mortality further increased to 42.5% and 85% during 24 and 48 hours of exposure, respectively. No mortality was observed for the control group during the 48 hour experiment.

Discussion

With the aim to follow the new strategy of integrated pest management focussed on agricultural greening together with food quality and safety we examined the *C. sinensis* essential oil potential for the use against one of the most serious insect pest of wheat, *O. melanopus,* under laboratory conditions. Our direct contact toxicity assay showed no effect of the oil on the *O. melanopus* adults. On the other hand, it revealed its remarkable larvicidal potential with the larvae mortality of 42.5% and 85% observed 24 and 48 hours after topical application, respectively (Table 3). Similar mortality rates have been reported by [21, 40] for some coleopteran beetles, nevertheless the active concentrations are incomparable due to different experimental designs used. The dose applied in this study (1uL of 1% solution) corresponds to approx. 8.45 µg/specimen (the density of 0.845 g/mL, given by the supplier (Sigma, 2014), was used for the calculation).

The essential oil composition obtained by GC/SMS (Table 1) is in accordance with literature data reporting d-limonene, myrcene, alpha pinene, linalool and sabinene amongst the main constituents. Also the d-limonene content of 89.49% is within the range previously reported (73.24 - 94.8%) [34, 33, 2, 18, 40].

The detailed examination of the *C. sinensis* essential oil effect on *O. melanopus* specimens was not the subject of this study. However, we can assume that the larvicidal activity might be partially caused by the larvae dehydration and surface distortion as has been observed by scanning electron microscopy on housefly larvae [18]. Although there was no direct insecticidal effect observed in our contact toxicity assay against *O. melanopus* adults, some previous studies indicate that the *C. sinensis* oil can have other anti-isect properties such as repellent or anti-oviposition activities as described e.g. in case of *Costelotrycha zealandica* White [24] and *Bemisia tabaci* Gennadius [30] respectively. Moreover, the oilmight perhaps be active against adults in vapour phase as in the fumigant bioassay of [21] against three coleopteran beetles, of which *Callosobruchus maculatus* belongs to the same family as *O. melanopus*.

An important factor, both in terms of food safety and the efficiency against crop pests, is the persistence of a pesticide in plants. The results of our persistence assay using d-limonene for the residue detection showed that

the *C. sinensis* essential oil evaporates very quickly from the treated plants (Table 2). The concentration of 0.01038 μL/g was detected by GC-MS 5 min after application (Fig. 2) which is below the minimum residue limit (0.01 mg/kg) established for substances that are not included in any of the annexes in EU regulations [8]. Thus it indicates *C. sinensis* essential oil presents minimum health risk and there is no need for protection period after application. Due to its low persistence it is also harmless to the environment and it is safe for non-target organisms if not directly exposed to the oil.

Although the low persistence can constitute certain disadvantage regarding to the pesticide efficiency, the results of our toxicity assay indicate that the larvicidal effect is irreversible once the specimen is exposed to the oil. Moreover, the oil might have also some pupicidal effect as in case of *Musca domestica* pupae [18] or some other anti-insect activity as already discussed above.

As was already mentioned the occurrence of this pest is subject to a suitable year. The harmfulness of adults is usually not significant and treatment against adults is not recommended. Significant yield losses are caused by the larvae of the last stage of development (75 % of the total quantity of damage throughout the larvae development). The larval density of 22-26 larvae per 100 stems of winter wheat can cause yield losses up to 4%. [31] whreas the density of 1 larva per stem can cause up to 12.65% yield loss which gives the economic threshold of 0.4 larvae per stem during the spike emergence to anthesis stages [4].

In conclusion, this is the first report on the use of essential oil against the serious cereal crop pest *O. melanopus*. Although the *C. sinensis* essential oil was not active against *O. melanopus* adults it exerted interesting larvicidal effect. Considering its very low persistence in treated plants and low toxicity and thus low negative impact on the environment, *C. sinensis* is very promising alternative to synthetic pesticides, suitable for implementation to integrated pest management and organic farming.

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Section – Rural Development

Outlet centre as a potential development impulse: a case study of the municipality of Voderady

MAREK CIVAN, ANDREJ SVORAD, ALFRED KROGMANN
Department of Geography and Regional Development
Faculty of Natural Sciences, Constantine the Philosopher University in Nitra
Trieda Andreja Hlinku 1, 949 74 Nitra
SLOVAKIA

marek.civan@ukf.sk

Abstract: The paper is focused on residents' perception of the newly established outlet centre located at the peripheral zone of the selected village. In the first part is a summarized theoretical aspect and literary source that is dedicated to the matter of interest with an emphasis on realized studies in Slovak and Czech geographical areas. Subsequently, the area of interest is picked up from various points of view, whereas the attention is concentrated on the main location conditions of placement of the target shopping destination. The core of the paper lies in the interpretation of the field survey results through which the features of the outlet center presence was researched. The obtained data is evaluated and synthesized using the Likert scale. Based on that are identified positive and negative aspects that the outlet centre has brought to the common population of rural settlements.

Key-Words: shopping tourism, outlet center, rural area, Voderady

Introduction

For a society of today, free time activities are tantamount and essential components of lives, as much as work and accommodation [1, 2]. Amusement, adventure and consumption have significantly become the initiators of interconnections between tourism and retail, what has resulted into the formation of shopping tourism. Shopping as an activity is not just the way of acquiring basic conditions for life, but the form of pleasure, as well.

For the satisfaction of needs to experience shopping, big shopping malls were created (for business and societal utilization) located at the outskirts of cities. There were many times the mean of revitalization of urban centers. Outlet centers also belong to shopping venues, where experience shopping runs because of their special large-scale form of retail. Within the term of goods, there are mostly offered fashion clothes and related accessories.

The first outlet centre was established in Pennsylvania in 1971. In 1995, there were 324 outlet centers in the USA, which represented that the market was fully covered. This phenomenon also came to Europe, but it took some time, whereas the first outlet store was opened in France in 1984.

An aggrandizement of this shopping destination affected the Slovak Republic in 2013, when the first outlet centre in the municipality of Voderady was opened. Its establishment was supported by positive experiences from outlet centers in Parndorf (Austria), which has become a long-term favorite shopping destination for customers coming from Slovakia. This form of retail has been brought to the Slovak population, but there is an assumption of captivation of some Austrian clients because of the restrictions that influence shopping facilities. The investment in Voderady has brought for inhabitants new experiences that are the matter of interest of this paper.

Many experts have mostly focused on research of development processes in urban areas. Among Slovak geographers, Trembošová [3], Trembošová and Tremboš [4] concentrated on the influence of retail on the improvement of urban features in the city of Nitra. Križan [5], Križan and Lauko [6], Križan and Bilková [7] paid their attention on retail relations in the capital of Slovakia, Bratislava. The connections within retail and their changes in area of the biggest cities in Eastern Slovakia (Prešov, Košice) were researched by Ferťalová [8] and Mitríková [9]. An impact of services and retail on rural hinterland of Bratislava was studied mostly by Lauko [10, 11], Lauko, Križan and Tolmáči [12]. The topic of features of shopping tourism between Slovakia and Austria and the example of the outlet center in Parndorf was the matter of research by

Civáň and Krogmann [13]. The geographers from Czech Republic explored mostly important regional city centers. The retail network of Brno was researched by Mulíček [14] and Kunc [15], whereas Szczyrba [16] paid attention on retail equipment of Olomouc. In consequent study, Kunc [17] concentrated on both of the mentioned cities and focused on shopping habits of the population. The complex study of retail presented Szczyrba [18] and Spilková [19], whereas Szczyrba, Fiedor and Kunc [20] devoted their research to retail and its position in the Czech countryside.

Trembošová [21] and Civáň [22] also pointed out many interconnections between two related branches (tourism and retail) that are very significant in our current society.

Material and Methods

The area of interest

The target area, where the field survey was realized, was the municipality of Voderady, which belongs from administrative point of view to the Region of Trnava (NUTS III level) and the District of Trnava (LAU I level). The number of inhabitants reached 1,424 people up to December 31, 2013[23]. Hence it belongs to the group of medium-sized rural settlements.

The accessibility is a very important element within the terms of localization of the outlet center for its potential customers. The most remarkable city is Bratislava (the distance takes 48 km using the D1 highway), but the selected shopping destination can be accessed from other regional city centers (Trnava, Trenčín, Žilina). Other potential clients may use the R1 expressway that connects cities from Banská Bystrica to Nitra and accesses the D1 highway near Trnava.

Methodology

The field research of inhabitants' perception of the outlet center was realized through a questionnaire survey during one day in the mentioned municipality. Švec [24] states that it is a research method used mostly for collective and relatively quick ascertaining of information about opinions, attitudes and findings of respondents to current or prospective reality. The identification of attitudes of the population living in the municipality of Voderady to the newly established outlet center in the peripheral zone was the aim of the presented survey. Ritomský [25] presents that formation of questionnaire and particular questions play a key role within this method of obtaining data. It was necessary to follow principles known from theory and praxis of empirical researches. The mentioned principles and facts are connected not only with the content, but also with the need of brief, obvious and understandable form of expressing the opinions. There is an interference point with the design of a questionnaire and principles of the questions' creation, such as completeness and unambiguity. The length of an interview and a workload of the interviewer in the area were other important features we need to pay attention to. The importance of a questionnaire and its consequent utilization is mentioned in studies made by Veselovský [26, 27, 28].

The questionnaire that the respondents faced is compiled from two main sections. The first one is focused on the identification of basic facts about respondents (sex, age, marital status, level of education, economic activity, monthly income and the length of dwelling in the municipality) and consisted just from closed-ended questions. The second part of the questionnaire was dedicated to obtaining attitudes of respondents to the mentioned outlet center. We used the Likert scale, which is considered as the one of the most used and reliable techniques in this way. The Likert scale consists of particular statements and there are five possible answers (strongly agree, agree, neutral, disagree, strongly disagree) for respondents, but each of them can pick only one answer for one question. To identify respondents on the scale were formed these 15 confrontational statements (abbr. S):

S1: Location of the outlet centre has helped publicize the municipality within the region.
S2: The outlet center has brought jobs mostly for inhabitants of the municipality.
S3: The land, where the outlet center is located, could be used more effectively for agriculture.
S4: The outlet center has caused the increase of traffic in the municipality.
S5: The outlet center has caused the increase of waste in the municipality.
S6: The prices of land, goods and services has become higher because of presence of the outlet center.
S7: The attendance of municipality of visitors from neighboring Slovak regions has rose after opening of the outlet center.
S8: The municipality economically prospers from the presence of the outlet center.
S9: The outlet center can pull more investors to the municipality or adjacent region in the future.
S10: The outlet center actively cooperates in the municipal events (e.g. cultural, sport events, etc.).
S11: The outlet center is mostly used by tourists and not by inhabitants of the municipality.

S12: I am used to choosing the outlet centre for spending my free time.
S13: The outlet center will be prospering and developing in the future.
S14: I consider the location of the outlet centre in the municipality as a mistake.
S15: The presence of the outlet centre has raised my patriotism to the municipality.

The final evaluation of the Likert scale was realized through the marking of the questions of every respondent. Each of the statements got a number of points (from 1 to 5) and their sum displayed the total score of each interviewed person. In this case, the man, who reached the minimal number of points (15) was extremely positive to the presence of the outlet centre. On the other hand, a respondent that acquired 75 (100%) points presented a strongly negative feeling to the outlet centre. All of the respondents were divided into 5 categories that showed their attitude to the outlet centre in general. Based on that, it can be distinguished as very positive (0–20% of total points), positive (21–40%), neutral (41–60%), negative (61–80%) and very negative (81–100%) attitude.

Results and Discussion

The researched sample consisted of 85 random respondents. Within the light of their basic information and structure, men (54) predominated over women (31). In terms of age structure can be summarized that our sample mainly copies a structure of the whole population. More than 75% of the people asked have been living in the municipality 15 years or longer. From the educational point of view, predominated respondents reached a secondary level of education.

The questions especially focused on the perception of the outlet center brought, in many cases, visible differences. The statement referred to publicity and propagation of the municipality was due to the outlet center belonging to the group of declarations that reached the majority share of agreement. More than 80% claimed that the presence of the outlet centre made their village more visible in the region. An affirmative opinion had more than 50% about the question of jobs for residents of the municipality. Approximately 60% of all interviewed people presented their stance that the land, where the outlet center can be found, could be used better and more effectively, such as for agriculture. More than half of the questioned people proclaimed that the process of the outlet center construction increased prices, especially the prices of land. Fewer than 70% of the respondents answered that the outlet centre can pull more investors to the municipality or its neighborhood in the future. The majority (70.59%) of the surveyed sample does not use the outlet centre for their free-time activities, but on the contrary, more than 65% do not consider the establishment of the outlet center as a mistake.

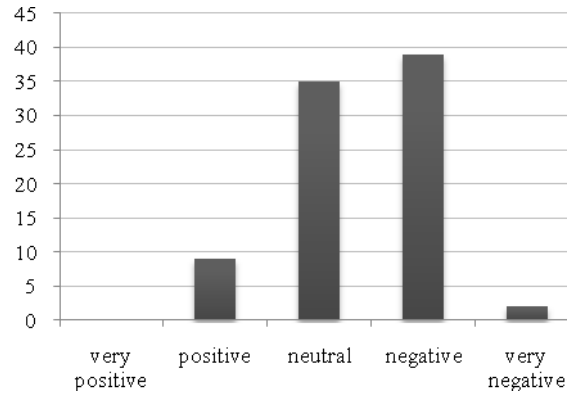
The proportional shares of inhabitants' answers on all statements are presented in Table 1.

Table 1 Inhabitants' reactions on the statements (%)

Statement	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
S1	63.53	20.00	5.88	7.06	3.53
S2	14.12	40.00	16.47	17.65	11.76
S3	47.06	15.29	11.76	16.47	9.41
S4	30.59	22.35	2.35	32.94	11.76
S5	10.59	16.47	20.00	25.88	27.06
S6	29.41	23.53	23.53	15.29	8.24
S7	15.29	24.71	20.00	32.94	7.06
S8	21.18	22.35	35.29	11.76	9.41
S9	17.65	52.94	17.65	9.41	2.35
S10	18.82	23.53	32.94	16.47	8.24
S11	29.41	24.71	16.47	23.53	5.88
S12	3.53	17.65	8.24	20.00	50.59
S13	12.94	30.59	28.24	21.18	7.06
S14	14.12	12.94	4.71	25.88	42.35
S15	4.71	25.88	15.29	34.12	20.00

The final results of the questionnaire survey realized, via the Likert scale, were that it showed neutral or negative attitudes of the interviewed inhabitants of the municipality of Voderady to the shopping destination located in their village. A negative attitude presented 39 of all the people, whereas 35 people of the sample perceived the outlet center in the neutral way (Fig. 1). Very negative feeling was contributed only by 2 respondents. Positive attitude was recorded by 9 people, whereas very positive attitude was not obtained. These final results reflect the situation that residents of the municipality have not been in a good mood about the presence of their shopping destination.

Fig. 1 Final attitudes of residents of the municipality of Voderady to the presence of the outlet centre



Conclusion

A boom of outlet centers hit Western Europe mostly in the last two decades of the last century. Slovak customers showed their favorability to this shopping venue by visiting the well-known outlet centers in Parndorf that is located near the Slovak and Hungarian borders. Some good conditions for the development of this shopping destination in Slovakia was formed. Not only did the outlet centre attract many customers from various regions, but first of all, it affected lives of ordinary people living in the municipality, where it is located. The realized questionnaire survey brought various attitudes of the population that showed some contrary features of perception of the outlet centre. Mostly older people, who cannot find a job in the outlet centre and also remember utilization of land for agriculture due to the quality of soil, did not present positive attitudes to the presence of the outlet center. Another group of people consisted of a population up to 40 years (students, employed people, mothers during maternity leave, etc.), who thought that the presence of the outlet center is a possibility for the municipality and its inhabitants. Each respondent answered at least one question in a negative way, what resulted to the fact of not very positive feelings about the outlet center. Looking to the future, we should think more intensively about the people, who get in touch with the outlet center on a daily basis; not only workers and management members that commute to the place every day, but especially ordinary people, who see the outlet center every day. The outlet center in the municipality of Voderady celebrates 1 year of existence (it was opened on October 30, 2013) and there is a future possibility that the residents of the municipality will change their mood about it, if they see more cooperation and interconnections between their everyday life and the presence of the outlet center.

The research of large shopping destinations' influence to rural settlements in Slovakia has not been finished yet. Hence we hope that the issue of outlet centers and their position can be the matter of future studies.

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The Changes of Czech Cultural Landscape in the Intermediate Countryside

VERONIKA DOSKOCILOVA, MILADA STASTNA, HANA VAVROUCHOVA
Department of Applied and Landscape Ecology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

veronika.doskocilova@mendelu.cz

Abstract: The Czech cultural landscape is a subject to permanent change, because it is permanently used and influenced by man. Cultural landscape associated with the local identity plays a key role in rural development. Therefore, it is important to document the changing landscapes for future generations. One type of Czech rural areas is an intermediate countryside as for example the Vysokomytsko microregion. The Vysokomytsko microregion is a voluntary association of municipalities in the Pardubice region.

Key-Words: cultural landscape, intermediate countryside, landscape heritage, land use, Vysokomytsko microregion

Introduction

Landscape which is permanently used and influenced by man can be considered as cultural landscape. This landscape has been in the current area of the Czech Republic since the Neolithic Age [1]. Therefore the cultural landscape is subject of constant change. It is relatively easy to analyse quantitative and qualitative changes in the landscape. But some investigations about social and mental aspect are rare. However just the mental changes and landscape perception are often most determining for a creation of local identity and by such a way for a stabilization of rural settlement [2]. Local identity associated with the cultural landscape plays a key role in rural development. This project aims to capture vanishing testimony about the cultural landscape and to retain it for the future generations.

Countryside and rural areas are important areas of Czech territory, but it is difficult to clearly define their boundaries. Basically there is no unified definition for the term countryside. The countryside is generally defined as a sparsely populated area where agriculture has an important function. Countryside can be defined by low number and density of the population in terms of demographics, by a specific type of buildings in terms of urban and by another way of life in terms of sociology [3]. The only universally accepted international definition is the OECD definition, which is based on population density. The upper limit of the population density is 150 inhabitants/km^2 for countryside. This limit is reduced to 100 inhabitants/km^2 in Czech Republic.

Fig. 1 Spaces typology for classifying of rural areas in the Pardubice region (Source: [4], edited by Doskocilova)

Municipalities with less than 2,000 inhabitants can be considered rural municipalities. There are more than 5,000 of these municipalities in the Czech Republic and their population is about 2.5 millions. The area of the cadastral territory of these municipalities forms ¾ of our country area. These are the reasons why the countryside in the Czech Republic must be taken as a significant area. Three types of rural areas are defined by typology of rural municipalities. The suburban countryside is found in vicinity of big cities and it is influenced by suburbanization and significantly increasing its population. The intermediate countryside is the average of developed municipalities farther away

from large urban centers with good transport connections. Remote countryside is defined in peripheral areas with unfavorable socio-economic characteristics of the population.

The cultural landscape went through many changes during the last seventy years. These changes can be measured in many ways, including comparative methods. In this sense, it is the last chance to experience the original mental image of the landscape.

Material and Methods

The project "Landscape memory as rural heritage" was launched in January 2014. Mapping of the changes in the cultural landscape of Czech Republic in the mental picture of their inhabitants is the aim of the project. The capture of vanishing testimony about the landscape and its preservation for future generations are the purpose. The model area was chosen with regard to the past landscape development. Therefore, each model represents a different type of the Czech cultural landscape. The selected model areas were chosen a case area at the level of microregions. Case study Vysokomytsko microregion should identify, analyze and permanently document the changes of Czech cultural landscape of the intermediate rural areas. Landscape structure was analyzed at the level of primary (natural), secondary (land use) and tertiary (landscape protection, socio-economic areas). This paper analyzes the landscape structure at secondary level.

Old map data were described based on the internet application of Geoinformatics Laboratory [5]. Land use data from the years 1845, 1948, 1990 and 2000 are from Database of long-term changes in land use of Czech Republic [6]. The data of the term from 2001 to 2013 which were used in the case study of Vysokomytsko microregion is from sources of Czech Statistical Office [7].

Currently field research is implemented focusing on landscape values and perceptions of the cultural landscape of its inhabitants. The initial information about the microregion development will be obtained using semi-structured interviews with current and former mayors and residents older than 65 years. The results arising from the interviews will be compared with historical sources. The obtained values and information about the territory will be passed to the youngest generation (pupils and students of local schools) through organized discussions, debates and exhibitions during the second year of the project. The final aim of the project is to prepare general recommendations for strategic development of communities and regions (the modern chronicle of the municipality).

Results and Discussion

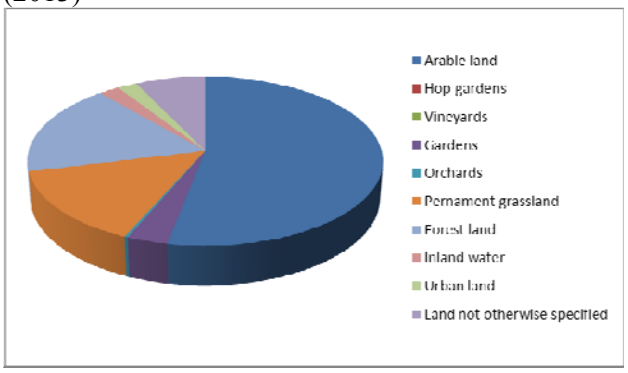
Vysokomytsko microregion is a voluntary association of municipalities in the Usti nad Orlici district in Pardubice region (see Fig. 2). The microregion was established on 8 March 2001, with an area of almost 20,000 ha. The microregion surface is hilly area with the average elevation about 350 meters above sea level. It is the warmest and driest areas in the Pardubice region. The microregion area falls into the Labe river basin. The Vysokomytsko microregion has the largest percentage of agricultural land in the Pardubice region. Arable land is covering more than half of the microregion (see Fig. 3). Forest area is quite small and widely dispersed, larger area of forests is in the north of the microregion. Permanent grass land covers almost one-fifth of agricultural land. There are not vine yards and hop fields in the microregion. During last years, there is an increase of developed and other areas. The microregion is ecologically unstable, ecological stability coefficient is 0.59.

Fig. 2 Place of the Vysokomytsko microregion in the map of the Czech Republic

Vysokomytsko microregion consists of 28 municipalities and there are more than 21,000 inhabitants but the population density is below average (113 inhabitants/km^2). Three-quarters of municipalities are villages with more than 500 inhabitants. There is just one small town – Vysoke Myto [8]. Vysoke Myto is the sixth largest city of the Pardubice region. Cadastral area of the municipality is a total of 4,402 ha. The population of Vysoke Myto is 12,436 inhabitants. Vysoke Myto is a municipality with extended powers. It means that this small town performs administrative duties for the inhabitants of the surrounding area. Vysoke Myto was founded by Czech King Premysl Otakar II. at place where an older settlement originally stood in 1262. There passed Trstenice trail, which

was an important trade route leading from Bohemia to Moravia. Vysoke Myto is a historic town with many interesting cultural monuments. In the center of town is the square of Premysl Otakar II. It is the largest square shape square in the Czech Republic. Vysoke Myto is situated at an altitude of 284 meters above sea level. The Loucna River flows through the town. There is breeding Chobot pond on the northeastern edge of the Vysoke Myto with an area of 49 ha.

Fig. 3 Land use of Vysokomytsko microregion (2013)



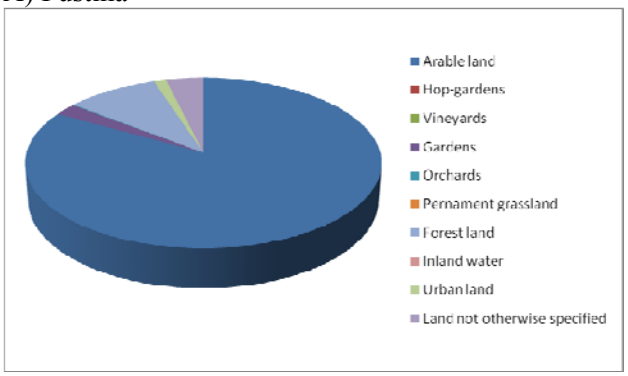
Two municipalities were studied in the microregion. There are the municipalities Pustina and Bucina. Pustina is located 7.5 km south of the Vysoke Myto. Pustina is one of the youngest municipalities in the Pardubice region. The first written mention of this small village dates from 1720. There are currently 67 inhabitants in the village. The total cadastral area of the village is 283 ha. Pustina is located at an altitude of about 450 meters above sea level, and it is one of the highest villages in the Vysokomytsko microregion. There are beautiful views of the surrounding countryside from the hill above the village. Bucina is located 7 km southeast of Vysoke Myto. There are 228 inhabitants in the village. The village area is a total of 383 ha. The first written mention of the village dates from the foundation charter of King Vladislav I of 1167. Although Bucina is located at a lower altitude (316 meters above sea level) than Pustina there are also good views of surrounding countryside.

The changes in the landscape of surveyed villages were examined on the basis of the surviving map data. Müller's map from 1720 presents a rough assessment of landscape structure. Bucina is already shown on this map. Pustina is not shown on the Müller's mapping, although Pustina is known this year since the first written mention. Comprehensive map works of the 1st, 2nd and 3rd Military Survey and stable cadaster served basic information about a landscape. The 1st Military Survey maps from the 18th century show the village Pustina for the first time. Forest areas are located mainly in the southwestern part of the cadastral territory, but there is also urban land. Bucina is unfortunately on the border of two map sheets within the 1st Military Survey. The top sheet is damaged at the bottom, so some information about the village is missing. Also this map shows the forest area in the southwestern part of the cadastral territory. The arable land is a large part of both villages area. The maps of the 2nd Military Survey from the first half of the 19th century show that forests disappeared in the southwestern part of the cadastral area and near the village Pustina. Forest stands in the southwestern and northwestern parts of the Bucina cadastral retained as arable land. There are no major changes on maps of the 3rd Military Survey. Forest areas are still as arable land around the village. Alleys are shown on the map around the main roads. The water surface can be clearly distinguished in the stable cadaster map of Bucina, These surfaces were located in the area. There is currently only fire tank in Bucina on the place where it was Louze.

The basic territorial unit in the Database of long-term changes in land use of Czech Republic [6] was created by linking of cadastre, depending on changing of cadastre's land area. For this reason, the Pustina cadastre was connected with cadastre of close village Repniky. Land use data from the years 1845, 1948, 1990 and 2000 can not be used. Bucina cadastre is considered a separate basic territorial unit in this database. In 1845 the arable land occupied 304.6 ha (80%) and forest land occupied 34.7 ha (9%) in Bucina. The arable land reached its peak of 319.6 ha (83%) in 1948, but forest land decreased by 6 ha. The arable land decreased to the 280.2 ha (73%) in Bucina during the following period of the communist regime. The arable land increased again by 19.1 ha (78%) during the transformation. This can be explained by a change in agricultural policy in the Czech Republic.

Statistics of land use have been kept since the beginning of the 21st century [7]. In 2001 the arable land occupied 230.81 ha (83%) in the Pustina cadastral area. Forest land occupied 26.11 hectares (10%). The urban land occupied only 3.52 ha i.e. 1% of the total area of cadastre (see Fig. 4A). In the same year the arable land occupied 299.26 ha (82%), forest land 29.25 ha (8%), and urban land 6.96 ha (2% - see Fig. 4B) in Bucina.

Fig. 4 Land use of villages from the year of 2001
A) Pustina



B) Bucina

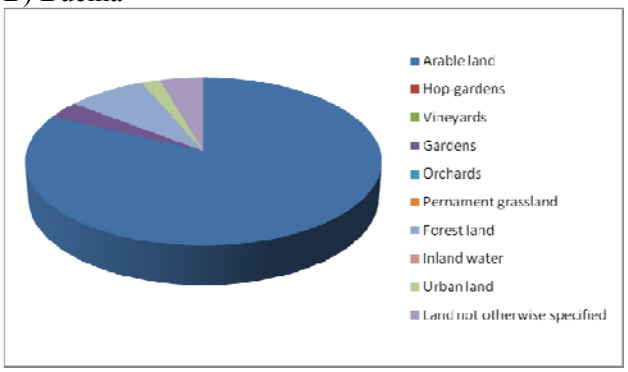
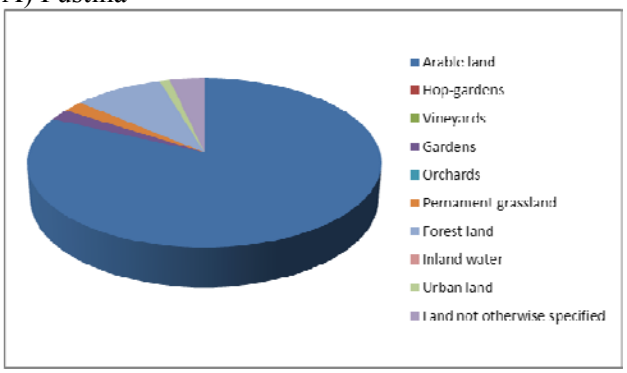
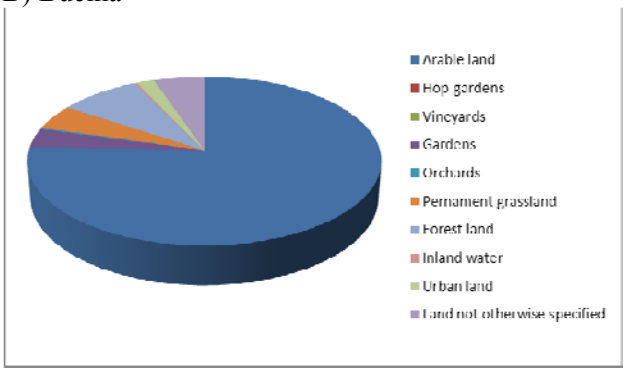


Fig. 5 Land use of villages from the year of 2013
A) Pustina



B) Bucina



Currently arable land occupies 230.9 ha (82%) of the cadastral area of Pustina (see Fig. 5A). Forest areas are located at the 9% of the area. Other categories of land use in the area either absent or are negligible. Arable land occupies 289.28 ha (76%) of the territory in the Bucina cadastre (see Fig. 5B). Forest areas represent only a small part (8%). Permanent grassland and other areas occupy both 5% of the cadastral territory. Other categories are zero or negligible.

Detailed information about land use of the Pustina cadastre is up to 13 years. In this period, the land use almost unchanged in Pustina. In contrast, changes are obvious in the land use of Bucina cadastre. Arable land decreased by 5.3 ha from 1845 to 2000. This area decreased by a further 10.2 ha for the past 13 years. The forest area decreased by 2.7 ha from 1845 to 2013. The largest increase belongs to the other areas and urban land in this cadastre in the category of land use.

Conclusion

The nature of this intermediate countryside did not change for last three centuries. The surrounding landscape of both studied villages is still used for agricultural farming. Forest land represents only a small part of the territory in comparison with arable land. Still almost everyone from the natives of the village finds something on the landscape that is missing or something will be missing the next generations. The aim of this project is just to keep at least the form of memories, image or photos.

The main project output will be modern chronicle of Bucina and Pustina communities. This chronicle will synthesize gained knowledge, memories and stories of local residents, archival video and audio documents and unique information about the Vysokomytsko microregion.

Another result of the project will be also a popularization of problems of microregion landscape and meeting of local residents of all ages (especially the elderly and children). The results of the project will serve to further promote of microregion and for the government too.

Acknowledgement

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Identifying Landscape values in a changing countryside of the Bystřicko micro-region: A Case study of the Rožná Village

HELENA LINCOVA, MILADA STASTNA, HANA VAROUCHOVA
Department of Applied and Landscape Ecology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

helena.lincova@mendelu.cz

Abstract: Czech cultural landscape was on the threshold of big changes in a beginning of the second half 20th century. These changes passed off predominantly without considering the consequences, therefore many of a landscape values have been hidden or even destroyed.
The contribution aims at the identification of landscape values in a post-mining [1] countryside of the Bystřice nad Pernštejnem micro-region. It is focused on the Rožná Village and its surrounding landscape, which was affected by many ways during last 70 years.
The methodology consists of following steps: to collect data about the Bystřice nad Pernštejnem micro-region in general, to focus on the Rožná Village in detail, to identify landscape values indoors and to compare them with a real situation verified in-situ.
The data used in our study were from these sources: ČUZK (The Czech Office for Surveying, Mapping and Cadastre), ČSÚ (The Czech Statistical Office), CENIA (Czech Environmental Information Agency) and department database.
To sum up, the post mining landscape in the Rožná Village has many varying values and most of them are connected with the primary landscape structure and its reflection to the both others. This impact together with the consequences of the mining leads to results in cultural, functional and natural values.

Key-Words: landscape changes, identifying values, post-mining countryside, Bystřice nad Pernštejnem micro-region, Rožná

Introduction

Bytřice nad Pernštejnem micro–region is situated in Vysočina region, close to the midpoint of Czech Republic. On the contrary to the location the micro–region is characterized by bad conditions of public transport and road network. Whole territory consists of 37 municipalities with total area of about 326 km^2. The Rožná Village is situated in a centre of this territory with an area of 12.87 km^2.

However the contribution is aimed at identify, evaluate and record values disappearing in last seven decades it is necessary to mention the whole history. As well as the whole micro–region the Rožná Village has been influenced by three main factors. Firstly, geological processes formed the surface to highlands landscape. Secondly, the Svratka river created many meanders and finally a human being changed the countryside in many aspects. The most significant conditions are these:

- The Perštejn family. They took over an agricultural landscape in 13th century. Nowadays, there are many ruins, which used to be as a summer residence by The Pernštejn Family.
- Mining. Consequence of an extensive medieval mining (silver, lead and copper) activities with impact of a recent history (uranium mining) are responsible for today´s conditions.
- Religion and Vernacular architecture. Sacral buildings and architecture based on local needs and construction materials recorded the spirit of the time.
- Transition. The Socialistic era and the Velvet Revolution have caused the biggest changes in a rural space during last 70 years. [2]

In reference to these facts the identification of the landscape values is quite difficult. As a first step towards the aim we have to deal with an in-depth analyse including evaluation of primary, secondary and tertiary landscape structures. The final synthesis leads to definition of "objective" landscape values.

Secondly we try to capture vanishing testimony in the inhabitant´s reflection. The results will be based on the comparison of "objective" landscape values using statistical data and "mental" landscape values hiding in the perception of the people.

The two-year project "*Landscape memory as a rural heritage – the changes of Czech cultural landscape in the mental reflection of its inhabitants*" started in January 2014. The aim of this paper is to present a partial research, which has been carried out till these days. The first step towards the final aim.

Material and Methods

The case study of the Rožná Village was considered in context of the Bystřice nad Pernštejnem micro-region in each of the partial analysis.

Analysis of the primary landscape structure.

This analysis was focused on natural conditions, which formed the local countryside to present form. Geology, morphology, pedology, hydrology and climatology characteristics were explored in this phase.

Analysis of the secondary landscape structure.

This phase was the main information source for the identification of cultural, social and functional values in evaluated landscape. History, settlement pattern and urbanization together with land use, population growth, socioeconomic and infrastructure conditions affect quality of life in many ways currently.

Analysis of the tertiary landscape structure.

Genius loci, landscape character, nature protection and traditions together with the primary structure were the basis for the determination of nature, recreational values, social and cultural values.

The final synthesis. Identification of landscape values in the case study area.

The results of the final synthesis concluded to following landscape value: cultural, social, recreational, functional and natural (Tab.1). Cultural values of the landscape were seen in spiritual and religious values, intangible values (like personalities, events, fairy tales), recognized cultural values (protected monuments of different ranks) and informal values like landscape composition, urban structure, etc. Social values were represented mostly by landscape suitable for meetings of people. Recreational values consisted in recreational zones, geo-parks, therapeutic values etc. Among functional values of the landscape anti-erosional, anti-flood measurements, landscaping could be named. Natural values were represented by landscape and nature protection of various scale. [3]

The data used in our study were from these sources: ČUZK (The Czech Office for Surveying, Mapping and Cadastre), ČSÚ (The Czech Statistical Office), CENIA (Czech Environmental Information Agency) and department database. [4]

Table 1 The partial analysis in relation to the "objective" landscape values, Helena Lincová, 2014 .

Type of analysis	The explored characteristic in the	Related landscape values
Primary landscape structure	Geology	Nature values Recreational values
	Morphology	
	Pedology	
	Hydrology	
	Climatology	
	Potential vegetation	
Secondary landscape structure	History	Cultural values, Social values
	Population growth	Social values
	Urbanization	Social values
	Settlement pattern	Cultural values, Social values
	Infrastructure	Functional values
	Socioeconomic	Social values
	Land use	Functional values
	Nature protection	Cultural values
Tertiary landscape structure	Genius loci	Nature values
	Landscape Character	Recreational values
	Traditions	Social values

Results and Discussion

The cultural values

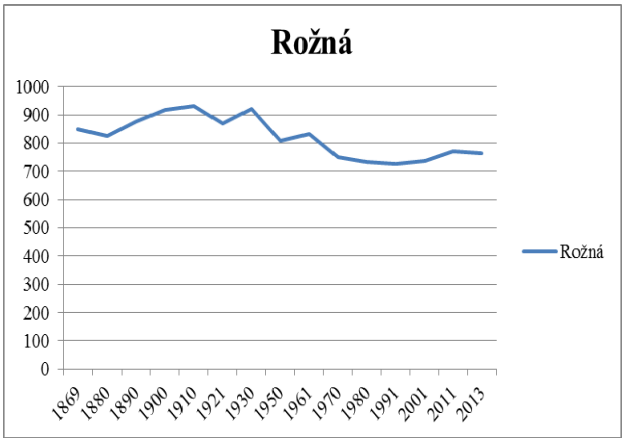
A rural countryside of the Rožná Village contains three different types of cultural values. Firstly, there is St. Havel Church with adjoining graveyard from 14th century. Secondly, the Hradisko Hill (Fig.1), a mysterious landmark situated on the right bank of the Nedvědička stream, which is closely attached to old building area. Hradisko is also a mineral deposit of lepidolit. Finally, there are two closed uranium mine.

Fig. 1 The Hradisko Hill with a closed uranium mine on the horizon, Helena Lincová, 2014

The social value

The quality of life in the Rožná Village is strictly connected with social infrastructure. The population growth (Fig.2) was predominantly influenced by new incomers in Josefov and Zlatkov cadastral areas. There are 765 inhabitants living in the whole municipality, which have a possibility to meet in a public library, a multifunctional playground and three restaurants.

Fig. 2 The population growth rate, Helena Lincová, 2014



The recreational values

The landscape reclamation made a contribution to a new recreation area along the Zlatkovský stream. Two multifunctional ponds with an accompanying equipment provide a public space usable by both, residents and tourists.

The functional values

The post-mining landscape of the Rožná Village plays a key role in determination of the functional values. Two sludge reservoirs gained a new function. The first one was reclaimed for the purpose of a solar power plan (Fig.3)t, which is situated on the top of slag heap. Nevertheless the second one has not been reclaimed yet, an ecological restoration has started to create a new informal recreation zone.

Fig. 3 The solar power plant, Helena Lincová, 2014

The natural values

A similar situation is repeating in this case. In the field of the formal values The Svratecká Hornatina Natural Park covers the east part of The Rožná Village area. On the contrary, an ecological succession (Fig.4) is under way in many places in the post-mining landscape.

Fig. 4 The post-mining landscape under an ecological succesion, Helena Lincová, 2014

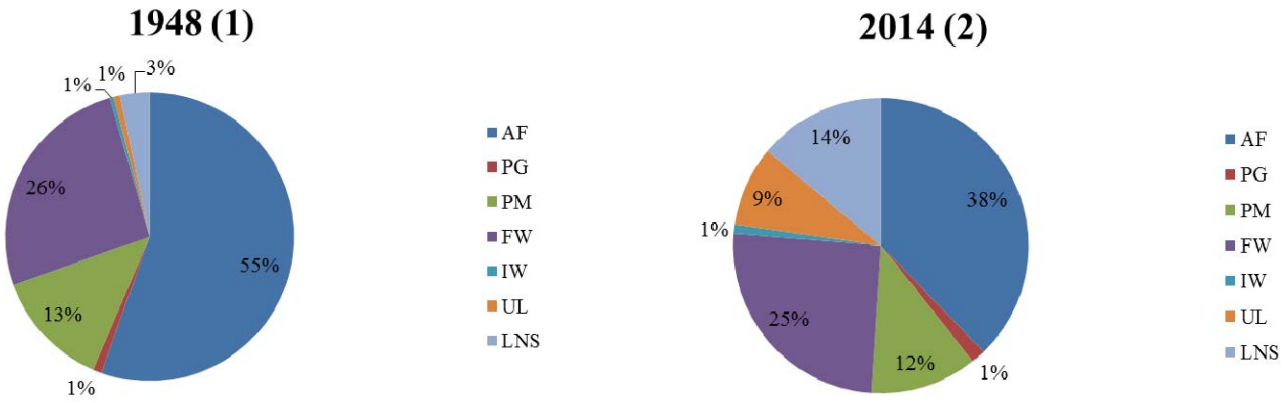
Conclusion

To sum up, the post mining landscape (Fig. 5) in The Rožná Village has many varying values. As we have seen, most of them are connected with the primary landscape structure and its reflection to the both others. This impact together with the consequences of the mining leads to results in cultural, functional and natural values.

These results show how much an individual and a holistic approach is essential. Therefore it is necessary to involve not only experts but also any responsible person, seniors 65+ and young people, as well. Take the second step towards the final aim.

Fig. 5 The Land use changes during last 7 decades, Helena Lincová, 2014

1) Situation in 1948, source: lucc.ic.cz, 2012 2) Situation in 2014, source: ČSÚ, 2014



Legend: AF– arable land, PG – permanent grassland, PM – pastures and meadows, FW – forests and woodlands, IW – inlad water, UL– urban land., LNS–land not otherwise specified

Acknowledgement

The study was realized with the financial support of the Technological Agency of the Czech Republic (TA ČR) as a part of the TD 020211 project.

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Current Condition of Electrical Engineering Industry in Individual Self-Governing Regions in Slovakia

MILAN MIDLER[1], ALENA DUBCOVA[2]
[1]Department of Ecology and Environmental Sciences
[2]Department of Geography and regional development
Constantine the Philosopher University in Nitra
Tr. A. Hlinku 1, 949 74 Nitra
SLOVAK REPUBLIC

milan.midler@ukf.sk

Abstract: The Slovak economy is generally regarded as a functioning market economy. Slovak electrical engineering industry belongs to younger and the most dynamically developing sectors of Slovakia.
Structural changes and foreign investments into the electrical engineering industry in 1989 led to its strong quantitative, qualitative and spatial changes. Consequently, growth and development of the electrical engineering industry varied among the individual self-governing regions of Slovakia. The article identifies, analyzes, compares and evaluates conditions of the electrical engineering industry at the self-governing level in Slovakia. Individual self-governing regions were allocated to the relevant category of development based on the typological analysis of selected geographical indicators.

Key-words: Electrical engineering industry. Regional disparities. Self-governing region.

Introduction

Industry in Slovakia plays an important role among economic sectors and significantly impacts the development of the overall economy.
The umbrella definition of industry covers all activities related to the extraction of raw materials, agricultural products and their transformation to finished products [1].

In the world, but also in Slovakia, industry has undergone a long development. In transition period after 1989 it becomes to the decline of industry. The biggest decline was observed in mechanical engineering industry. Part of the labour force was absorbed in tertiary sector, other part was registered as unemployed [2].

Currently, automotive, metallurgical and electro technical industry belong to the most dominant industries in Slovakia. The electrical engineering industry (EEI) is one of the most dynamically performing industrial sectors in Slovakia. Today, EEI is one of the main pillars of national industrial growth. EEI was significantly growing mainly after the World War II. Foundations of high and low voltage electrical engineering associated with the production of components for power stations, power supplies, motors, wires, insulators, television, radio apparatus and lighting have been shaped in this period.

Because of loss the COMECON market, ineffective and lagging production, EEI led as a sector with no potential for further growth. Since then EEI absolved extensive structural, quantitative and qualitative changes. After 1989, EEI production was focused on production of cable harnesses [3].
The reduction of the number of stuff and number of EEI companies was affected by economic crisis in 2008. Today, the production of electrical engineering products continually develops to the next production phase where implementation of new innovative technologies plays an inevitable role.

Production of electrical engineering equipment includes a wide range of simple as well as highly complex products. According to SK NACE Rev. 2 [4] EEI is defined as a summary of all divisions of industrial sectors.

26 – production of computer, electronic and optical products,

27 – production of electrical equipment.

Changes in EEI after 1989 were reflected also in the uneven spatial distribution of individual electrical engineering production sites in Slovakia.
Uneven spatial distribution of electrical engineering industry remarkably affects the development and growth of regional disparities.

A growing number of scholars with diverse backgrounds investigate and analyze regional disparities. Consequently, the definition and interpretation of regional disparity is inconsistent and highly diversified.
An important source of theoretical knowledge on the classification of regional disparities provide following scientific papers:

According to Viturka [5] regional disparities are defined as inequalities, differences and heterogeneities.

Hančlová, Tvrdý [6] confirm that the term regional disparity is relatively intensively used term. Scholar literature and scientific practice define it differently.

Regional disparity is understood as the distance between regions in abstract metrical space that can describe one selected descriptor of region or the entire group of these descriptors, statically as well as dynamically.

Matlovič, Matlovičová [7] characterize that analysis of relevant development factors is a basis for a definition of regional disparities in Slovakia. This means the identification of key impacts on regional development such as definition of suitable spatial units, selection of suitable rations as well as statistical instruments and scales that allow comparisons in time and spatial horizons.

Molle [8] explains that policies of European Union are closely connected to cohesion. The amount of disparities defines the ratio of cohesion shortage. Disparities' development is usually compared to the achieved level of living standard in members' countries, regions and social groups.

The OECD definition of regional disparities [9] has an important limitation. It is focused only on economical phenomena of regional disparities.

Vorauer [10] considers regional disparities as a problem or adverse events. On the contrary, regional disparities can be regarded also positively if the further development of the individual region is based on comparative advantage.

The level of EEI development in individual self-governing regions in Slovakia was defined by the typological analysis of selected geographic indicators: number of production plants, average evidence number of employees (AENE), turnover for own products and services (turnover), AENE per 1 production plant (PP), AENE for 1 km^2, AENE per regions' inhabitants, AENE per employed in industry in the region.

Material and Methods

The regional allocation to the relevant category was achieved as follows. Firstly, data values were grouped to five levels of development.

Secondly, the districts were allocated to five levels of EEI development based on the final indicator's value. Subsequently level of the indicators was counted by arithmetical average of regions. Level 1 was assigned to regions with the smallest classifications level of development, level 5 was assigned to regions with the highest classification level.

Evaluation of selected indicators is available in Table 1 and displayed in Fig. 4.
Type 1 with the least developed EEI
Type 2 with below average developed EEI
Type 3 with average developed EEI
Type 4 with above average developed EEI
Type 5 with the most developed EEI

Importance of electrical engineering industry in Slovak regions

The regional disparities in allocation, structure and performance of EEI are highlighted based on the comparison of selected geographic indicators presented in individual self-governing regions. Spatial definition of the region was carried out according to the current classification of statistical territorial units at eight self-governing regions of Slovakia.

Fig. 1 describes the spatial allocation of achieved level in **number of electrical engineering plants** in 2012. Statistical Office of Slovak Republic was the source of relevant information [11a], [11b]. Trnavsky self-governing region (TSR) had with 28 production plants the most dominant position of the division production of electrical equipment in 2012. The lowest number of production plants in the analyzed division was localized in Trenciansky self-governing region (TNSR) with 8 production plants.

The greatest level of representation of the division production of computer, electronic and optical products was in TSR with 15 production sites. 8 production factories were located in the Nitriansky self-governing region.

The lowest level of representation of the analyzed division was in Banskobystricky self-governing region (BBSR) with only 2 factories.

The growing importance of electrical engineering industry is definitely proved in the analysis of the ratio of the number of EEI production plants to the overall number of industrial production plants in Slovak Republic. The ratio was growing from 7% in 2008 to 8% in 2010 and to 8.6% in 2012.

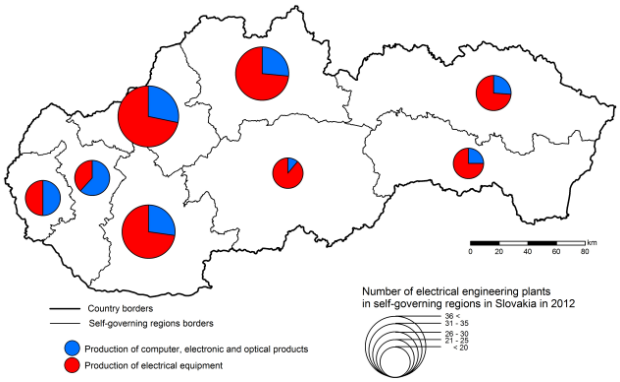
The highest observed increase in the number of production plants between 2008 and 2012 was recorded in the division production of electrical

equipment. Here the number of plants grew from 118 in 2008 to 139 plants in 2012.

The number of production plants in the division production of computer, electronic and optical products for the same period of time was stagnating on the level of 65 factories.

The comparison of different levels in increase in the number of plants between the individual self-governing regions showed that the highest increase was documented in regions in West Slovakia, such as TNSR, Zilinsky self-governing region (ZSR) and NSR. The lowest increase in the number of production plants was evidenced in BBSR and Kosicky self-governing region (KSR).

Fig. 1 Number of production plants in electrical engineering industry by self-governing regions in Slovakia in 2012



The average evidence number of employees depends on number of electrical engineering plants

Although the number of EEI production plants in Slovakia is continuously growing, the ratio of employed people in EEI to the total industrial employment has been constantly falling since 2008. Statistical data was taken from Statistical Office of Slovak Republic [13a], [13b]. In 2008, 49, 908 employees were working for one of the EEI production plants, resulting in 11.67% of total industrial employees. In 2012 the number of people employed in EEI decreased by 11, 577 employees. The number of employees in EEI in 2012 was 38, 331, resulting in 10.33% of total industrial employees. The economic crisis in 2008 is considered as one of the main reason for the fall in number of employees in EEI. Moreover, changes in statistical methods in 2009 influence the results as the production of electrical cables was transferred to the category of production of cars and accessories. As a result, EEI recorded a fall of 27, 000 employees. Furthermore, the significantly growing importance of new innovations and technological progress that leads to substitution of labour force by machines influence the decrease in the number of employees in EEI.

The decrease in the number of employees can be seen also in the individual divisions of EEI. In 2012 in the division production of electrical equipment were employed 23, 702, which was 5, 072 employees less than in 2008. Division production of computer, electronic and optical products with 14, 629 employees in 2012 recorded a decrease in 6, 505 employees compared to 2008.

The spatial allocation of the number of employees in EEI shown in the Fig. 2 describes that the highest level of number of employees in EEI in 2012 was achieved in TNSR with 11, 354 employees. It results in 3% ratio on overall industrial production in Slovakia. NSR a TTSR employed approximately the same amount of employees on the level of 6, 200 employees. In addition, the named regions had also similar 1.6% ratios of EEI employment on the overall employment in Slovakia. In 2012, NSR recorded a downturn in the number of employees of 1, 969 compared to 2010. At the same period of time, TTSR reported a decline of 894 EEI employees. The lowest levels of number of employees in EEI plants were evidenced in BBSR and Bratislavsky self-governing region (BSR) where the level of 2, 000 employees was not reached.

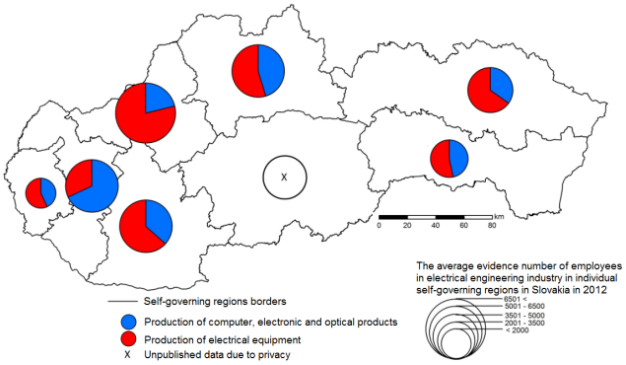
In 2012, TTSR documented 4, 224 employed in the division 26. TNSR, ZSK and NSR recorded approximately 2, 400 employees. Lower level of number of employees in the division production of computer, electric and optical products was noted in KSR, BSR and Presovsky self-governing region (PSK). The dominant role in the division production of electrical equipment had TNSR with 8 948 employees. In NSR 3, 942 employees were employed. In 2012 ZSR recorded a decline of 178 employees compared to 2011. Lower levels of number of employees in the division production of electrical equipment were found in PSR with 2, 302 employees and with 1, 698 employees in KSR. TTSR recorded 1, 988 employees which was 795 employees more compared to 2010.

Industry growth is determined by turnover for own products and services in the electrical engineering industry.

The indicator turnover for own products and services in the electrical engineering industry shows the overall growth of the sector. EEI plays an important and irreplaceable role in Slovak industry. EEI sales in 2012 compared to other economic acitivities in industrial production achieved in the division 26 with the volume of 5, 751, 253 thousands € the third place and in the division 27 with the volume of 2, 325, 108 thousands € the tenth place. The EEI market share increased from 13% in 2008 to 15.4% in 2009. Due

to the impacts of global economic crisis, the turnover share declined to 11.5% in 2012.

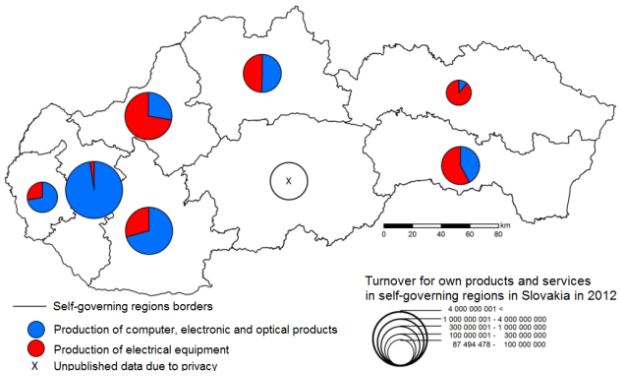
Fig. 2 The average evidence number of employees in electrical engineering industry in individual self-governing regions in Slovakia in 2012



The highest levels of turnover achieved the EEI in TTSR with 4, 115, 902 thousands €, followed by TNSR with 1, 453, 825 thousands € and NSR with 1, 100, 602 thousands €. The lowest levels of turnover were recorded in PSR with 87, 494 thousands €. The level of achieved turnover in PSR suggests that the EEI factories in the region make use of less technologically demanding production processes with low added value and higher demand for non-qualified labour force. Turnover decrease in 2012 compared to previous periods of time recorded NSR, KSR, PSR, BSR a BBSR.

In 2012, TTSR was dominated by turnover in the division 26 with 4, 019, 071 thousands €. Relatively high levels of turnover with 778, 386 thousands € were achieved in NSR. In BSR were prevailing turnover levels in division 26 with 182, 536 thousands €. The lowest amount of turnover in division 26 was evidenced in PSR with the level of 39, 667 thousands €. The lowest amount of turnover in division 27 was achieved in BBSR with 3, 113 thousands € in 2010. TNSR in 2012 reached the highest turnover level with 1, 052, 189 thousands € in the less technologically demanding division 27. Relatively high levels of turnover recorded NSR and PSR in 2012 when the turnover amounted to more than 300, 000 thousands €. The lowest turnover levels of division 27 were noticed in BSR and TTSR with amounts lower than 100 000 thousands €. BBSR's turnover for the division 27 in 2010 registered 93, 228 thousands € [13a, 13b]. Spatial description of the achieved level in turnover for own products and services in EEI are shown in Fig. 3.

Fig. 3 Turnover for own products and services in electrical engineering industry by individual regions in Slovakia in 2012



Results and Discussion

Evaluation of the electrical engineering industry in self-governing regions in Slovakia

The highest EEI development level (type 5) was recorded in TNSR that currently belongs to the most developed industrial regions in Slovakia. EEI also belongs to prosperous segments of the region. EEI achieved in all evaluated indicators except from turnover the best evaluation. Good positioning of TNSR influence production of electrical equipment with a focus on the production of modules for the automotive industry and the production of electric motors. The important status of TNSR is underpinned by the EEI share on the number of industrial production plants in the region that increased from 9.3% in 2010 to 9.7% in 2012. The EEI share on the employees in industrial production was stagnating around the level of 17.0%.

The most important electrical engineering companies in TNSR are: Emerson, a.s., Nové Mesto nad Váhom with 1, 396 employees. Other important companies are Hella Slovakia Signal-Lighting, s.r.o.; Leoni Slovakia, s.r.o.; Delta Electronics, s.r.o.; YURA Corporation Slovakia, s. r. o.; Askoll Slovakia s. r. o; Elster s. r. o.; Power - One s.r.o.; VACUUMSCHMELZE, s.r.o.; AU Optronics s.r.o.. To the regions with above average developed EEI (type 4) belong NSR and TTSR. The EEI production did not play such an important role in these regions as it does today. The EEI importance increased due to the market entry of foreign investors from Japan and South Korea. From the economic structure point of view, TTSR followed by BSK belong to the most industrially developed regions in Slovakia.

The EEI share on the number of production plants in TTSR increased from 7.3% in 2010 to 8.3% in 2012. The increase of the number of production plants correlated with the market entry of subsuppliers for Samsung. Also production plants producing electrical equipment for automotive industry were growing in number. The

EEI share on employees in industrial production in TTSR decreased between 2010 and 2012 from 14.9% to 13.3%. However, the EEI share on salaries in industrial production increased in TTSR from 91.3% to 96.3%. Salary increase can be the result of downsizing of low-salary employees and employment of better paid employees in the production of computer, electric and optical products. TTSR achieved high development level in all indicators except from the number of production plants whereas the indicator average evidence number of employees per 1 production plant recorded level 5. This disparity is caused by Samsung that in 2 production plants employs a significant amount of employees. The most important electrical engineering company in TTSR is SAMSUNG with the production in Galanta and Voderady. Other important companies are Elektronika Slovensko, a.s. Trnava; OMS, s.r.o., Dojč.

Table 1 Evaluation of selected indicators in 2012

Self-governing region	Number of production plants	AENE	Turnover	AENE per 1 PP	AENE per 1 km^2	AENE per regions' inhabitants	AENE per employed in industry in the region	**Overall level**
Bratislavsky	2	1	2	1	3	1	1	**2**
Trnavsky	2	4	5	5	4	4	4	**4**
Trenciansky	5	5	4	5	5	5	5	**5**
Nitriansky	4	4	4	3	3	4	4	**4**
Zilinsky	4	3	3	3	2	3	3	**3**
Banskobystricky	1	1	3	2	1	2	1	**2**
Presovsky	3	2	1	3	1	2	2	**2**
Kosicky	1	2	3	4	2	2	2	**2**

Abbreviations: AENE - average evidence number of employees; Turnover - turnover for own products and services; PP- production plant

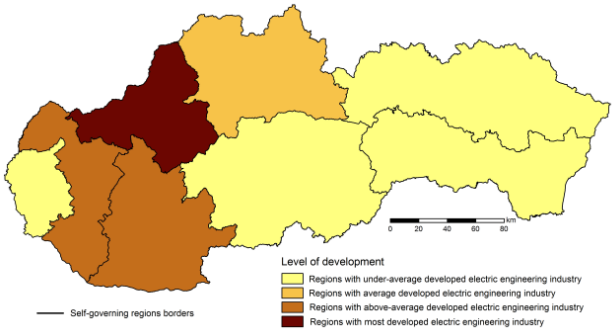
NSR is characterized from the economical point of view as industrial-agricultural region. Electrical engineering industry in NSR has never recorded such a high development level as it does today that is mainly influenced by the market entry of foreign direct investments. EEI share on the number of production plants in NSR was stagnating at 10.6% during the observed period of time which is derived from the new entry of subsuppliers in the production of electrical equipment. It is possible that the mother company finished cooperation with some subsuppliers in the division 26 and implemented their processes in-house. NSR achieved in all observed indicators an average and above average level. The most important EEI Company in NSR is Foxconn Slovakia, s.r.o. To other important companies belong ICS Industrial Cables Slovakia, s.r.o., Nitra; Osram, a.s., Nové Zámky; SE Bordnetze – Slovakia, s.r.o., Nitra; KROMBERG & Schubert s.r.o.; SEWS Slovakia, s.r.o.; Danfoss Compressors, spol. s.r.o.; Hefra Vráble s.r.o.; GLOBO EASTERN EUROPE, s.r.o.; Ryoka Global Europe s.r.o.; Daidong Slovakia s.r.o.; Farquell Nitra, s.r.o..

The average EEI development level (type 3) was achieved by ZSR. EEI has a relatively important role in ZSR and its importance is still growing. EEI in ZSR recorded an average level in the majority of evaluated indicators. The above average level was documented by the indicator number of production plants and below average level by the turnover indicator. The most important electrical engineering companies in ZSR are: Avex electronics, s.r.o., Dolný Kubín; Elteco, a.s., Žilina; Eltek, s.r.o.. Liptovský Hrádok; Klauke Slovakia, s.r.o.. Dolný Kubín; Panasonic Industrial Devices Slovakia, s.r.o., Trstená.

Type 2 with below average EEI development includes BSR, BBSR, KSR and PSR. The EEI development in BSR with the industrial orientation on services has almost no influence on the economic indicators of the region. The most important EEI companies in BSR are: BEZ Transformátory, a.s., Bratislava; Hella Innenleuchten-Systeme Bratislava, s.r.o., Bratislava; PPA Controll, a.s., Bratislava; Siemens, s.r.o.; Universal Media Corporation /Slovakia/, Bratislava; WHIRLPOOL SLOVAKIA spol. s.r.o.. The bad position of EEI in BBSR is derived from the evaluated indicators that were on the lowest level of scale except from the indicators, the average evidence number of employees per

1 production plant and per region's inhabitants which were on the below average levels

Fig. 4 Development level of self-governing regions in Slovakia in 2012



BBSR is a direct contrast to TNSR based on the EEI development. The most important EEI companies in BBSR are: H.E.S., s.r.o.; ZVT - PRINT, a.s.; Elba, a.s.; Brother Industries, Ltd.; Yura Eltec Corporation Slovakia s.r.o. Rimavská Sobota. KSR belongs to regions with the most developed industrial production. EEI does not play a significant role in KSR confirmed by the evaluated data. The most important EEI companies in KSR are: BSH Drives and Pumps s. r.o.; Molex Slovakia a.s.; Yazaki Wiring Technologies Slovakia, s.r.o.; Panasonic AVC Networks Slovakia, s.r.o.. PSR region is located outside of the main transportation and industrial ways. Therefore, it is a marginal region with shortage on financial resources for development. Final evaluation of electrical engineering industry supports the above mentioned facts. Relatively high evaluation was recorded in PSR only in the EEI share on the average evidence number of employees. Turnover documented the lowest value. As a result, the EEI in PSR has relatively low added value. The most important factory in PSR is Whirpool Slovakia, s.r.o., which is established in Bratislava. The important electrical engineering companies in PSR are: TESLA STROPKOV, a.s.; Elcom, s.r.o.; TATRAMAT - ohrievače vody, s.r.o.; Křižík GBI, a.s.; Hengstler. s.r.o.. Kežmarok; Regada, s.r.o., Prešov; Embraco Slovakia s.r.o. Kompresory Spišská Nová Ves.

Type 1 regions with the least developed EEI do not include any of Slovak regions.

Conclusion

Due to structural changes after 1989, electric engineering industry was regarded as a sector without perspective, technologies, knowledge and financial resources. Attracting foreign investors with their financial capital and technologies caused turnaround in EEI development. As a result, sector without foreign investments does not achieve such a significant development.

One of the first employers in the field of EEI were investors focusing on the production of wiring harnesses. Production of wiring harnesses and insertion of circuit boards are found in many cities. In recent years, due to required low wages and high operational performance, many foreign companies have left Slovak Republic. Today, this type of production is located only in areas with high level of unemployment rate.

The correlation between electric engineering industry and automotive industry is another factor of distribution of electric engineering industry in Slovakia.

The growth of EEI production for automotive industry leads to an increased number of international companies in Slovakia. Additionally, new companies specialized in massive production of LCD and LED panels, televisions, monitors, media players and satellite receivers set up their subsidiaries in Slovakia. These production facilities decreased dependence of EEI on automotive industry. Small companies producing electric motor systems, transformers, lamps, light sources and memory components have significant presence on market. The business crisis forced EEI companies in Slovakia to optimize their production processes and invest in more efficient machines, technologies and innovations.

EEI is unevenly distributed in Slovakia. The majority of companies are located in Western Slovakia close to cities Trnava and Nitra. Other locality with significant impact of EEI is Middle Váh valley with two significant concentrations of EEI companies. The first group is created by companies operating in Trenčín, Nová Dubnica and Dubnica nad Váhom. Second group is created by companies in Nové Mesto nad Váhom, Piešťany and Stará Turá. Rest of Slovakia is not that important for EEI. Further development of EEI in Slovakia requires building up and modernising infrastructure of research and development departments, innovation and human resources and thus creating the conditions for a development of knowledge-based economy. It is necessary to increase the use of local and regional resources provided by EU. Irreplaceable position of EEI development in Slovakia has foreign direct investment and its importance for modern technologies, innovations and new knowledge.

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Local action groups – entities of rural development

SVATAVA NUNVAROVA
Department of Regional economics and Administration
Faculty of Economics and Administration
Masaryk University
Lipová 41a, 602 00 Brno
CZECH REPUBLIC

nunvar@econ.muni.cz

Abstract: Rural development is carried out by local action groups. This paper introduces the entities concerned with local community development. It specifies what conditions must be met so that they can be established. This paper explores the ways the local action groups work and implement the LEADER method, which is typical of community-led local development. Also the difference of the local action groups and voluntary associations of municipalities is mentioned.

Key-Words: local action group, strategies of local development, LEADER, Rural development programme of the Czech Republic, rural development

Introduction

Most of the territory of the Czech Republic is rural. Regardless of the methodology used for the definition of this space, the countryside makes up about 75% of the area of the Czech Republic and about 25% of the population live there (more on definition and categorization of the countryside, see e.g. [1], [2]). Rural areas are no longer associated with farming only; therefore, rural development is a complex topic that cannot be simplified. Among others, the European Union has brought rural development together with the Leader method, which is implemented by local action groups (LAG) in the specific areas. LAGs are new structures that have not often been discussed in the Czech Republic, e.g. [3]. This paper aims to introduce these entities that carry out rural development through local partners working in the communities. The aim is therefore to identify and briefly introduce the conditions for the existence and functioning of the local action groups in the Czech Republic and their possible effect on rural development.

Material and Methods

LAGs are a relatively new way of cooperation of the entities at the micro-regional level in the Czech Republic. Their emergence in the Czech Republic is related to the preparation and implementation of the EU Common Agricultural Policy (CAP Rural development has been one of its priorities since 2000 when the EU adopted Agenda 2000, declaring rural development as a priority of the CAP for the first time.

Rural areas form a significant part of the European Union (almost 90% of the total area of the member states). The effort to preserve the cultural character of the landscape which is located in the surroundings of rural settlements has led to the implementation of the idea of rural development by LAGs. The landscape located between cities and villages is not only a space for agricultural production, but also a place for other activities, for example it is a place where we can spend our leisure time. However, rural areas should also provide their inhabitants with a suitable social and economic environment for their life so that they stay and are interested in a full functioning or development of the territory. [4] The concept of the farmer has changed as well. The farmer is not only a person or a company engaged in crop and livestock production. In a broader perspective, farmers are also foresters and fish farmers (fishermen). The farmer has always been the key person in the country. Nowadays, the farmer is no longer just a producer of food and raw materials for other sectors of the economy, but also a person closely involved in the provision of additional rural functions, such as protection and maintenance of the landscape and the environment, the development of other businesses directed towards the use of historical, natural and cultural potential of rural areas (typically in the field of tourism). The ability to develop rural areas through communities living

there and entities working there is one of the outcomes of this change in understanding the countryside, which has led to a respect of the broader context and stopped relating the countryside to farming only.

The significance of agriculture as a multifunctional economic activity related to rural development is manifested in the fact that the development of rural areas has been excluded from the EU policy of economic and social cohesion and has become a part of the CAP since 2007. The CAP itself focuses on the functioning of the market for agricultural commodities, support for farmers and their production, conservation of the landscape and rural development by LAGs.

Results and Discussion

A LAG is a community of people who actively contribute to the development of their microregion. The group is based on the principles of partnership. Their priority is the use of community methods for strategic planning of the territory development, which consists in involving all stakeholders of local development in the creation and implementation of the microregion strategy. The financial resources for the implementation of the territory development strategies are obtained from the EU budget and national programmes. It is also important that LAGs use the LEADER method (hereinafter referred to as Leader).

The Leader method comes from the European Union. The EU works based on the principle of programming, i.e., its policies are implemented through programmes. Therefore, also the Czech Republic uses various programme documents to implement EU policies. The Rural Development Programme (RDP) of the Czech Republic is a programme document that outlines the national priorities within the framework of the CAP. The RDP defines the criteria that must be met for the formation and operation of a LAG, above all, so that they can use the resources from EU funds for their existence and development of the defined territory.

The parameters of local areas are defined with regard to the territorial and demographic specifics of the Czech Republic. The LAGs founded should meet the following conditions:

- a geographically homogeneous area: a small socially cohesive territory which has for example typical traditions, a local identity, a sense of belonging or common needs and expectations. Geographically, such a territory need not match with an established territorial arrangement of the public administration [5];
- the number of inhabitants from 10,000 to 100,000 outside cities with populations of over 25,000 (The Ministry of Regional Development provides an upper limit for local partnerships of 150,000 inhabitants in its methodology for the creation of a Community-led Development Strategies [6]. The Ministry of Agriculture works with a limit of 100,000 inhabitants [7]);
- the participation of public administration representatives in the LAG is at most 50% (also concerns the managing authority), the other half is made up of representatives of entrepreneurs and non-profit organizations;
- the members of the LAG must have the residence in the given microregion or they must perform their activities there;
- the LAG must have the statute, regulations and organizational structure issued and approved, and must be registered;
- the LAG has to discuss and approve the strategy for the given LAG territory;
- use of the Leader method. [8]

The local areas in concern are thus characterized by a small size, homogeneity and cohesion; there is no need to take account of the territorial arrangement of the public administration, and there are typical common traditions and identity as well as common needs and expectations.

The LAGs are communities where the representatives come from the public, private and non-profit sectors. The number of representatives of the public sector is limited to a maximum of 49% so that the LAGs are not just another form substituting activities of associations of municipalities but are forced to repeatedly and systematically encourage other entities to work towards the development of the microregion. Other cooperating entities can be citizens, non-profit organizations (cultural, social and environmental), representatives of the private business sector (craftsmen, farmers, company owners, trading associations, professional organisations and unions), clubs (fire brigade, Sokol - a Czech sports organization, women's associations, children's groups, groups of young people, and more). In the process of decision making, all the partners in the group have an equal voice, irrespective of their size. The involvement of other than public representatives is successful to a varying degree in the practice. The public sector representatives are usually more active and more involved (typically the mayors of municipalities).

The LEADER Method

LEADER ("Liaison Entre Actions de Développement de l ' Économie Rurale", which stands for 'linking of activities of the development of rural economy') denotes linking of activities developing the economy of rural areas. Leader was founded in 1991. It was originally called the European Union Initiative and gradually evolved, similarly to the CAP with which it is closely linked now. Its aim was to strengthen the development potential of rural areas using local initiatives and skills by promoting the know-how acquisition in the process of local integrated development and the dissemination of know-how to other rural areas. [9] It was applied three times as an initiative in EU countries: Leader I (1991–1993), Leader II (1994–1999) and Leader+ (2000–2006). In addition, the member states or EU regions had separate Leader programmes with their own funding allocated at the EU level. In 2007 the distribution of resources by the Leader method was included in one of the CAP axes.

We can find mentions of first LAGs in the Czech Republic since 2002 in the context of their support within the rural renewal program. Leader+ was implemented in the first EU programming period 2004–2006 the Czech Republic participated in. It was funded from the EU budget within the framework of the Rural Development and Multifunctional Agriculture Operational Programme. This programme was to support the creation of the first LAGs and test the application options of the Leader method for the development of rural areas. It continued in the years 2004–2008 by a separate national programme Leader CR, which was financed from the budget of the Czech Republic. Since 2008, LAGs activities have been financed from the Rural Development Programme of the Czech Republic (Axis IV Leader). It was allocated five percent of the total financial allocation of the Rural Development Programme (RDP) of the Czech Republic. This remains similar in the programming period starting in 2014: the implementation of the RDP will newly involve programmes managed by other ministries, namely the Integrated Regional Development Programme (managed by the Ministry of Regional Development and funded by the European Regional Development Fund), the Operational Programme Employment (managed by the Ministry of Labour and Social Affairs and funded by the European Social Fund) and the Environment Operational Programme (managed by the Ministry of the Environment and funded by the European Regional Development Fund and the Cohesion Fund). Through LAGs, the RDP will endeavour to achieve the modernisation of agricultural and food industry enterprises and investments in forestry. Financial resources go to the RDP from the European Agricultural Fund for Rural Development.

The territory development policy is going to be understood more comprehensively by 2015. The development of rural and metropolitan areas is to be interwoven and they should complement each other. The development of rural areas will continue to be implemented through the LAGs and the strategic documents they create. The strategic documents that LAGs are creating in the current programming period are called "Strategy Community Led Local Development" for the programming period 2014–2020 (SCLLD). Metropolitan areas should use the Integrated Territorial Investments (ITI) for their development; similarly to LAGs, these combine financial resources across priority axes of operational programmes and integrate them into one tool that focuses on specific problems of a specific territory.

A prerequisite for the Leader method to work is that "the effectiveness of the strategies increases if the decisions at the local level are made and implemented by local entities, clear and transparent procedures are used, and support and the necessary technical assistance for the transfer of good practices is provided by the relevant public administration authorities" [9].

The Leader is characterized by the "bottom-up" approach, in which the community members are involved in decision making about the territorial strategy and the choice of priorities that are to be monitored in the local area. The principle is to call local residents and entities to take over the leadership and involve in the local development. However, even the "top-down" combination seems to be appropriate, in which the cooperation with the central and regional public administration representatives may bring achievement of better general results. The cooperation can take a form of e.g. an appropriate and adequate support or a methodological guidance and providing examples from the practice.

How LAGs work

The main LAG activity is creating a territory development strategy through a combination of the expert and the community methods. The strategy is submitted together with the application for support within the RDP (or other subsidy programmes). However, a LAG is only allowed to apply for a financial subsidy if it meets the pre-defined

standards (i.e., to act in a territory that meets the given parameters, to bring together partners of varied nature, to have the prescribed authorities, and to work in compliance with the specified legal forms and business regulations; for more, see [7]). If the LAG is successful in the process of fundraising, it can receive financial resources for its work (its existence), as well as for the implementation of the strategy (the committed financial allocation for the territory development). The LAG then selects the projects of the final recipients that are supported. The LAG is thus a 'small grant (implementation) agency' which distributes the financial resources for projects carried out in its territory with the purpose that is appropriate and demanded for the LAG's territory development strategy. In addition to the support for the projects, the LAG can also implement its own projects. It is its task to choose the projects that meet the real needs and at the same time lead to an increase in the local competitiveness. A secondary LAG activity is to look for subsidy calls suitable for the implementation of the projects of its members in various programmes and funds, and to provide assistance with the processing, submission, implementation and accounting of the projects. The LAG can also participate in the education of its members.

Each LAG elects its organisational structure; the elections of the authorities and their competences are governed by the methodology (see [7]). The names of the authorities as well as their specific tasks are different. Generally, the LAG authorities include those that perform decision-making, control, inspection, selection, monitoring, and executive functions.

The highest authority is the assembly of all the partners involved; and it is here that the public sector may not have more than 49% of votes. The competences of the highest authority include the establishment of other obligatory authorities (decision-making, inspection and selection) and the selection of their members and it decides on the admission or exclusion of LAG partners. Further, its major tasks include the approval of the local development strategy, the LAG budget and the project evaluation and selection methods. The managing/decision-making authority (e.g. the committee or the council) elects the chairperson from their ranks. Also this authority may have a maximum of 49% of the members from the public sector. The inspection authority primarily focuses on the compliance with management principles and legislative requirements. Once a year, it submits a report to the highest LAG authority on the results of its inspection activities. The inspection authority is also responsible for the monitoring and evaluation of the SCLLD (i.e., if and how the indicator and the evaluation plans are met). In addition, the LAG has the selection committee which preselects the projects on the basis of a predetermined (by the highest authority) objective set of criteria and draws up the order of their implementation based on their contribution to the goals and objectives of the SCLLD. [7]

The executive functions are exercised by e.g. the secretariat or the manager and his/her collaborators. The minimum number of employees to perform the basic functions is two (a manager and an administrative assistant). "In the countries that have longer experience with the Leader method the LAG executive teams usually have 4–5 people" [9].

The legal forms used nowadays for LAG existence are generally: public benefit institution, society, interest association of legal entities, and institute. [10] A society is the most frequently used form - 63% of all LAGs. 33% of the local action groups are public benefit institutions. The other forms make up the remaining 4%; there is only one registered institute (LAG Brdy). LAGs in the Czech Republic are associated in The National Network of Local Action Groups of the CR. This network associated 166 LAGs out of the total 179 existing (i.e. 93%) in 2014 [10]. The network primarily represents an informational and methodological support for the existence of LAGs. It brings together LAGs and represents them in negotiations with public authorities. It cooperates with foreign entities that use the Leader method. Membership in the network is charged, each LAG paid a membership fee of 10,000 Czk in 2014 [10].

Voluntary Associations of Municipalities (AoM) are another form active in rural development. They operate using a platform completely different from the Leader method. The territories that can form AoMs have other restrictions. They need to be contiguous and there is no size limit. AoMs must comply with other regulations of functioning and financing stipulated by the Act on Municipalities (no. 128/2000 Coll. as amended) and other related regulations (e.g. no. 250/2000 Coll. on budget rules of territorial budgets, as amended). There are various kinds of AoMs, for example those with specific or non-specific purposes. Those with non-specific purpose usually aim at a general development of their territory. Representatives of the member municipalities, usually the mayors, make decisions on the development of the territory in the microregion. The resources they use to develop their territory originate either in the budgets of the member municipalities, or subsidies they gain

based on the submission of projects to different programmes and funds. AoMs need not (but can) involve other stakeholders in the decision-making about their development. This decision making is purely within the framework of the public administration through a public vote, i.e. the representatives elected by citizens. A LAG can be territorially coincident with an AoM. However, the LAG can associate more AoMs operating in its territory. The reverse does not apply. An AoM can only cooperate with a LAG as only municipalities can be members of AoMs.

Conclusion

The Leader method enables rural communities to shape their own future actively. Local action groups form the basis of its application in rural areas. The main tasks of LAGs are to define and implement the local development strategy, decide on the allocation of the available financial resources and manage them. One of the important tasks of LAGs is the acquisition and distribution of financial resources for their microregion based on the actual needs. For this reason, the local development strategy needs to be prepared and approved. The strategy is prepared using a combination of the expert and the community methods. The territory development is defined and implemented by the "bottom-up" approach, which is used in the preparation and implementation of the strategy.

The integration of appropriate and beneficial plans for rural areas into a single strategy (SCLLD) allows for the implementation of many high-quality projects that would not otherwise be attainable through standard ways of EU funds administration. These are often less expensive projects, providing various synergistic outputs that are not usually included in the standard structures of operational programmes. During their existence, LAGs have already allowed an implementation of many projects in which they operated as the managers of public funds. However, their role also includes being animators of the events in the region, communication channels, coordinators of cooperation and centres of microregions, who mobilize the potential of the territory and people living or working in it.

LAGs represent a revolutionary change for the development of microregions. The preparation of subsidy programmes is in the hands of the public administration, subsidies are provided by public authorities and requests mostly come from individual, isolated applicants. The Leader method changes this and supports the emergence of inter-sector partnerships, which themselves allocate the funds for the development of their territories (LAGs are private persons). In order for a LAG to be successful, a partnership must first be established and its members must agree on the focus of its development strategy. The strategy then forms the core of the applications for funding local applicants' partial projects. The local applicants are addressed by a public call. The key role of the LAGs is the mobilization of the development potential of the microregion where it is operational. [1]

Community led local development through LAGs is evaluated positively. The Leader program has brought significant improvements in the services in the country. Moreover, there have been improvements in the quality of life, diversification, the environment, the integration of groups of women and young people. The value added to local products has increased. As a side effect, new work positions have been created. [9]

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The assessment of the sludge lagoons reclamation plans in the area of the uranium deposit Rožná

ANETA PAVLU[1], VALERIE VRANOVA[2]
[1]Department of Applied and Landscape Ecology,
Faculty of Agronomy
[2]Department of Geology and Pedology,
Faculty of Forestry and Wood Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

anetapavlu@seznam.cz

Abstract: The article is aimed at an assessment of the reclamation plans of the sludge lagoons K1 and K2, which arose as a result of mining and chemical treatment of uranium ore in the deposit area Rožná. The area was explored and soil samples were taken from the locality of the sludge lagoons and near surroundings in the autumn of 2012. Then the samples were analyzed in the geological laboratory. An interesting succession of vegetation of different ages was found in the K1 and K2 area, so it was recommended to let the part of this vegetation stand to be developed, without human intervention (reclamation). Results of the laboratory biochemical analysis showed the sufficient microbial content in the soil samples from stands with succession of vegetation. On the other hand, low nitrogen content in the mineral form was detected for all the studied surfaces. The reclamation proposal should include filling the sludge lagoons with an inert material, building a cover layer and isolating the layer of bentonite and subsequent grass seeding and planting shallow-rooted shrubs. After the examination of the reclamation proposal documentation, the project was assessed as appropriate with some comments on the choice of shrub species and the project realization. It is also important appropriately to integrate the sludge lagoons body into the landscape. A possible future "social" use of the area after reclamation, for example public recreation or educational trails, was also suggested.

Key Words: sludge lagoon, reclamation, deposit Rožná, uranium ore, soil sample

Introduction

The sludge lagoons named K1 and K2 arose as a result of mining and chemical treatment of uranium ore in the deposit area Rožná. The deposit is located close to the town of Bystřice nad Pernštejnem, in Bystřice nad Pernštejnem micro-region (Vysočina Region, the Czech Republic). The deposit exploration and mining began in 1956. Rožná is currently the only uranium ore deposit being mined in Central Europe. [2] Its share in the world production of uranium was 1.3 % in 2003. The most important uranium deposits are located in Canada, the United States of America, Australia and South Africa. [4]

The sludge lagoon is a natural or artificially created area on the earth's surface, which is used for permanent or occasional storage of mainly hydraulically deposited sludge. In the sludge lagoons, solid matter (sludge) with the water used in production cycle is stored.

Land reclamation includes different measurements and adjustments, which are necessary for the renewal of soils devastated by natural or human activities. This usually includes a technical and biological phase. The biological phase has the task to create production land that would facilitate the plants growth and fauna life in the shortest possible time. [11].

Closing technology of K1 and K2 is the same in principle and consists of reshaping and filling the sludge lagoon body to the designed shape, building of a sealing layer, covering the layer and biologically appropriate layer (soil) over the entire body, and grassing and planting shallow-rooted shrubs. The sludge lagoon closure and overlap aims at removal dustiness, shield gamma radiation, decrease of radon consumption rate, significantly reduces the infiltration of rainwater into the sludge lagoon body, and finally integrate the anthropogenic structure into the surrounding landscape. Insulation and covering layers are:

mineral seal bentofix, geotextile, drainage layer, cover layer of an inert material, biological layer (soil or pond mud). [15]

The subject of the study is to assess the reclamation plans of the above mentioned sludge lagoons. The basis for this assessment is reclamation plans documentation study, field survey in the locality and an analysis of the collected soil samples.

To realize a research of the published knowledge of uranium ore mining and subsequent reclamation was the first goal. The second objective was aimed at the study of the K1 and K2 reclamation plans documentation. The work deals mainly with biological reclamation proposals and with the impact of this reclamation on the environment. The third objective consisted of an evaluation of the field survey and a realization of soil samples from the sludge lagoons locality and near surroundings, where, successional processes are in progress for a number of years. The fourth objective was found in the laboratory analysis of soil samples (soil physical and biochemical properties) and their appropriate statistical evaluation.

Material and Methods

The field research in the area of mines and sludge lagoons was performed in the autumn of 2012. Soil samples were taken from the sludge lagoons K1 and K2 and near surroundings. A mixed soil sample was taken from places on the K1 dam, where successional vegetation with white birch (*Betula pendula*) and Scots pine (*Pinus sylvestris*) was found, specifically a soil sample from the place covered with white birch and another soil sample from the spot covered with Scots pine. One sample was also taken from the place where the reclamation experiment was in progress. Other soil samples were taken from the K2 locality, specifically one sample from the place overgrown with grass - mostly Wood Small-reed (*Calamagrostis epigejos*), and one sample from the white birch standing at the K2 dam. Also, one soil sample was taken from agricultural land under K1. At all the sites intact soil samples for the determination of soil physical properties were also taken. The following soil properties were studied: content of nitrate (μg $NO_3 \cdot g^{-1}$), ammonium nitrogen (μg $NH_4 \cdot g^{-1}$) and microbial carbon ($mg \cdot g^{-1}$); the protease (μg L-tyrosine$\cdot g^{-1}$), urease (μg NH_4-N$\cdot g^{-1}$) and catalase (ml $O_2 \cdot 5\ g^{-1} \cdot\ 15\ min^{-1}$) activity, pH, density ($g \cdot cm^{-3}$), water and dry matter content (%), porosity (%) and maximum capillary capacity (%).

Laboratory analyses were performed according to the methods available in the literature. [13, 17] For statistical analysis of laboratory data three repetitions of the same sample were always performed, so it enabled using of average values and also creating graphs of divergence by means of Statistica software.

Results and Discussion

Interesting successional vegetation was found on the K1 and K2 dams. The artificial reclamation was not recommended, but leaving the succession locations and monitoring their (where it is possible). However, there is also an expansion of expansive Wood Small-reed (*Calamagrostis epigejos*) visible, which extrudes other types of herbs. The oldest stand logically occurs on the lowest terrace of the dam, while the grass species dominate on the upper terrace. Among the trees there is a continuous grass cover.

Results of the laboratory analysis showed a sufficient microbial content in the soil samples. The presence of microorganism in anthropogenic soil is necessary for starting the biochemical and biological processes relevant to plant growth. Sufficient content of microbial biomass was demonstrated by the authors, who have dealt with a different type of sludge lagoon in Třinec (Moravian-Silesian Region, the Czech Republic). The authors evaluated the presence of microbial biomass as high to very high, the highest in the oldest terrace of the dam. [9] The author Lencová [7] evaluated in her study higher content of microbial biomass in grassy areas that were mowed compared to not mowed areas, which can suggest that the controlled succession (management) is better for the microbial activity in the soil. The results of this work, however, can not confirm nor deny this trend. The controlled succession can be recommended in the areas left without artificial reclamation and regular lawn mowing in the areas with reclamation.

In all areas examined, however, low content of mineral nitrogen was found, which is used in large volume by plants, but its source is essentially only degradation processes of organic matter in the soil. According to Králová et al. [5] low content of ammonium nitrogen is found in the surface soil layers, especially in winter. Low detected ammonium nitrogen values could therefore be supported by the late sample collecting and the small depth. The low nitrogen content is logically followed with low content of ensyme urease in all the samples. This corresponds with the results of analysis carried out on the sludge lagoon in Třinec

(another type of sludge lagoon), where a very low ureolytic activity on all the terraces was found. [9] On the monitored sites steep decline in dependence on the content of ammonium nitrogen and nitrate towards the youngest terrace was noticeable. According to the author team it can be said that ureolytic activity is directly dependent on the quality of the vegetation cover and humus content. This corresponds again to the results presented, where the oldest stand on K2 with apparent developed continuous vegetation contained the highest ureases values. The urease activity may be easily affected by contamination by foreign substances, pH, substrate concentration, temperature and agricultural management, so it is not very appropriate for determining the quality or degradation of soils. [10] Studies have shown that ensyme urease is sensitive to toxic concentrations of heavy metals. Other studies of soil samples collected from various horizons showed a decrease in urease activity with increasing soil depth. The differences were attributed to the decline of organic matter in the soil with increasing depth. Generally, the activity of urease increases with increasing temperature. [8] The urease bound to soil organo-mineral complexes is more stable than the urease in the soil solution. These complexes are highly resistant to denaturing agents, for example extreme temperatures. On the other hand, urease in soil solution can be rapidly proteolytically degraded. This suggests that much of the soil ureolytic activity is included in urease, which is stabilized by the organic and mineral colloids. A higher content of soil colloids means increment of ureolytic activity and vice versa. [1] Low urease activity in the surveyed areas could be partly related to the late collection (low temperature), insufficient soil colloids content or heavy metals content, which have not been subject of the analysis, but we can assume this content on the sludge lagoon K1 and K2 location.

The analysis also showed adverse properties of the reclamation substrate without vegetation (reclamation experiment), which should be seeded as soon as possible to avoid soil degradation. The activity of catalase in soil samples was assessed as medium to high, except of the reclamation experiment.

The protease activity was assessed as high, except of the reclamation experiment, where the values were five to ten times lower, which supports the autor's statement that the values of protease activity are at a low level in areas without vegetation or with only sparse vegetation. However, the given values can not confirm nor deny the autor's argument of the proteolytic activity independence on stand age. The statement that the proteolytic activity is higher in areas with at least partial plant cover can be agreed.

The physical parameters showed that the examined soils are generally structurally satisfactory and water-holding but actual water content of the samples was relatively low. Areas without continuous vegetation cover may suffer from desiccation. The agricultural land soil sample (we can say the most natural sample, but also human-influenced) showed no major differences from the other samples in the monitored parameters. Biochemical and physical parameters of anthropogenic soils are, of course, different from natural soils but over time can lead to a "succession" in the soil, and these values may begin to equalize.

Table 1 Nitrate content in soil samples

Sample	Nitrate Content ($\mu g\ NO_3 \cdot g^{-1}$)	Nitrate Content Evaluation [14]
1 (K1 birch)	0,110	low
2 (K1 pine)	0,088	low
3 (field)	0,163	low
4 (reclamation experiment)	0,089	low
5 (reclamation experiment)	0,137	low
6 (K2 grass)	0,036	low
7 (K2 grass)	0,087	low
8 (K2 birch)	0,093	low

Recommendations for practise

First, the main insulating layer will be constructed, which is necessary to avoid environmental contamination. That is an inescapable fact and therefore it will not be possible to leave the locality covered with succession vegetation or plant trees (forestry reclamation), because tree roots would destroy the insulating layer. It is only possible to plant shallow- -rooted shrubs and grasses (meadow grass species). Nevertheless, there are also spots where the insulating layer is not required (foreground of the sludge lagoon K1), so it was suggested not to reclaim artificially, but to leave some areas evolve. This conclusion was supported by the laboratory analysis, which showed high microbial activity in the soil on the successional stands.

In the reclamation plan six species of shrubs for planting were proposed, namely hazel tree *(Corylus avellana*), common privet (*Ligustrum*

vulgare), barberry (*Berberis vulgaris*), black currant (*Ribes nigrum*), European Spindle tree (*Euonymus europaeus*) and blackthorn (*Prunus spinosa*). [12] Hazel tree and common privet were recommended as appropriate to plant on the location. Other species were not recommended, due to either climatic or other reasons (demands for land quality or tendency to expansion). On the contrary, other shrubs were recommended, specifically dog rose (*Rosa canina*) and hawthorn (*Crataegus laevigata*). These shrubs are appropriate with climate and soil on the locality, and in addition they can fulfill an aesthetic function. Dog rose is frost-resistant and can withstand dry habitats. Hawthorn is climate undemanding, and able to withstand dry and acidic substrates. Thermophilic species are not appropriate for the site due to a colder climate. Expansive or not indigenous shrubs are not appropriate, either. [16]

In the case of areas intended only for biological reclamation (foreground of the sludge lagoon K1), where the insulating layer is not needed, it is possible to agree with Grohmanová [3], that during the reclamation some perspective places should be left to the succession, because the occurrence of rare and interesting species of plants and animals is documented there by biomonitoring.

Lacková [6] proposed the sludge lagoon reclamation plan as a "socio-ecological" plan, which combines nature conservation requirements and social requirements, which may be considered suitable objectives that should guide proposed K1 and K2 reclamation in Rožná.

Pic. 1 Sludge lagoon K2 (source: author's archive)

Conclusion

Uranium industry can be understood as a certain controversial topic due to the potential risk arising from the operation of nuclear power stations and nuclear weapons production. But if we consider only the Rožná deposit and consult on this issue purely as a resident settled in the region, we can see the mining which is not devastating hundreds or thousands hectares of our land, but we can see a significant employer in the region, for whom currently approximately one thousand employees work. We can expect an increase in unemployment in the region, when the mining is reduced and terminated in the end, so we can also expect some negative social effects.

It is true that the natural landscape of the area was changed by human activities, we can say it is agro-industrial landscape now, with an intensive human use. The largest share of that is apparently carried by inconsiderate field uniting in the second half of the last century and the uranium mining in the area at the same time. Uranium ore mining certainly has some environmental influence, but it does not currently have any major impact on the worsening of environment quality, because the legal limits on the releasing water, dust and other risk factors are respected, and there are also preparing decontamination and reclamation works being prepared.

The sludge lagoons, which arose as a result of mining and chemical treatment of uranium ore in the deposit area Rožná, are really an important consequence of human activity in the landscape. Their negatives and positives were mentioned in this article. These water surfaces mean unquestionably some risk, but they also contributed to the increase of biodiversity in the landscape, there are a lot of species of birds and some rare species of plants. Their dams are covered with successional vegetation of herbs and trees, but most of these places will have to be destroyed because of technical and biological reclamation. In fact, it will be necessary to build isolation and covering layer over the entire body of the sludge lagoon to avoid contamination of the environment. In areas where the isolation is not necessary, it was proposed to leave some areas for controlled succession (reduction of expansive Wood Small-reed etc.). This is supported by the laboratory analysis, which showed a high microbial content in the soil samples from stands with succession of vegetation. It would be wrong to destroy a lot of years of succession on the surface and in the soil.

The analysis further showed, inter alia, a low content of mineral nitrogen, which is used in large volume by plants, but the source is essentially only degradation processes of organic matter in the soil. Plants from the *Fabaceae* family could help

increase the content of nitrogen that is acceptable for plants. Analysis also showed adverse properties of the reclamation substrate without vegetation, which should be seeded to avoid soil degradation as soon as possible.

The sludge lagoons reclamation, which will progress in several phases in the following years, will be completed after the end of mining deposits and will be one of the last very important activities in the area. Precise and correct realization of the reclamation is necessary to avoid the environmental contamination. However, the sludge lagoons K1 and K2 will remain officially water constructions of the 2nd category and will be continuously monitored and maintained. Reclamation proposals of K1 and K2 are prepared and were analyzed in detail in this work. Other tree species for biological reclamation were recommended, while some of the proposed species were not recommended. Some important principles for proper planting were also recommended. The possible social use of the locality in the future was also outlined, for example recreation or nature trail. With respect to the environment and landscape engineering it is particularly important to avoid all the environment components contamination, to use appropriate indigenous trees, to ensure stability of the entire body enclosed sludge lagoon and appropriate integration of these anthropogenic structures to landscape.

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The influence of tourism on the southern and northern region of South Moravia

KATERINA STONAWSKA, PAVLINA THONNOVA, MILADA STASTNA, ANTONIN VAISHAR
Department of Applied and Landscape Ecology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

katerina.stonawska@mendelu.cz

Abstract: In the South Moravia there are some areas that can be more oriented on tourism. The paper is focused on two areas. One area is located in the north, in the Moravian Karst (case municipalities Jedovnice and Křtiny) and second is located in south, in the Lednice-Valtice area (case municipalities Lednice and Valtice). Both regions are important tourist centers in the region, also in the Czech Republic. The Lednice-Valtice area is inscribed on the UNESCO list and its landscape are under protection by Natura 2000, by Special Area of Conservation and by the Ramsar Convention. In the north is famous and most important karst area in the Czech Republic and Central Europe. The employment rate of people working in tourism in these regions is about 5.3 %. The catering and accommodation facilities in Lednice and Valtice are in operation year-round in all cases, in Jedovnice and Křtiny are 25 % of them operating only in the summer season. The main reason of tourists to visit Jedovnice and Křtiny is vacation, particularly summer holidays and the most preferred reason for visiting Lednice and Valtice is a wine tourism. The most residents expressed a quite strong support for tourism development. Findings in this paper is based on our questionnaire survey, own investigations and data from the Czech Statistical Office.

Key-Words: tourism, accommodation facilities, community development, Moravian Karst, Lednice-Valtice area

Introduction

The tourism sector belongs to the largest component of the national and global economy, even in the Czech Republic. Tourism brought a significant contribution to the economy of tourist destinations, but also to their socio-cultural development. Being a labour-intensive domain, tourism industry has proven its ability in creating and maintaining jobs even when other industries are in decline (Mahika, Răvar, 2014).

In the Czech Republic there are some areas that may be more oriented on tourism. Such areas that bigger part of their activity is oriented to the tourism may face problems but at the same time can have benefits more than others (Šťastná, et al., 2014).

A major problem of tourism in the Czech conditions is its seasonality. It is given by the natural conditions and lore. The South Moravia has relatively weak opportunities for the development of winter tourism (in terms of winter sports). For the most part it is reliant on the summer holiday period, despite the fact that some forms (gastronomy connected with the culture of wine) are enabled a virtually year round. The pitfall is a general habit of Czechs to be engaged in tourism, especially in the summer months.

The potential for rural tourism is evenly distributed in the South Moravian Region (Zvara, 2010). However, we can find considerable differences in individual tourist regions, especially between their cultural and historical value.

For the needs of tourism, the South Moravia is divided into 5 natural tourist areas. The paper is focused on two areas – located in the north Moravian Karst (municipalities Jedovnice and Křtiny) and south located the Lednice-Valtice area (municipalities Lednice and Valtice).

Southern region is above all Lednice-Valtice area

The southern area is very popular not only for domestic tourists but also for foreign tourists. Lednice-Valtice area is the largest man-modeled territories in Europe ("Garden of Europe") and in 1996 was inscribed on the UNESCO list.

Since 2003 it is a part of the Biosphere Reserve called "Dolní Morava". Biosphere Reserve Dolní

Morava is the youngest and smallest of the Czech Biospheric Reservations, however it is the only one in the Czech Republic which has twice UNESCO status. It is not even on a global scale too common.

There are the set of six ponds and the largest Moravian pond called Nesyt. Around the pond Nesyt is occurred salt-loving flora. The ponds are also protected as bird area (NATURA 2000), Special Area of Conservation and as a wetland of international importance by the Ramsar Convention. There is one of the most important bird sanctuaries in the country (Kmet, 2011).

The surrounding of Lednice ponds is a very popular tourist site. There are many paths or trails for hikers. The route between forest, meadows and ponds is surrounded by a variety of romantic historical buildings, which in the past belonged to the Liechtenstein family. Near the Lednice Castle is a greenhouse and labyrinth with geometrically cropped shrubs and minaret with views of the nearby Lednice-Valtice area.

The region is popular for tourists not only because of the beautiful nature, but also because of the history. Lednice castle charmed even the filmmakers who enjoy shooting there. This is evidenced also by attendance of visitors. A total of 330,000 people visited the Chateau Lednice last year. It was the highest attendance ever (Lindnerová, 2013). This attendance has been surpassed in August. Chateau Lednice is the most visited monument in the Czech Republic. Preservationists estimate that the park annually visited by about 1.5 million people (Šupálek, 2014).

This southern region has very beautiful nature, charming romantic architecture, unmistakable genius loci, which makes it one of the most visited tourist regions in the Czech Republic.

Lednice and Valtice lies in the hottest area in the Czech Republic. A typical altitude ranges from 170 to 250 m above sea level, so quite favorable for touring all groups of tourists (parents with strollers, seniors or small kids on bikes).

Northern region is mainly Moravian Karst

The attractiveness of the northern area in terms of tourism is high and means the significant potential for the development. It is the most famous and most important karst area in the Czech Republic and Central Europe. More than 300,000 visitors is visited this area annually (Hromas, 2013).

In contrast with the southern area there is an average altitude 450 m above sea level and there are no significant ponds here. However, tourists can find a wide variety of karst phenomena, as well as valuable species of flora and fauna, which are protected and monitored by experts in terms of the impact of climate. Seven basic climatic characteristics are being measured at the specialized automatic climatological stations built in years 2006-2008. The highest mean air temperature was measured at the station Sloup in 2009. This locality shows the highest variability of air temperature due to its position. The highest relative air moisture (about 85 %) is reached in the bottom of the dry valley (Rožnovský et al., 2010).

Most of the area of interest lies in the Moravian Karst. The Moravian Karst is declared as a protected landscape area from 1956. The forests cover almost 60% of the area and there are more than 1,100 caves and five of them are open to the public. It is a cave Punkevní, Sloupsko-šošůvské caves, Balcarka cave, cave Výpustek and Katřinská cave. This cave was also monitored by experts in terms of the impact of microclimate. The result was that there is no significant negative effect of attendance on Kateřinská cave microclimate. During normal operation does not exceed carrying capacity of the environment (Středová et al., 2013). The cave system called collectively Amaterská cave is the longest cave system in the Czech Republic (Mackovčin, 2007).

In this example we can see, what even the scientific literature demonstrates - that only nature can be considered a commodity that can be used for the production of tourism experiences - signifying attractive places for tourists leading to increased tourist numbers and employment (Byström, Müller, 2014).

Material and Methods

The selection of the area and case municipalities

The selection criteria for inclusion municipality in the survey was a qualified estimate of active tourism (estimate intensity of tourism activities, taking into account several factors – size, density of population, degree of seasonality). Another criterion was a relative frequency of range occurrence of the text in guides, where the municipalities are characterized as a tourist destination (in Czech and also foreign guides). We also considered the importance of recreational functions, which is referred in the Atlas of tourism in the Czech Republic (Vystoupil, 2006).

Based on this reasoning, we selected two municipalities from southern and northern area. These municipalities are considered as a center of tourism or they are attractive for tourist.

The selected areas of interest are shown in the Figure 1 by green dial.

Fig. 1 The location of studied area on the map of the South Moravian Region

Source: Czech Statistical Office and own additions, 2014

The survey and obtained data

The survey was conducted through questionnaires and interviews.

Questionnaire was distributed via online questionnaire system Survio (http://survio.com). The questionnaire constructed a team associates on the project. Pilotage was conducted before the start of the research. Questionnaire found out the state of tourism in the area (the economic situation of workers in tourism, attendance, capacity, development of tourism in the village, promoting tourism in the area, etc.) through 33 open and closed items. The invalidity of questionnaires eliminates the permeability of the items. The providers of catering and accommodation services workers and tourist information centers and other authorized persons, competent in the field of tourism, filled the questionnaire. We approached all of them in case municipalities by e-mail or by personal interview.

The answers were converted into electronic format using MS Excel and processed by using standard statistical methods.

Other data were obtained on the basis of own investigations or from the Czech Statistical Office.

Results and Discussion

Questionnaire survey

The questionnaire survey was carried out from April to October 2014. Respondents were contacted by email and telephone. Those respondents, who prefer a personal meeting, completed a questionnaire in paper form and we transferred it into electronic form. To complete 33 items in questionnaire did not take more than 15 minutes. Rate of return was about 30%. Some respondents did not want to cooperate on research, because of high frequency of research carried out on various topics. Total has been contacted 34 providers of catering and accommodation services workers and tourist information centers and other authorized persons in Jedovnice, 9 businesses in Křtiny, 81 firms in tourism in Lednice and 87 enterprises in Valtice.

Tourism in southern and northern region of South Moravia

For the case study were selected municipalities Lednice, Valtice (southern region) and Jedovnice and Křtiny (northern area). If we had evaluate the potential of communities in tourism, it seems that most important is the ability to accommodate visitors. A major problem may be low capacity of beds, which do not cover the demand of tourists.

The numbers of accommodation facilities with their capacities are recorded in Table 1. As we can see, the difference of accommodation facilities in Lednice and Valtice is only one company, in Křtiny is 12 accommodation facilities less than in Jedovnice.

The selected municipalities are tourist localities, which becomes evidenced by the employment rate of the economically active population working in tourism. The employment rate of people working in tourism is even larger than in people working in agriculture, where the rate is 2.2% (Ministry of Agriculture, 2013). And the Southern Moravia is traditionally agriculturally oriented. Even if there are only 3 accommodation facilities in Křtiny, employment rate in tourism takes similar values, as in the other municipalities.

Table 1 Case municipalities

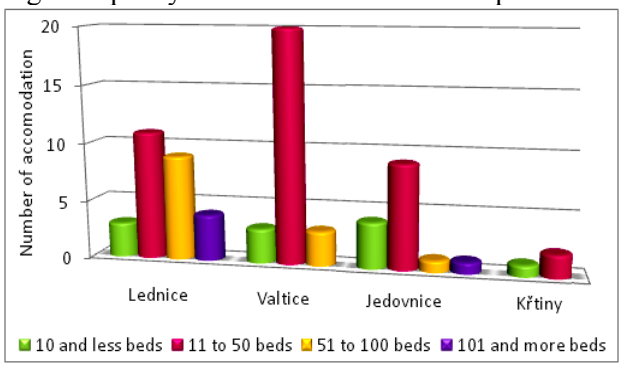
Case municipality	Number of accommodation facilities	Capacity of accomodation	Working in tourism
Lednice	27	1,387	5.95 %
Valtice	26	682	5.48 %
Jedovnice	15	692	5.59 %
Křtiny	3	66	4.24 %

Source: Czech statistical office and own investigation, 2014

Lednice is very popular not only for Czech visitors but also for tourists from abroad. It also illustrates the fact that the Lednice has the largest capacity of beds in accommodation facilities (1,387 beds) of all surveyed municipalities. Valtice and Jedovnice reported a very similar bed capacity (Tab. 1), although in Jedovnice is smaller number of accommodation facilities. Accommodation facility

with medium capacity of beds (from 11 to 50 beds) predominated in all the areas (Fig. 2). Accommodation facility with 101 and more beds cannot be found in Křtiny and Valtice. It is mainly for the reason that in Valtice is more family suites offering accommodation for lower income groups. While in the Lednice, which is spa town, is located more hotels and higher category accommodation.

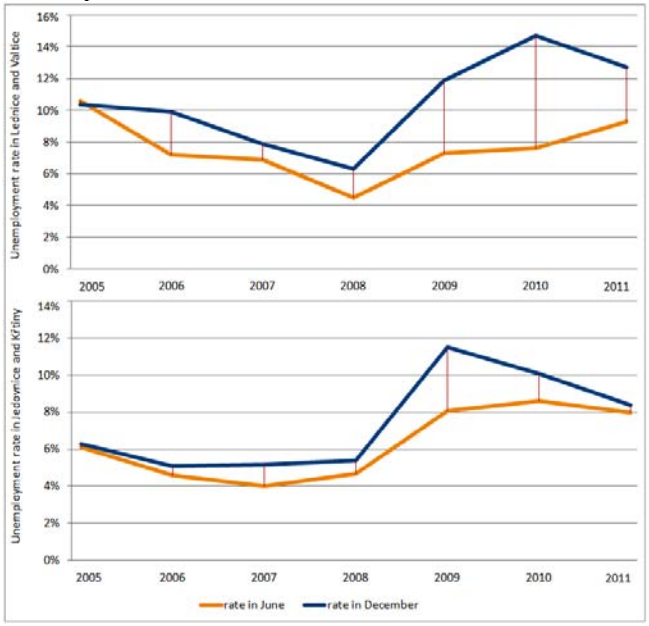
Fig. 2 Capacity of beds in the case municipalities



Source: data from own investigation

Catering and accommodation facilities (approached by our survey) in Lednice and Valtice was in operation year-round in all cases. The reasons of it are various cultural events (wine exhibitions) that are held throughout the year. In Lednice support it even year-round opend spa. However, 75% of respondents in Jedovnice and Křtiny are in operation year-round and 25 % of them only in the summer season.

Fig. 3 Seasonal unemployment rates in municipalities

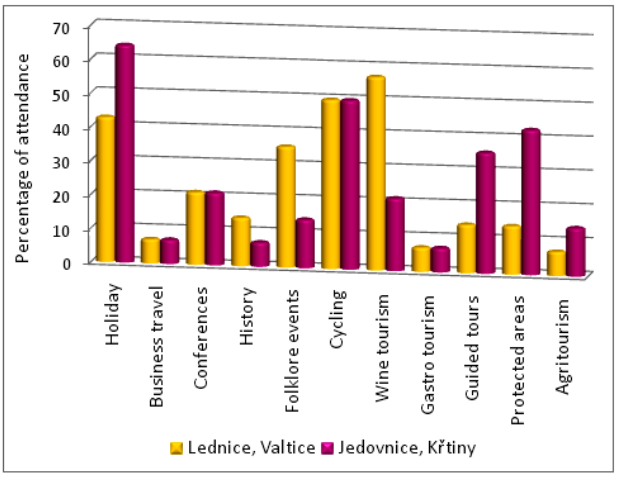


Source: Czech statistical Office, own elaboration, 2014

Although 1/4 devices work only seasonally in Jedovnice and Křtiny, but seasonal unemployment is higher in Lednice and Valtice, where tourist facilities in operation year-round (shown in Fig. 3). In Lednice draws a distinction in seasonal unemployment up to 7.1% in 2010. That may be due to a greater number of agricultural workers in southern Moravia. Nevertheless Dorobanţu et al. (2013) sees tourism as one of the economic activities with most significant potential to generate future growth and employment

Based on the questionnaire survey, it was found that the main reason for visiting northern region is vacation, particularly summer holidays (64%) as we can see in Fig. 4. Instead of the most preferred reason for visiting Lednice or Valtice is a wine tourism (57%). As was mentioned earlier, these festivals and events related to wine have a tradition in this area. Figure 4 shows noticeable difference on surveyed areas - the biggest difference in order of visiting is a wine tourism (36% difference), more disparity was determined on visiting protected area. A large part of northern interest area is located in the Moravian Karst, and that is the biggest attractiveness of this region. Due to cycling go 50 % of visitors on both areas. The southern area is suitable for less demanding recreational cycling of all age groups, and the northern area is for cyclists seeking adventurous ride by diverse terrain.

Fig. 4 Reason for visiting



Source: data from own investigation

Promotion of tourism and marketing

Municipalities are aware of the natural and historic potential of landscape and they put the importance of tourism and they are trying to foster the development tourism in their region – they particularly co-organized cultural events and take care of the development cycling paths. Their efforts

are do not purport only to tourists or locals, what is good. According to Knifton and Warn (2010) local events and festivals which are originally intended for locals can be highlighted by tourist participation. The authors also point out that the services built primarily for tourists (such as cultural and sports facilities, shops, etc.) may be used by the local population too, which will contributes to its sustainability.

Most respondents (90%) consider tourism as a carrier for the development of the municipality. Addressing respondents agreed that one of the positive impacts of tourism on the area is that "... employees come from the municipality, thereby promoting employment in the village."

Some respondents are concerned about excessive tourist pressure on the landscape, which is in both studied areas protected, and they do not wish to be focused so much on tourism. Ryan (2013) in his study finds that, on average, countries that promote biosphere reserves as tourist destinations tend to have better environmental records than countries that do not.

Shapley's research (2014) with 262 households and also our research detected, that most residents expressed a quite strong support for tourism development.

Our research also showed that marketing and advertising cannot be target only the Web or leaflets promotion. In the surveyed municipalities rely more on direct experiences and references "tourists extend their gained experience beyond the borders of visited areas."

Conclusion

Although it might seem at first glance that it is impossible to compare the tourism in southern and northern parts of South Moravia, it can be said, that both areas use its potential very well. Both regions are different; nevertheless the cycling is a common link. A well-established network of cycling routes could be found as a development means of studied municipalities.

The positive impact of tourism is the availability of services to citizens and better infrastructure in the villages.

Tourism in the Czech Republic is tied to the holidays and summer months. Seasonality is therefore a significant problem, which is reflected not only unemployment rate but also in the daily life of the municipalities.

We also identified mild tourist inflation in both areas. Nevertheless, the tourism is a core industry in both areas, which brings profit to municipalities, employment and cultural life.

Acknowledgement

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Conditions for ecotourism development – Lessons learned from Protected Landscape Area Dunajské luhy and National Park Duna-Ipoly

ANGELIKA TAMASOVA
Department of Landscape Ecology
Comenius University in Bratislava, Faculty of Natural Sciences
Mlynská dolina, 842 15 Bratislava
SLOVAK REPUBLIC

tamasova@fns.uniba.sk

Abstract: This article is dedicated to ecotourism in cross-border areas and presents the lessons learned from two case-study areas in the Slovak Republic and in Hungary. The study compares the Slovak and the Hungarian territorial nature and landscape protection systems and protected area management categories, and evaluates ecotourism activities in two areas – in Protected Landscape Area Dunajské luhy in the Slovak Republic and in National Park Duna-Ipoly in Hungary. Furthermore it provides proposals for the improvement of ecotourism to encourage cooperation between protected areas in cross-border regions.

Key-Words: ecotourism, rural development, nature conservation, cross-border areas

Introduction

Ecotourism is nowadays a well-known concept. The number of protected areas offering activities in harmony with sustainable principles grows day by day. Ecotourism ('eco' as in ecological) is founded primarily on a specific interest in the natural history of a region [1]. Sirakaya, Sasidharan, and Sonmez refer to ecotourism as a type of tourism that does not expend resources, is educational and focuses on undeveloped and undervisited natural areas [2]. The purpose of such tourism is to protect natural areas, and understand and appreciate the natural and social culture of the target location by the involvement of local residents [2]. Protected areas are prevailingly situated in the countryside, surrounded by rural areas. They have the potential to attract visitors, which may be especially significant in remote, less visited cross-border areas, as the area of Slovak-Hungarian border. This article presents the lessons learned from two case-study areas from the Slovak Republic and Hungary. We investigated ecotourism activities in a cross-border context in two protected areas, which were Protected Landscape Area (PLA) Dunajské luhy in the Slovak Republic and National Park (NP) Duna-Ipoly in Hungary (see Fig. 1).

Protected Landscape Area Dunajské luhy or Danube floodplains is one of the youngest of the 14 protected landscape areas in the Slovak Republic. It was established in 1998 and consists of five separate parts in the Danube Lowland, stretching from Bratislava in the north-west, following the Danube and the borders between the Slovak Republic and Hungary to a river island called Veľkolélsky ostrov near to Komárno. The biggest part is Žitný ostrov, the largest river island in Europe. Altogether, it protects 12,284 hectares of floodplains, wetlands and numerous water bodies, such as lakes, oxbow lakes, ponds, and streams. [3] National Park Duna-Ipoly is the ninth national park of Hungary established on 60,314 hectares in 1997, and includes the Pilis, Visegrád and Börzsöny Hills, the undisturbed sections of the Ipoly Valley as well as parts of the Szentendre Island on Danube. The park includes areas in Budapest, Pest County, Komárom - Esztergom County and Fejér County. It protects mountainous regions, caves carved in limestone and river floodplains of the Danube and Ipoly. [4]

Fig. 1 The location of Protected Landscape Area Dunajské luhy (PLA DL) in the Slovak Republic and National Park Duna-Ipoly (NP DI) in Hungary

Material and Methods

While developing the study on ecotourism in cross-border and protected areas various methods were used. First of all information on Slovak and Hungarian territorial nature and landscape protection systems and protected area management categories was needed. The necessary data were extracted from the Act no. 543/2002 Coll. on nature and landscape protection in the Slovak Republic and Act no. LIII/1996 on nature conservation in Hungary. A comparative analysis about legislation was carried out and two case-study areas were chosen; PLA Dunajské luhy in the Slovak Republic and NP Duna-Ipoly in Hungary. It was an important requirement to choose protected areas located near to the Slovak-Hungarian border. PLA Dunajské luhy and NP Duna-Ipoly are both situated near the border and connected indirectly, by the Danube. Information on ecotourism activities were gathered at the Directorate of PLA Dunajské luhy and at the Directorate of NP Duna-Ipoly. Afterwards the current situation was evaluated. Due to occasional cooperation of these protected areas, the proposals for cross-border cooperation in the region were drafted.

Results

The results of this study can be divided into two sections. The first section deals with the comparative analysis of the protected area management categories in the Slovak Republic and in Hungary. The second section provides information on the current situation of ecotourism in the two case-study areas.

Comparison of the protected area management categories

This part of the article refers to the comparative analysis of the protected area management categories in the Slovak Republic and Hungary. Obviously it is not possible to analyse and compare these systems without providing some information on International Union for Conservation of Nature (IUCN) protected area management categories. The IUCN defines a protected area as a clearly defined geographical space, recognized, dedicated and managed though legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values [5]. The IUCN protected area management categories are a global framework for categorizing the variety of protected area management types. There are six categories in use: strict nature reserve (Ia), wilderness area (Ib), national park (II), national monument or feature (III) habitat/species management area (IV), protected landscape (V) and protected areas with sustainable use of natural resources (VI) [6]. The names of the categories used by IUCN do not necessarily reflect the names used at national levels in the Slovak Republic and in Hungary (see Table 1).

To gain information about Slovak and Hungarian systems two legislative regulations connected to this field were analysed in detail. In the Slovak Republic Act no. 543/2002 Coll. on nature and landscape protection is applicable, while in Hungary Act LIII/1996 on nature conservation is in force. Act no. 543/2002 Coll. on nature and landscape protection from the Slovak Republic considers that territorial nature and landscape protection is a special treatment or care of the state and its citizens of wild flowers, wild animals and their communities, habitats, ecosystems, minerals, fossils, geological and geomorphological formations, as well as land use systems. Nature and landscape protection is realized mainly by limited and guided interventions into nature and landscape, by support and cooperation of the owners and users of land, and by cooperation with public authorities. [7] The third part and first chapter of the Act no. 543/2002 Coll. is dedicated to the territorial nature and landscape protection. According to this act territorial protection involves the nature and landscape protection in the territory of the Slovak Republic or in its part. The territorial protection specifies five levels of protection. For each level of protection different limitations and restrictions are declared. Sites hosting natural habitats of European interest or natural habitats of national interest, and habitats of species of European interest or habitats of species of national interest and habitats of birds including migratory species, important landscape elements or areas of interest, can be designated protected areas. [7] The categories of protected areas in the Slovak Republic are the following ones: protected landscape area (§ 18), national park (§ 19), protected site (§ 21), nature reserve or national nature reserve (§ 22), nature monument or national nature monument (§ 23), protected landscape element (§ 25), protected bird area (§ 26) and municipality protected area [7]. If required the nature protection body designates protective zones for a protected area. In the Slovak Republic all natural caves and natural waterfalls are nature monuments or national nature monuments. [7]

Table 1 Overview of the protected area management categories defined by IUCN, applicable in the Slovak Republic (SR) and in Hungary (HUN)

Organization/ Country	IUCN	SR	HUN
Category	strict nature reserve (Ia)	nature reserve or national nature reserve (§ 22)	nature conservation area (§ 28/1)
	wilderness area (Ib)	-	-
	national park (II)	national park (§ 19)	national park (§ 28/1)
	national monument or feature (III)	nature monument or national nature monument (§ 23)	natural monument (§ 28/1)
		protected landscape element (§ 25)	
	habitat/species management area (IV)	protected site (§ 21)	protected bird area NATURA 2000 network
		protected bird area NATURA 2000 network (§ 26)	
		areas of European interest NATURA 2000 network	areas of European interest NATURA 2000 network
	protected landscape (V)	protected landscape area (§ 18)	landscape protection reserves (§ 28/1)
	protected areas with sustainable use of natural resources (VI)	-	-
	-	municipality protected area	-

The mission of territorial nature and landscape protection according to Act LIII/1996 on nature conservation in Hungary is to identify which natural values and areas deserve strict protection out of geological, hydrological, botanical, zoological, scenic, cultural, historical or any other public interest; to determine the dangers threatening protected natural values or areas; to prevent or avert any damage to protected natural values and areas, and to reduce or to eliminate damage already occurred; to conserve protected natural values and areas for the present and future generations, and, if necessary, to restore them or ensure their maintenance. [8] It shall be a priority to meet the society's need for a healthy and aesthetic natural environment; to foster the traditions of nature conservation and improve its achievements; and to protect, conserve, maintain and enhance Hungary's natural values. The third part of the Act LIII/1996 contains the provisions for the special protection of natural values and natural areas in Hungary. There are no levels of protection in Hungary. A special, stricter protection of a natural value or natural area can only be ensured by taking special measures. In this case the natural value or natural area can be declared to strictly protected. Article 28 states that protected natural areas may - according to the comprehensiveness, objectives, national and international significance of the protection - be categorised as national parks, landscape protection reserves, nature conservation areas and natural monuments. [8] The territory of all national parks shall be classified in natural, managed and demonstration zones. According to the Act LIII/1996 in Hungary all springs, bogs, caves, sink-holes of sinking streams, salt lakes tumuli and earthen fortifications are protected as protected areas of national importance. [8]

In both country protected bird areas (the equivalent of Special Protection Area-SPA), areas of European interest (the equivalent of Special Area of Conservation -SAC) and their protective zones form a part of the coherent European network of protected areas, the NATURA 2000. It aims at conservation of a favourable status of both natural habitats of European interest and species of European interest. The arrangement of these protected areas is very similar in the Slovak Republic and Hungary and stems from the European

Union's Habitats Directive (92/43/EEC) and Birds Directive (2009/147/EC).

Ecotourism in the selected case-study areas

This part of the article describes and compares the arrangement of ecotourism in PLA Dunajské luhy and NP Duna-Ipoly, evaluates the current situation, and provides proposals for the improvement of ecotourism to encourage cooperation between protected areas in cross-border regions.

Ecotourism in PLA Dunajské Luhy and NP Duna-Ipoly

In the Slovak Republic State Nature Conservancy of the Slovak Republic is in charge of all protected areas. It operates directorates of national parks and PLA, which are in charge of the management of protected areas. [9] One of them is the Directorate of PLA Dunajské luhy, which has three offices, in Bratislava, in Dunajská Streda and in Komárno and has many obligations connected to nature conservation and other fields. This study focuses just on area connected to ecotourism (infrastructure, environmental education, projects, publications, information availability etc.). In relation to infrastructure PLA Dunajské luhy unfortunately does not have an information centre or a conveniently located facility for working with the public. It is possible to obtain leaflets, information materials or publications directly at the office of the directorate, at the office of BROZ or at regional information centres of NATURA 2000 areas. [10] Due to lack of finances the network of educational locations and educational trails is growing slowly. Most of the time the nearby municipalities or non-governmental organizations initiate and replace information panels, maps and playgrounds to inform visitors about the unique sights of the protected area (eg. Šúr, Podunajské Biskupice, Číčov etc.). Regarding environmental education employees of the directorate provide professional explanations with a focus on nature and landscape protection to visitors of the protected area; propose, build and foster educational trails, and operate other facilities connected to nature protection. There is an opportunity for schools to order lectures connected to nature protection in LPA Dunajské luhy directly from the directorate. At the weekends there is also a possibility for field visit in form of bird-watching, botanical and geological walks organized seasonally. [10] Non-governmental organizations, for example Regional Association for Nature Conservation and Sustainable Development (BROZ) realize plenty of activities in the area. The dissemination of information about events is very poor. There is no common website or platform, where the activities of the directorate, non-governmental organizations and other partners could be summarized and published.

The Ministry of Rural Development of Hungary supervises the directorates of the national parks in Hungary. These directorates are independently operating central bodies managing not just the area of the national parks, but also the area of landscape protection reserves and natural monuments situated in their area of competence. [11] Regarding ecotourism at NP Duna-Ipoly, the Directorate of NP Duna-Ipoly has a separate Department of environmental education and ecotourism, which coordinates the programs and activities and is responsible for the tourist information office "Green Dot", visitor centres, information centres, educational sites, educational trails and outdoor schools. [12] Financial resources for the projects and activities are drawn from funds such as European Union's Life +, the Program of cross-border cooperation between Hungary and the Slovak Republic (HUSK), Swiss Funds, programs like BioEuparks, Periurban Parks and Hungarian funds Új Magyarország and Új Széchenyi Terv. [12] Since 2009 the infrastructure for ecotourism has been gradually constructed and completed. Now, in 2014 it contains the following elements: tourist information office located in Budapest, three visitor centres, educational and information centres, marked hiking trails, educational locations, 16 educational trails and two outdoor schools. Educational trails can be visited with the employees-guides of the directorate every weekend. The directorate organizes courses for teachers on nature protection, environmental education and guiding in nature. Everyone is invited to participate in voluntary activities, which are aimed at the restoration of marked hiking trails and their tourist signs, waste collection, removal of invasive species, frog rescue activities and bird nest guarding. [13] Publications about the NP Duna-Ipoly provide a clear message and simple information for the public. They are available at every visitor and information centre. Every year the annual program of the national park is issued. In every three months the directorate issues the magazine of the national park "Cincér". Furthermore, those who are interested in programs and activities can register on the website of the national park and receive weekly electronic news letter via e-mail. The directorate of the national park is very active on Facebook. [13] The brand-new website dedicated to ecotourism in the national park was launched last year. The website provides information about protected areas, on-going projects, educational localities and educational

trails. It is possible to download publications and materials connected to each site. The only weakness of the website is that it provides information only in Hungarian language. [14] Directorate of NP Duna-Ipoly is also aware the fact that in case of ecotourism the carrying capacity of the ecosystems has to be taken into consideration. Before the development of the ecotourism activities the area of the national park was mapped and divided into sensitive parts called core areas, areas suitable for tourism and protective zones. Specified limits for the ecosystems are applied, which in practice means some limitations in space and time in the protected areas. These restrictions are implemented carefully, so visitors have access to important areas without compromising the protection of the valuable localities. [12]

Proposals for cross-border cooperation between the case-study areas

There are already existing initiatives regarding cross-border cooperation between PLA Dunajské luhy and NP Duna-Ipoly. Both protected areas are part of the Network of protected areas on Danube, called DANUBEPARKS. Moreover the State Nature Conservancy of the Slovak Republic and the Directorate of NP Duna-Ipoly has an agreement of cooperation, in which cooperation areas are summarized in 14 bullet points. This agreement relates mainly to the field of species conservation and monitoring, but also involves parts about the development of cross-border ecotourism and environmental education between the two protected areas.

PLA Dunajské luhy as well as NP Duna-Ipoly both separately and jointly have a great potential for ecotourism. The Danube river connects these protected areas and offers many opportunities for water activities like daily or longer boat tours. [15] The international Danube cycling route crosses the area of PLA Dunajské luhy. NP Duna-Ipoly also has a potential for the development of cycling activities. Another possibility to connect the two case-study areas is by developing existing cycling infrastructure between them. By creation of programme and service packages for different target groups (eg. package of school trips, water sports package, package for families, cycle tour packge etc.) it would be possible to increase the number of visitors in these protected areas. [15] PLA Dunajské luhy and NP Duna-Ipoly are located in close proximity to capital cities of the Slovak Republic (Bratislava) and Hungary (Budapest), which provides further opportunities. Their potential should not be ignored. It would be useful to organize training courses for the employees of Directorate PLA Dunajské luhy and Directorate Duna-Ipoly, for teachers and other guides performing in the region, both from the Slovak and Hungarian side on nature protection and environmental education. Establishing cross-border relationships between primary and secondary schools and youth exchanges within the frame of environmental education could increase the public interest in visiting cross-border protected areas. The implementation of the proposed measures, however, is highly dependent on the available financial resources.

Discussion

In this part of the article we would like to focus on the two main topics of the study. Firstly, the similarities and differences of the territorial nature and landscape protection systems and protected area management categories are presented. According to the results of the comparative analysis the territorial nature and landscape protection system is similarly adopted in the Slovak Republic and in Hungary. The subjects of nature and landscape protection are the same. Legislation connected to this field is slightly different. Regarding territorial nature protection there are five levels of protection in the Slovak Republic. The whole territory of the Slovak Republic is provided with the first level of territorial nature protection according to § 17 [7]. Second level of protection is valid in protected landscape areas, third level for eg. in national parks etc. The fifth level is the strictest level of protection. There are no levels of territorial nature protection in Hungary. It is possible to designate and declare unique protected natural areas of national importance as strictly protected [6]. Both countries adopted the IUCN protected area management categories, slightly modified and implemented them into their national legislation. In the Slovak Republic there are three extra management categories, it is possible to establish a protected site, protected landscape element and municipality protected area. Protected site is an area, usually up to 1,000 ha with existence of natural habitats of European interest or natural habitats of national interest in which favourable status of these natural habitats depends on human economic activities [7]. A significant landscape element fulfilling a function of a biocentre, a biocorridor or an interactive element, especially of local or regional interest may be designated as a protected landscape element [7]. A municipality protected area is a relatively new category in the Slovak Republic, it is a locality, usually up to 100 ha, with cultural, scientific, ecological, aesthetic or landscape significance and is

operated by a municipality [7]. According to § 31 there is a possibility to declare private protected area with protective zones in the Slovak Republic [7]. In Hungary there are four protected area management categories, approximately equivalent to IUCN categories and categories applicable in the Slovak Republic. The arrangement of NATURA 2000 network is very similar in the Slovak Republic and Hungary and stems from the European Union's Habitats Directive (92/43/EEC) and Birds Directive (2009/147/EC).

The second part of the study addresses ecotourism in the Slovak Republic and in Hungary. According to the collected information and own experience we evaluated and compared the arrangement of ecotourism in two case-study areas, PLA Dunajské luhy and NP Duna-Ipoly. The Slovak side has a great potential and suitable conditions for these kind of activities, yet the ecotourism is still in its infancy. PLA Dunajské luhy doesn't have a coherent concept of ecotourism development. Few measurements connected to ecotourism have been implemented for eg. in surrounding municipalities under tourism development activities. Regarding infrastructure and information dissemination there is still a room for improvement. Establishment of at least one visitor centre in the protected landscape area is necessary. Launching a website or other common information site for potential visitors about the activities would also be useful. In Hungary the Directorate of NP Duna-Ipoly has successfully implemented the concept of ecotourism development in the last six years. The necessary infrastructure for ecotourism activities was completed recently. The directorate proudly promotes the opportunities in the area of the national park and attracts more and more visitors every year. In our study the arrangement of ecotourism in a landscape protected area and in a national park was investigated. However, we must take into consideration that these protected area management categories are similar, but not the same and not equivalent. A protected landscape area in general has less financial supply than a national park. Not to mention that our case-study areas are located in different countries with different financial mechanisms. Consequently the results of the study might be slightly distorted. In the future, we would like to prepare another comparative study regarding the management of national parks in the Slovak Republic and Hungary to get more accurate and clearer picture.

Conclusion

The situation in the National Park Duna-Ipoly in Hungary is a positive example of a functional ecotourism. This area may become a suitable inspiration for the Slovak side, because it is possible to adapt ideas from abroad and implement in the area of Protected Landscape Area Dunajské luhy or in other protected areas. Ecotourism integrates several positive impulses necessary for sustainable development of protected areas. It particularly increases the environmental awareness of visitors but also local inhabitants, who improve their relationship with the surrounding environment. Another important dimension of ecotourism is the fact that it contributes to rural development of cross-border regions.

Acknowledgement

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Attractiveness of the Vranovsko micro-region as a tourist locality

ALONA VASYLCHENKO, ANTONIN VAISHAR
Department of Applied and Landscape Ecology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

alona.vasylchenko@seznam.cz

Abstract: The aim of this paper is to explore tourism as an alternative way for development of the Vranovsko micro-region. Most popular forms of tourism characterized for this region and main attractive places are described in this paper. Research was conducted to detect current economic situation of providers of tourism services, their problems, threats and opportunities for future development. For this purpose providers of tourism services from two municipalities of Vranovsko micro-region were asked to fill out the questionnaire. It was found, that all respondents consider, that tourism is a perspective way for development of their micro-region.

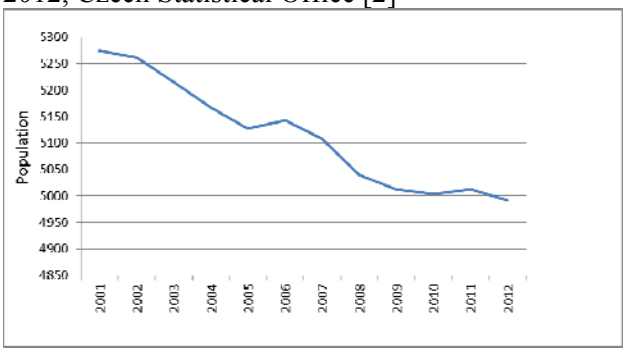
Key-Words: - Vranovsko micro-region, tourism, Podyjí National Park, Water reservoir Vranov

Introduction

Vranovsko micro-region is situated at Znojmo District in the south-western part of the Czech Republic and its area is 21,975 ha. This micro-region borders with Austria and its center is Vranov nad Dyjí. The association of municipalities of Vranovsko was created 7 September, 1998, and it consists of 21 villages, namely Bítov, Chvalatice, Korolupy, Lančov, Lesná, Lubnice, Onšov, Oslnovice, Podhradí nad Dyjí, Podmyče, Stálky, Starý Petřín, Šafov, Štítary, Šumná, Uherčice, Vranov nad Dyjí, Vratěnín, Vysočany, Zálesí and Zblovice [1].

Villages of Vranovsko are small, they have limited own activities and they are situated far from all regional centres both in Czech and Austrian sides. Because of these factors the micro-region has emigration tendencies [1]. As it is seen in the graph 1, population of the region decreased. Now the population is 4,992 inhabitants [2]. So population has declined of 5% for the last 10 years.

Fig. 1 Population development in the years 2001 - 2012, Czech Statistical Office [2]



That's why it is necessary to look out an alternative way for the development of Vranovsko micro-region. Tourism can be one of the solutions for this problem.

Tourism is already an important feature of the rural economy in Europe. EuroGites (the European rural tourism umbrella group) calculated that tourism supports more than 1.300.000 direct and indirect jobs in Europe, and generates more than 100.000 million EUR in gross income each year [3].

Moreover rural tourism has positive impacts on [4]:

- reversing of depopulation (employment, attracting in-migration);
- new skills and training for inhabitants;
- housing (the building sector grows);
- service retention (tourism increases the local market for services);
- employment opportunities for women;
- environment (justification of rural tourism provides the conservation of Europe's rural landscapes, ecosystems, built environments, communities and cultures);
- socio-cultural relations (the recognition and celebration of past ways of life);
- creating a focus for regional planning.

The micro-region disposes with conditions for such forms of tourism as sightseeing tourism, water recreation and cycling.

The Water reservoir Vranov is a popular place for water tourism there. Tourists have possibilities for recreation, water sports, fishing and swimming. Many camps and guesthouses are situated here.

National park Podyjí is the next attraction of the micro-region. Poodyjí represents the smallest national park in the Czhech Republic [5].

Vranovsko micro-region is attractive for tourists not only by its flora and fauna but also by plenty of cultural-historical monuments, such as castles (The chateau in Vranov nad Dyjí, The Bítov castle, The ruins of castles Cornštejn and Frenštejn) and remains of mill (Lesná, Uherčice).

Village Vratěnin got a title of Village of the Year in 1996 and it was declared a rural reservation zone. Vratěnin has 24 protected historical monuments.

So Vranovsko is attractive for tourists both by its nature and by cultural-historical monuments and has a large potential in this field.

Material and Methods

Tourist infrastructure in micro-region is not completely developed. Basic comparably good standard elements for accommodation and meals are located in Vranov nad Dyjí, Bítov, Chvalatice, Podhradí nad Dyjí and Štítary. But supplementary and entertainment services are here in lack. Information sector actively develops. Vranovsko's Tourist information center (TIS) is situated in Vranov nad Dyjí.

The highest number of tourist accommodation facilities are located in Vranov nad Dyjí, its number is 14. Summertime pensions prevails here, they have 50 rooms as maximum. Additionally three three stars hotels can be found here. There are Hotel under Castle, Castle Hotel and the biggest one Military sanatorium Dyje with maximum capacity of 100 rooms.

Three pensions, three cottage settlements, two tourist accommodations and one camp are located in Štítary, together it is 12 objects. Nine accommodation objects works in Bítov, there are 1 three stars hotel (Hotel Bítov), 2 pensions, 1 camp and 3 cottage settlements. Total number of collective accommodation establishments in Oslnovice is 6, they consists of one pension (Pension Under castle), three cottage settlements and other accommodations.

Lančov, Podhradí nad Dyjí, Chvalatice, Lesná a Vysočany have from 1 to 5 collective accommodation establishments.

The highest number of catering objects are concentrated in Vranov nad Dyjí (U Vodnáře, Hotel's restaurant, Under Castle, Parník, Formosa Restaurant, Country salon, Castle's Hotel Restaurant, Herold restaurant, Pážecí dům Restaurant, Přehrada restaurant, Brusel restaurant, Titanic Restaurant).

For evaluation of the current situation of Vranovsko's tourism potential two municipalities were selected. There were Vranov nad Dyjí and Bítov (fig.2).

Fig. 2 Overview map of Bítov and Vranov nad Dyjí (Znojmo district), Czech Statistical Office [6]

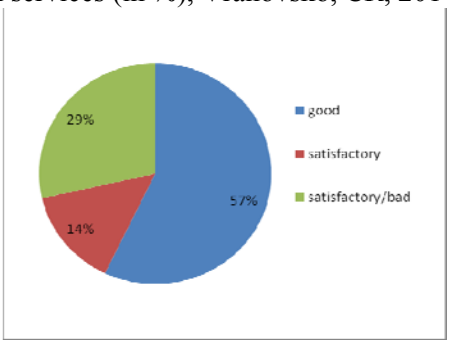
Questionnaire survey of accommodation and catering objects was carried out in these municipalities. The questionnaire contained questions about long-term economic situation of providers, the structure of visitors and reasons of visiting, main problems and opportunities for future development. The total number of asked subjects was 64. And only 7 agreed to answer questions (mainly there were accommodations - pensions).

Within the project not only questionnaire survey was done, but also interviews with providers of tourism services were carried out.

Results and Discussion

According to the survey, which was done from June till September 2014, more than half of providers of tourism services found their long-term economic situation as good (fig. 3).

Fig. 3 Long-term economic situation of providers of tourism services (in %), Vranovsko, CR, 2014

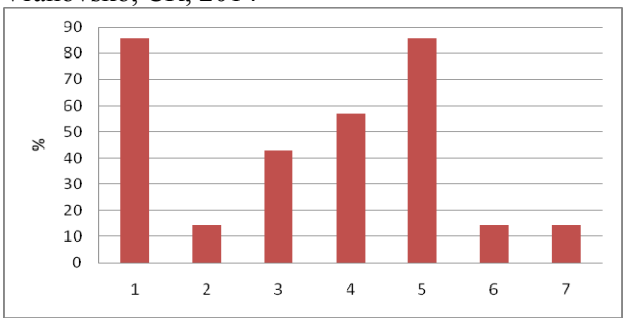


In the same time almost 30% of respondents determine their situation between satisfactory and

bad. These results have been plotted on the pie chart.

Respondents consider, that higher percent of Vranovsko's visitors are families with children and active visitors (for sports - hiking, biking). Next important groups consist of pensioners and students. The structure of Vranovsko's visitors shows chart (fig. 4).

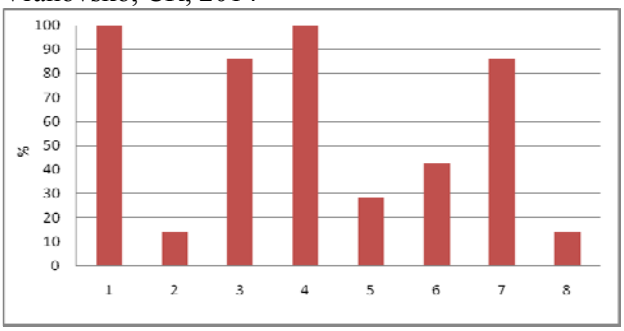
Fig. 4 The structure of Vranovsko's visitors (in %), Vranovsko, CR, 2014



Legend: 1 – families with children, 2 – economically active people, 3 - young people – students, 4 – pensioners, 5 - active visitors, 6 - passive visitors, 7 - cottagers.

Next chart presents main reasons to visit Vranovsko. So as it is seen in figure 5, people visit this region because of holiday, protected areas (National park Podyjí), biking and historical monuments.

Fig. 5 Reasons why tourists visit Vranovsko (in %), Vranovsko, CR, 2014



Legend: 1 – holiday, 2 – conferences and seminars, 3 - history, 4 – biking, 5 - wine tourism, 6 - sightseeing tourism, 7 - protected areas, 8 – visit concrete accommodation or catering object.

During interviews with providers of tourism services it was found, that the main problem of tourism in this region is its extreme seasonality. There are no conditions for the winter tourism in the area.

Owners of pensions in Vranov nad Dyjí complained, that parking isn't here. And only one large parking is situated in 500 m from the Castle. Also playground for children is absent in the town. The problem relates to the insufficient place in a relatively narrow canyon where the commune is situated.

Expected reconstruction of the dam crest represents one of the main threats. This would lead to stop of transit from the Camp Vranov beach to town and to Castle in Vranov nad Dyjí for 2-3 years. Similarly a limitation of the shipping evoked by the disagreement between the operator and the landscape protection could hinder the connection between two most visited attractiveness (Vranov chateau and Bítov castle).

Moreover providers of tourism services are afraid of economic crisis.

The high quality of environment of all tourist destination is perceived as the main strength whereas the subsidies into tourism is considered as a mayor opportunity.

In the same time respondents think, that permission of water tourism on the river Dyje would improve the situation. And all the service providers agreed, that tourism is a perspective for the Vranovsko micro-region.

Conclusion

Vranovsko is a micro-region, which consists of 21 communities. It is a periphery region and because of that it has emigration tendencies and the population of region decreases. However one of the main goals of Association of Vranovsko's communities is development of tourism in region, so it can be a good solution.

The peripheral nature of the micro-region is caused not only by the distance from regional centers but also by the position on the borderland. The border was a part of the iron curtain for almost 40 years. It was like the end of the World that time. Even today the border is hardly impassable due to the national conditions (relatively deep canyon of the Dyje River and due to the highest level of the landscape protection within the National Park Podyjí/Thayatal on both sides of the border.

Last historical development of the micro-region was loaded with the post-war ethnically based population exchange. The relationship of the new settlers to their villages, regions and neighbors created for a long time step by step. Moreover the Austrian communes on the opposite side of the borderline are partly in a similar situation. That is why certain hopes in cross-border collaboration have been not fulfilled. The quality of social capital is reflected by the fact that no Local Action Group has been created in the micro-region till this time.

To recapitulate, the main tourist attractions of Vranovsko are the Water reservoir Vranov, National park Podyjí and historical monuments (the chateau in Vranov nad Dyji, the Bítov castle, State chateau Uherčice, the ruins of Cornštejn and remains of mills).

To evaluate tourism situation in Vranovsko the survey of providers of tourism services was done. From the survey follows, that Vranovsko is visited mainly by families with children and active visitors. The main reasons for visiting of Vranovsko are holiday, protected areas (National park Podyjí), biking and historical monuments.

Nevertheless more than 57% of respondents have good long-term economic situation, they have many complications and threats. For example, tourism in region has extremely seasonal character, parking and playground for children are absent in Vranov nad Dyjí and many problems are connected with protection of river Dyje. At the same time respondents hope for business support of tourism in Vranovsko.

To conclude, the survey and interviews suggested, that Vranovsko micro-region is a perspective tourism area. But due to the seasonal limitation and some other problems, it is not possible to expect that the micro-region could survive from the tourism only. Following activities could be recommended: agriculture on relative fertile soils (but with relatively high productivity, which results in decreasing jobs), energy production from renewable sources, elaboration of local products. All these branches are not able to ensure sufficient employment in the micro-region.

Unfortunately, although Vranovsko is strictly rural area, there are no conditions for the farm tourism development [see e.g. 7]. There are almost no family farms which are a necessary prerequisite for its development.

That is why the depopulation will probably continue. It could change into structural demographic changes (emigration of young and educated people and immigration of seniors) which should result in the aging. Also villages (especially the smallest and less accessible) could change from living settlement into concentration of second homes (cottages), later used for permanent residences. It would be accompanied by attenuation of service – which would be unpleasant for the tourism development.

The aging could cause a development of services for seniors. The problem consists in the fact that seniors often perceive tourism as an undesirable element.

On the other hand, the peripheral position protects the borderland settlements from intensive changes of rural to urban character [8].

In any case, next development of the Vranovsko micro-region is worth for further investigation. As the most peripheral micro-region in the South Moravian Region, Vranovsko could be a case study of possible negative manifestations and consequences of the peripheral nature.

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Impact of tourism on the structure of housing in the microregion Termal

MICHAELA ZONCOVA, ALENA DUBCOVA
Department of Geography and Regional Development
Constantine The Philospher University in Nitra
Tr. A. Hlinku 1, 949 74 Nitra
SLOVAK REPUBLIC

michaela.zoncova@ukf.sk

Abstract: The paper deals with analysis on development of housing structure in rural municipalities. Houses in rural space were built in order to fulfil a particular function (e.g. agricultural function). Whereas rural space fulfilled mainly productive, agricultural function in past, buildings were also adapted to this function, there was not any emphasis on visual aspect. Nowadays, rural space has changed to multifunctional system as a consequence of the decline of agriculture importance and new functions are coming to the fore.
In the study area of microregion Termal function of tourism comes to the fore, which also has influence on the structure of housing. Based on data from The Population and Housing Census (1991, 2001 and 2011), we looked over the development of building-up of houses in municipalities of microregion Termal, where we identified a strong building-up period and then we analysed the number of unoccupied houses and its trend in the period. Increase of the number of unoccupied houses is caused by the increase of share of houses which are determined for recreation. The tourism development has caused a change of visual side of rural houses and newly built houses do not fit into the former compact architectural concept. This disturbs the image of rural landscape and elements typical for cities get into rural space (e.g. multi-storey houses). Statistical data were analyzed also in terms of spatial allocation, which we presented using the cartographic methods. The aim of this paper is to point out changes in the rural housing, which has been forced to diversify their functions.

Key-Words: housing, rural landscape, tourism development, rural functions

Introduction

The landscape is a dynamic system that varies in time and space. Rural landscape is a specific type of landscape that was perceived as a symbol of agriculture and rural area had initially the productive function. After 1989, there was a decline of agriculture and a collapse of cooperative farms, consequently. Rural landscape and rural municipalities were forced to change their character and perform also other functions.

A new phenomenon gains ground – rural diversification, i.e changeover of agricultural (productive) rural space to multifunctional system that tries to take advantage of its potential and move capital to different activities. According to Šarafin [1] rural area passed into the world of globalization, it became part of a new dimension of economic processes. Agriculture stopped to fulfill the most important role in the rural space and the rural space was divided into suburban, recreational and stagnant.

Development of industry and concentration of population in cities has resulted in abandonment of the rural houses, which were often located far from the workplace, especially in areas with dispersed settlement. Existing houses in municipalities, which lost its original economic function and had the prerequisites for the development of recreational activities, began to use for an individual recreation [2]. As a consequence of changes in the transformation and post-transformation period, housing in municipalities also undergone significant changes, as in the field of quantitative, qualitative, physical and spatial structures, too [3]. Period of socialism and ideology of rural-urban coequality significantly facilitated to replace the old buildings with new ones, peculiarity capitulated under the influence of expressive diversity of new houses [1].
According to Gajdos [3] presents rural area considerably differentiated type of settlement. On the one hand, there are municipalities where improvement of quality of housing was managed with the help of funds, but on the other hand, there are municipalities where the insufficient attractiveness for living appears.

Since the second half of the 19th century, high building-up is typical for rural municipalities, which are located in economically developing regions. It is directed to the reconstruction and modernization of existing buildings and reflects into the boom of building-up new residential houses. It is also reflected in investing to improvement of infrastructure facilities. By the lower level of economic activities in municipalities, these activities decrease and reflect into the reconstruction and modernization of houses. These activities are reduced in troubled regions, too [4]. Changes in the structure and condition of the housing are one of the consequences of transformation of monotonous, agricultural rural area to multifunctional system. Change of the function of rural area also reflected in state of houses and residential function is supplemented with recreational function.

Visual scene of rural landscape is also changed, where on one hand, there is the building-up of new houses and on the other hand, there occurs deterioration of old houses causing contrast in the rural housing. There occur also changes in architecture of houses. In the past, rural area had unvaried character, where was visible an architectural consistency of buildings. Over the long-term influence by man- householder on the surrounding countryside there established relatively stable typical rural landscape with a specific landscape impression. Characteristic relation with the environment, modesty in the pretensions, the criterion of functionality, quality and durability were typical signs of rural areas. The result was artistically, aesthetically and functionally balanced architecture. In consequence of pseudo-revolutionary trends in evolution of society and tendency to "approach rural area to city", there was not respected any realities and necessities of life of the rural environment. The continuity of building development was significantly disturbed. During the 20th century, the traditional rural architecture becomes the symbol of poverty and backwardness in the inhabitant's set of priorities [5]. The integration of new buildings to existing buildings and to landscape in such style which will not disturb visual aspect of rural landscape is very problematic.

Material and Methods

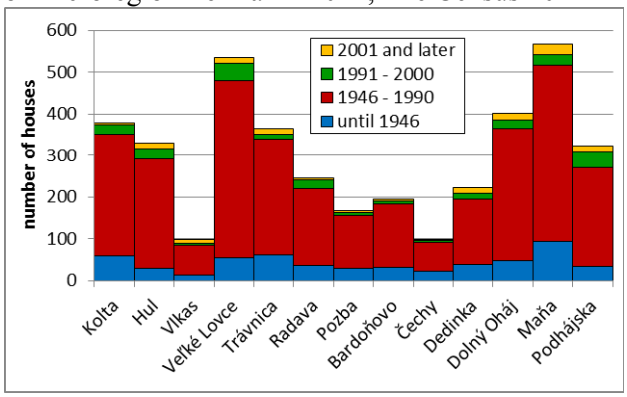
To identify changes in the evolution of the structure of houses in municipalities of microregion Termal, we used statistical methods, where we analyzed data from the Population and Housing Censuses of 1991, 2001 and 2011 [1]. By this twenty-year period, we are able to analyze the changes that have occurred in the transformation and post-transformation period. In this paper we look over the age of houses in rural area, the share of unoccupied houses of the total number of houses and share of the houses determined for recreation. Unoccupied house is defined as house determined for housing, where any dwelling was not permanently occupied during the Census. The data were expressed also spatially using cartographic methods, concretely using ArcGIS 10.1. Consequently, we analyzed information and searched for a correlation between changes in the structure of housing and the increasing importance of tourism in the observed micro-region. Microregion Termal is the area of interest, which consists of thirteen communities - Radava, Podhájska, Bardoňovo, Travnica, Bohemia, Hul, Pozba, Dolný Ohaj, Veľké Lovce, Dedinka, Kolta, Vlkas and Maňa. In terms of regional classification, microregion is part of Nové Zámky district and Nitra self-governing Region. In the study area of the microregion there is an increase in importance of tourism. Podhájska is center of microregion and it is well-known because of thermal swimming pool and thanks to it journal TREND ranked Podhájska to 4th place of the most attractive thermal swimming pools and aquaparks in Slovakia in 2013 on the ground of income.
These development trends had an impact on the character and scene of the rural landscape in the microregion, but also functions of housing.

Results and discussion

Development of building of houses

Based on statistics from the 2011 Census, we can analyze the age structure of houses in rural municipalities of microregion Termal.

Fig. 1 The age structure of houses in municipalities of microregion Termal in 2011, The Census 2011



Age of houses in municipalities had an influence on the scene of municipalities, because many old houses under the influence of time left abandoned

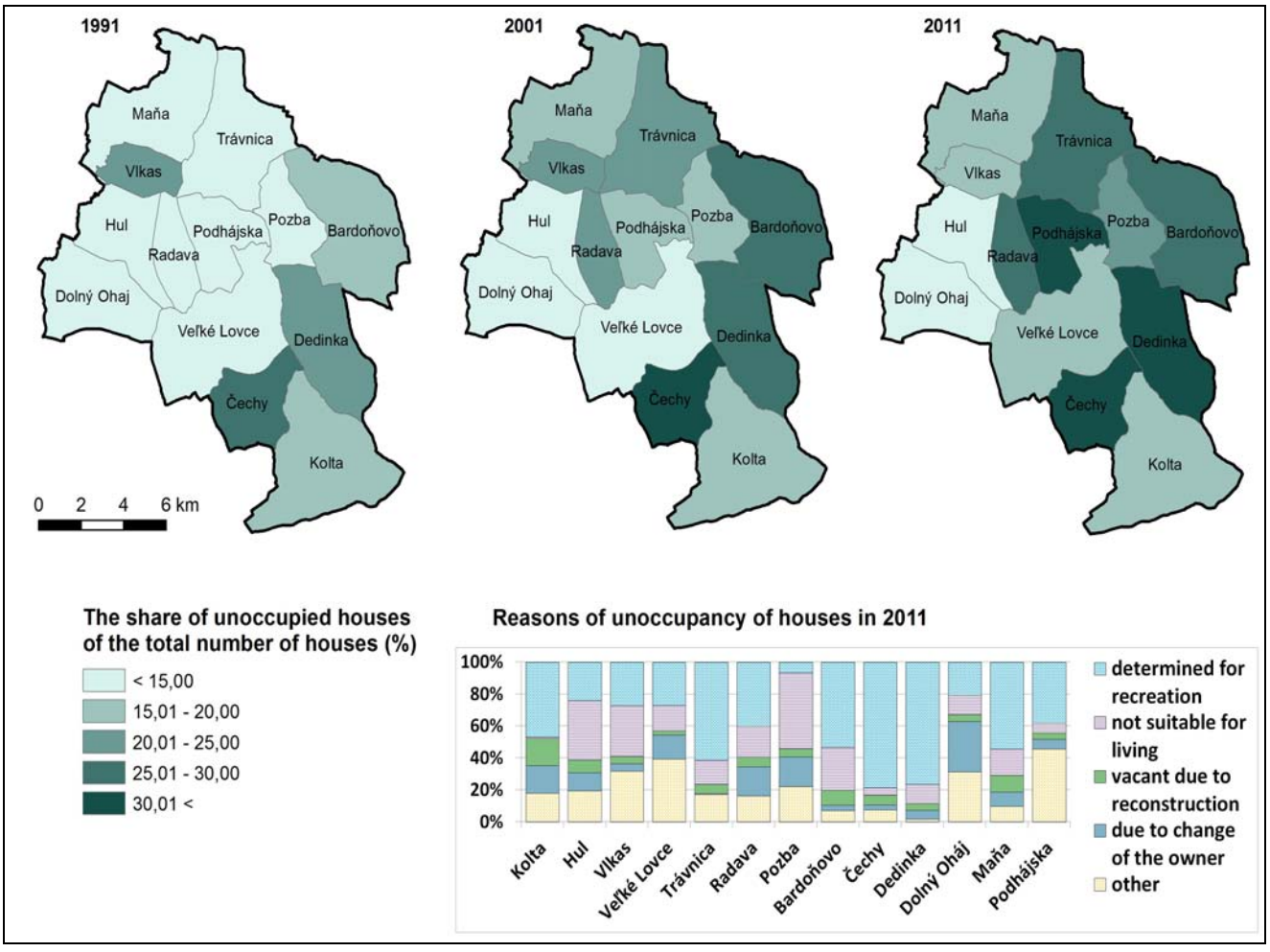
and they deteriorated. Instead of reconstruction of old houses the building-up of new houses began. The largest building boom started in the years 1946 – 1990, i.e. in pre-revolutionary period (Fig 1). In all observed municipalities approximately 2/3 of houses were built at this time. In the post-revolutionary period, there was a recession in building-up of new houses. In the monitored municipalities only 10% of the houses in average were built in this period. The most newly built houses can be found in the municipalities of Veľké Lovce, Podhájska and Maňa, where more than 50 new houses increased in each municipality. At least new houses were built in the municipalities of Čechy, where only 7 new houses were built since 1991. This fact is also related to the size of municipalities. Percentage provides an undistorted view and therefore the most new houses of the total number of houses are located in the municipality of Podhájska (16.1%), Vlkas (14.3%), Dedinka (12.2%) and Hul (12.1%), what indicates the development of municipality and interest of population to live in these municipalities.

There appears the building-up of new houses on "greenfield", but in the municipalities there are many dilapidated houses that distort the landscape scene and they are disruptive. Džupinová and Pšenka [7] dealt with research of the impact of tourism on development and changes in the structure of housing. They focused mainly on individual objects of recreation, so called "second housing". In analysis of the objects of second housing, they focused exactly on the category "unoccupied houses, determined for recreation", which was examined in the population and housing censuses of 2011.

Development of unoccupied houses

In the process of transformation low social and economic situation appears in many rural municipalities. It accelerated to the other problems in the last decade of the last century. It was leaving young people to work, aging of population and dilapidation of rural houses.

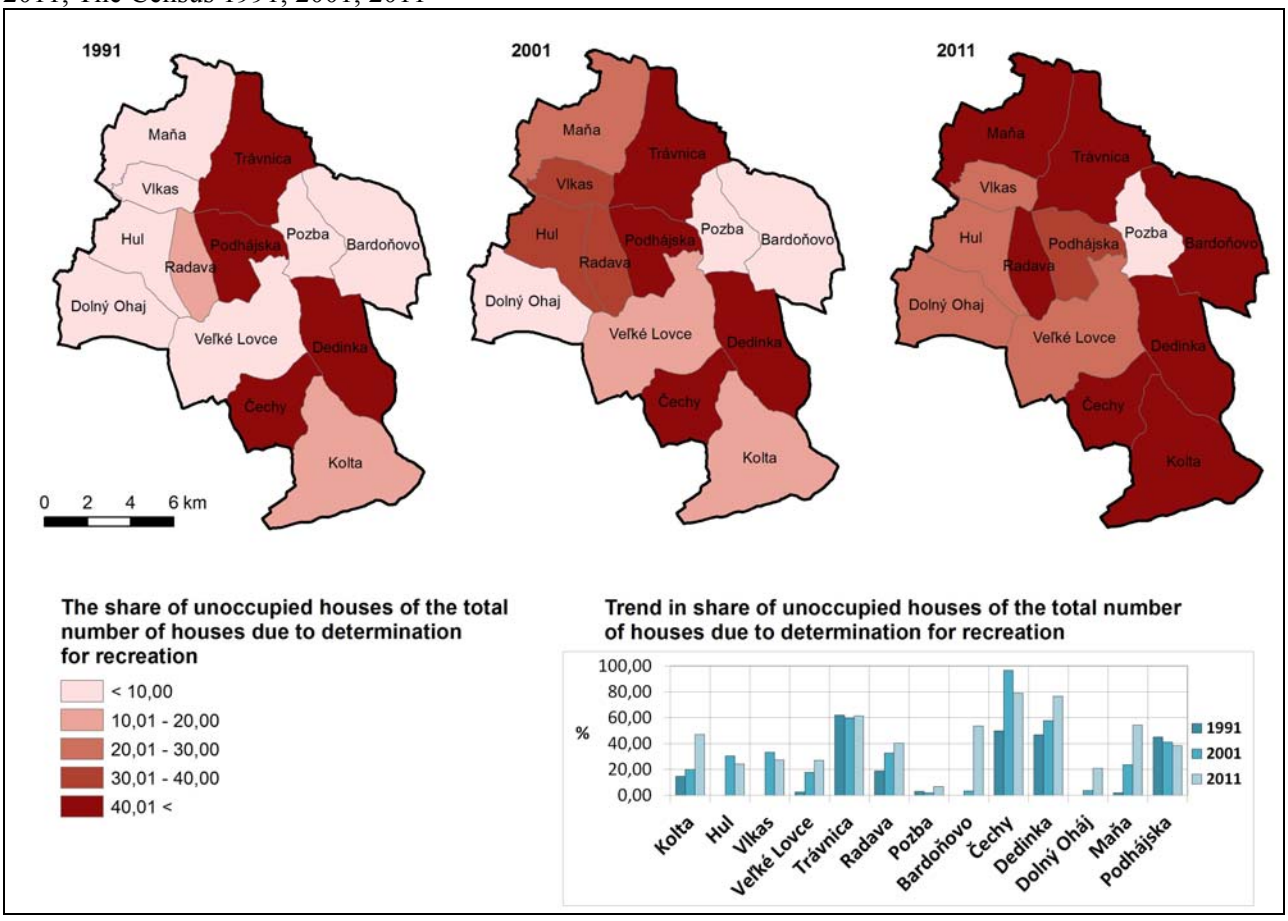
Fig. 2 Development of unoccupied houses in microregion Termal in 1991, 2001 a 2011, The Census 1991, 2001, 2011



Kuldová [8] states untidy areas and private parcels, neglected houses, manufacturing buildings, dilapidated monuments formerly important elements of tourism for the main problems of rural areas after 1989. In terms of the structure of housing fund, we can follow the development of the number of unoccupied houses that may be unoccupied for various reasons – due to change of owner, because of determination for recreation, vacant due to reconstruction, unsuitability for living, upon approval of building, due to inheritance or court action or for other reasons. Development of share of unoccupied houses of the total number of houses in municipalities based on data from The Census 1991, 2001, 2011 is shown in the Fig. 2. In the observed twenty-year decade is a high increase of unoccupied houses noticeable in some municipalities. In 2011, in municipalities of Čechy, Dedinka and Podhájska for more than 30% of the houses were unoccupied.
In municipality of Čechy is this fact noticeable during the whole observed period, where the share of unoccupied houses reached in 1991 value of 21.1%, in 2001 it was 26.4% and in 2011 it achieved a value of 34.4%.

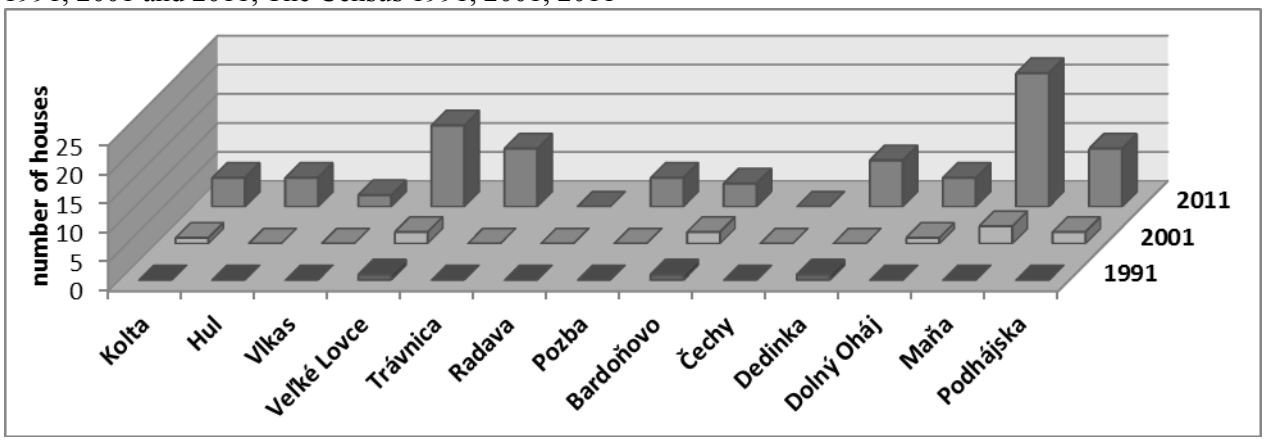
Houses were not vacant because of dilapidation, but because they were determined for recreation. The idea of "second housing" comes to the fore i.e. partial transformation of residential function of municipalities to the recreational function. Houses in rural area are starting to use as "weekend" housing and a cause of this phenomenon may be an "escape" of the working population from the busy cities to peaceful countryside. Municipality of Čechy provides a calm environment with potential enjoyment as in winter (local ski tow), as well as in summer (local pond, nearby aquapark in Podhájska). An interesting phenomenon is also evident in the municipality of Podhájska, where the share of unoccupied houses raised and in 2011 reached 32.4%. However, this is not because of dilapidation, but it may be caused by the development of tourism in the municipality and with the consequent demand for accommodation services. Hardly any house determined for recreation is not in municipality of Pozba, where houses are unoccupied because of unsuitability of living. Development of share of unoccupied houses, which are determined for recreation, is shown in the Fig. 3.

Fig. 3 Development of number of unoccupied houses due to determination for recreation in 1991, 2001 and 2011, The Census 1991, 2001, 2011



In the Fig.2, there is a noticeable increase in the number of unoccupied houses, but on the Fig.3 there is striking increase in the number of unoccupied homes due to determination for recreation. It follows that in the observed area does not occur to dilapidation and degradation of houses, but there appears a transformation of function of houses. Houses determined for recreation since 1991 have been concentrated in municipalities of Trávnica, Podhájska, Dedinka and Čechy and in 2011 the concentration also increased in municipalities of Maňa, Radava, Bardoňovo and Kolta. The opposite phenomenon is visible in the village Podhájska, where you can see a decrease in houses for recreation. But it is a very low drop, but it can also be caused by increases in competition in the village tourism. The opposite phenomenon is visible in the municipality of Podhájska, where you can see a decrease in number of houses determined for recreation. Nevertheless it is a very low decrease, but it can also be caused by increase in competition in tourism. Objects of individual recreation recorded a decrease of their significance as a result of the increase of the number of providers of accommodation in the municipality. Based on the internal data of aquapark in Podhájska, this aquapark visit about 400,000 visitors a year. Because of it is necessary to provide accommodation services to visitors not only in newly built hotels and guesthouses, but private accommodation comes to the fore. The inflow of new functions to the rural area brings also a change in the appearance of houses. Houses does not fulfill initially only functionality, but comfort and visual aspect comes to the fore. Result of efforts to bring urban elements to the rural area is the construction of multi-storey buildings, too. Based on statistics, this phenomenon is also visible in the observed area of microregionTermal (Fig. 4).

Fig. 4 Development of number of three- and multi-storey houses in municipalities of microregion Termal in 1991, 2001 and 2011, The Census 1991, 2001, 2011



In the Fig. 4 a high increase of multi-storey houses in 2011 is noticeable. In 1991 there were a multi-storey houses only in the municipalities of Veľké Lovce, Bardoňovo and Dedinka. Other municipalities had still rural appearance. Ten years later, there were added multi-storey houses in other municipalities, but still it was not as radical as in 2011. The most multi-storey houses were built in the municipalities of Maňa, Veľké Lovce, Trávnica and Podhájska, where there are currently 10 and more of these houses. In Maňa, there were 23 multi-storey houses in 2011 and 11 of them had 5 or more floors. Such high houses architecturally fit not into the rural landscape, where a large proportion of houses have only one or two floors. In the rural areas small "housing estates" form, those are typical for the urban environment. Not only that, the height of the houses changes, but changes their visual aspect, too. The emphasis puts on first impression, it means that the house should be striking, and different from others, but this is disruptive element in unified nature of rural area. Impact on the visual aspect of the houses also has a growing importance of tourism in the microregion, where houses determined for the accommodation try to attract a costumer in the competition network also with its appearance. Illustrative example we can see on Fig. 5, where are houses determined for accommodation for tourists.

Fig. 5 Accommodation in Podhájska, Google streetview, 2014

Those houses do not fit among the surrounding houses on one side with appearance, but also with the height and with its function, too. By this style of architecture a uniform atmosphere of rural community is not kept and municipality is becoming an architecturally fragmented area.

Conclusion

Rural area changed a lot in last decades, what influenced its internal structure and appearance. The greatest development of building-up of new houses in rural area was in 1946 – 1990, when the most part of houses was built. Subsequently, there was a sharp decline in building. Built houses began to dilapidate under the influence of migration of population to cities and instead of reconstruction of the old houses; new houses were built on "greenfield". As the rural space is gradually losing its primary agricultural function, the appearance of the countryside is changing under the influence of the inflow of new functions. Although a growing number of unoccupied houses is in the study area, a large percentage of these houses are determined for recreation. The municipality is losing permanent population, because people do not have in these objects a place of permanent residence and use them only for recreational purposes. In the observed area tourism develops successfully, because of the location of the aquapark in the municipality of Podhájska. Residential function of houses is partly converted into a recreational function under the influence of increasing competition of accommodation's owners and they are forced to attract the visitors, even by visual aspect of houses determined for accommodation. In the past, the typical low buildings have primarily served the purpose and functionally have been determined for the population engaged in agriculture and now they relegate into the background and modern houses of different shapes, colors and sizes a lead come to the fore. Instead of maintaining the uniformity of rural architecture, there occurs the fragmentation and the influx of new elements. Approval of new houses in the municipalities should underlie also to the approval of aesthetic side to fit harmoniously to earlier houses and the original character of the countryside should be kept.

Acknowledgement

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Section – Food Technology

Detection of di-n-butyl phthalate and di-2-ethylhexyl phthalate in packages of meat products

SONA BOGDANOVICOVA[1], ALZBETA JAROSOVA[1], JOSEF KAMENIK[2]
[1]Department of Food Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Department of Meat Hygiene and Technology
University of Veterinary and Pharmaceutical Sciences, Brno
Palackeho 1/3, 612 42 Brno
CZECH REPUBLIC

sona.bogdanovicova@mendelu.cz

Abstract: The study is focused on determining di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) in materials used for packaging meat products. 80 packages were analysed with a sample of about 10 cm^2 taken from each of them and then subjected to double analysis (i.e. a total of 160 analyses). The determination of phthalates was conducted using high-performance liquid chromatography (HPLC) with the Zorbax Eclipse C8 column and UV detection at a wavelength of 224 nm. The concentrations ranged from 0.19 to 39.52 $\mu g \cdot dm^{-2}$ for DBP and from 0.01 to 103.33 $\mu g \cdot dm^{-2}$ for DEHP. The objective of this study was to determine whether the content of the monitored phthalates in packages of meat products was in compliance with the limit provided in the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact and whether or not it was posing any serious health risk for consumers.

Key-Words: – PAE, package, phthalic acid esters

Introduction

Phthalic acid esters (PAE/phthalates) are members of the group of organic environmental contaminants. Virtually ubiquitous compounds, the reason for their high production, hence their rampant spread in all the components of the environment, are suitable physico-chemical properties due to which the substances are used as plasticisers, i.e. substances improving the mechanical properties of plastics. The U.S. Environmental Protection Agency (EPA) has classified six phthalic acid esters as priority hazardous pollutants. These include dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), di-2-ethylhexyl phthalate (DEHP), di-n-octyl phthalate (DOP) and dibutylbenzyl phthalate (BBP) [1]. Since phthalate-based plasticisers are not firmly bonded by covalent bonding in the material, they slowly release into the surrounding environment by volatilisation, leaching or migration [2]. Large amounts of phthalates are released not only during use, but also when handling plastic waste (storing at landfills, incineration etc.) [3]. Toxic and most abundant phthalates include di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DBP) that are of lipophilic nature. As a result, they accumulate in fatty tissues. Events of chronic toxicity have demonstrated adverse effects of PAE – teratogenicity, spermiotoxicity, nephrotoxicity, hepatotoxicity and carcinogenicity, as well as detrimental effects on membrane function. Due to the health impact in humans, the penetration of phthalates into the environment should be controlled and the exposure reduced [4].

The materials with which food comes into contact are the main source of food contamination [5]. These have to meet the requirements of the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact, one that defines a migration limit and a specific limit.

The migration limit (ML) refers to the maximum amount of packaging components that are released into a food or into a food simulant during the leaching test per unit of area of the plastic package or per unit of weight of the food or food simulant. Plastics and plastic products shall not release their ingredients into the food in a quantity greater than 60 $mg \cdot kg^{-1}$ of the food or food simulant. The limit of aggregate migration per unit of area was set at 10 $mg \cdot dm^{-2}$ of the surface of the material/product.

The specific migration limit (SML) is the maximum allowed quantity of the substance migrating from the package into the food, onto the food or into the simulant. For food simulants, the SML is 30 mg·kg^{-1} for BBP, 1.5 mg·kg^{-1} for DEHP and 0.3 mg·kg^{-1} for DBP [6].

Material and Methods

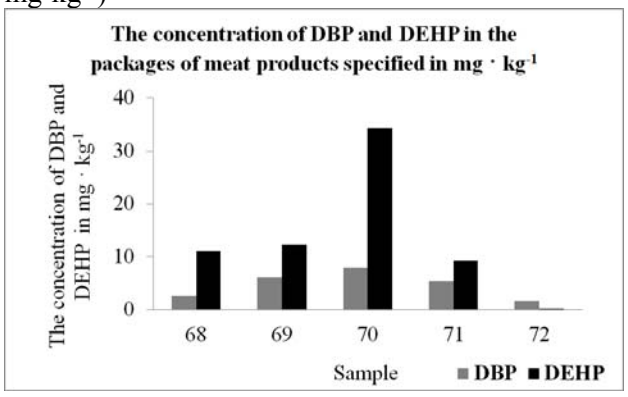
Meat product packages were provided in cooperation with the Veterinary and Pharmaceutical University in Brno and analysed at the Department of Food Technology, Mendel University in Brno. A sample of about 10 cm^2 was taken from each package (n = 80) and then subjected to double analysis (i.e. a total of 160 analyses were done).

The samples were leached in a mixture of solvents *n*-hexane: dichloromethane (1:1) for 72 hours and then extracted three times (60, 30 and 30 minutes). The combined extraction proportions were filtered and evaporated using a rotary vacuum evaporator; then there was a final drying with nitrogen. Subsequently, the extract was transferred into vials using hexane (5 ml) and centrifuged. The upper portion of the extract (1.5 ml) was removed and there was a final drying with nitrogen. The samples were re-centrifuged; the upper layer of the extract was removed (1.5 ml) and subjected to final drying with nitrogen as well. Subsequently, acetonitrile was added into each of the vials to a volume of 1 ml. If an extract was coloured or turbid, it was purified using sulphuric acid. Phthalates were determined by the HPLC method with UV detection at a wavelength of 224 nm, using the column ZorbaxEclipse XDB-C8 150 x 4.6 mm, 5 µm (Agilent Technologies, USA). The quantity of the sample sprayed on the column was 10 µl. The resulting concentrations were computed based on the calibration curve in the AgilentChemstation for LC and LC/MS systems software. The range of the calibration curve was from 1.06 to 106.00 µg·ml^{-1} for DBP and from 1.01 to 100.50 µg·ml^{-1} for DEHP. The correlation coefficient was 0.9999 for both DBP and DEHP. The limit of detection was 0.05 µg·ml^{-1} for DBP and 0.11 µg·ml^{-1} for DEHP. At the final stage, the results were statistically processed using Microsoft Office Excel 2007.

Results and discussion

The resulting concentrations of DBP, DEHP and DBP + DEHP are specified in µg·dm^{-2} of sample area and are shown in Table 1. The DBP concentration values in the samples ranged from 0.19 to 39.52 µg·dm^{-2} of sample area and DEHP concentrations ranged from 0.01 to 103.33 µg·dm^{-2} of sample area. The highest DBP + DEHP concentration observed was 127.28 µg·dm^{-2}. The analysed packages complied with the limit of the aggregate migration per unit of area referred to in the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact (max. 10 µg·dm^{-2} of the surface of the material/product). With regard to the specific migration limit for DBP (max. 0.3 mg·kg^{-1} of the food simulant) and DEHP (max. 1.5 mg·kg^{-1} of the food simulant), five of the packages analysed would be not meeting the above regulation. These values are provided in mg·kg^{-1} and shown on Fig. 1 and involve samples 68, 69, 70, 71 and 72 – coloured textile covers that are designed for packaging boiled meat products and contained phthalates in the printing colours. In the previous studies, we demonstrated the variability of phthalate content in printed and non-printed portions of the package. The concentration in the printed portion was in most cases higher, probably due to the addition of PAE in printing colours. In the case of migration of the monitored substances into the packaged products, these packages would not be meeting the specific migration limits set by the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact.

Fig. 1 The concentration of DBP and DEHP in samples exceeding the specific migration limit (in mg·kg^{-1})



Legend: DBP = di-n-butyl phtalate, DEHP = di-2-ethylhexyl phtalate.

Foods and raw materials can be contaminated by means of plastic equipment used for the processing or by migration from the packaging material and from the printing ink [7]. Our analysis found elevated concentrations of phthalates in five packages of meat products. Phthalic acid esters are lipophilic organic compounds, with the migration of these from the package to the packed food is influenced by a number of factors, including the type of the polymeric

Table 1 The average concentration of DBP, DEHP and DBP + DEHP in the packages of meat products specified in μg·dm^{-2}

Sample	μg·dm^{-2}			Sample	μg·dm^{-2}		
	DBP	DEHP	DBP + DEHP		DBP	DEHP	DBP + DEHP
1	1.32	5.81	7.13	41	39.52	8.88	48.40
2	3.17	5.80	8.97	42	0.28	11.47	11.76
3	4.27	25.14	29.41	43	22.73	6.24	28.97
4	2.41	1.52	3.93	44	1.54	9.75	11.29
5	7.38	12.36	19.74	45	1.17	14.16	15.34
6	6.31	12.06	18.37	46	3.11	3.59	6.70
7	3.49	14.74	18.24	47	1.12	14.06	15.18
8	0.88	6.78	7.66	48	3.66	2.17	5.83
9	0.99	12.47	13.46	49	0.62	3.79	4.41
10	0.58	10.43	11.01	50	6.88	2.29	9.16
11	0.99	5.95	6.93	51	20.62	6.37	26.99
12	2.69	3.38	6.07	52	0.92	1.84	2.77
13	3.18	2.17	5.35	53	0.36	3.13	3.49
14	0.49	5.36	5.85	54	2.55	2.97	5.52
15	12.59	8.74	21.33	55	0.71	10.52	11.22
16	17.75	2.14	19.89	56	0.22	2.91	3.14
17	25.13	5.43	30.56	57	2.37	6.96	9.33
18	0.72	1.98	2.70	58	0.35	0.84	1.19
19	0.70	1.48	2.18	59	0.19	2.10	2.30
20	0.75	1.51	2.25	60	11.47	4.11	15.58
21	2.67	12.44	15.11	61	0.80	3.37	4.18
22	3.22	10.31	13.52	62	1.81	9.47	11.28
23	0.64	4.26	4.89	63	0.57	2.18	2.75
24	0.83	5.11	5.94	64	0.83	3.80	4.63
25	1.81	3.67	5.48	65	1.35	2.59	3.95
26	0.43	5.57	6.00	66	0.25	2.53	2.78
27	1.83	21.35	23.18	67	0.32	2.72	3.04
28	1.08	13.62	14.70	**68**	**4.35**	**19.10**	**23.45**
29	1.44	16.18	17.62	**69**	**8.26**	**16.79**	**25.05**
30	1.13	22.68	23.81	**70**	**23.95**	**103.33**	**127.28**
31	1.43	0.28	1.70	**71**	**15.09**	**26.54**	**41.63**
32	1.48	0.08	1.56	**72**	**5.26**	**0.30**	**5.56**
33	2.59	0.01	2.60	73	0.33	2.35	2.68
34	1.89	1.04	2.93	74	0.33	4.20	4.53
35	1.25	1.03	2.28	75	15.76	2.07	17.82
36	1.95	11.59	13.54	76	19.69	0.16	19.85
37	0.46	4.85	5.31	77	0.91	0.34	1.26
38	0.60	5.45	6.05	78	0.65	0.14	0.79
39	2.51	24.45	26.96	79	0.31	0.25	0.56
40	2.85	13.71	16.56	80	0.62	17.74	18.36

material, the type of food, temperature, contact duration, etc. Phthalate leaching is enhanced as the fat content in the food increases. Possible migration of DBP and DEHP from the analysed packages is further monitored for a product of the "Gothaer salami" type, depending on the packages used and storage period.

The presence of phthalates in packaging materials and the possible migration of PAE from the package into the food have also been demonstrated by studies of other authors.

Shuangling and Kangquan [8] found that the migration of DEHP from a PVC film into meat increased with increasing temperature and time, with the maximum being reached at 90˚C and 30 minutes of exposure. The aggregate migration limit (60 $mg \cdot kg^{-1}$) was exceeded for all of the time and temperature combinations studied, except that of 10˚C and < 41 hours, where migration was not observed.

Xue et al. [9] analysed 13 printed materials that are in contact with foods. For comparative purposes, four blank samples were analysed that were not printed. The results show an elevated content of phthalates in printed materials compared with those without printing. For this reason, eight types of colours used for printing of packages were tested to demonstrate that they were the main source of contamination. It was confirmed that printing colours are the main source of the examined substances that could cause a risk to food safety.

Guo et al. [10] studied the migration of DEHP from a packaging film into ham sausages that were relatively low in fat. The content of DEHP in the products was considerably decreasing with increasing distance from the surface. The concentration of DEHP in the packaging film was 8.7 $mg \cdot g^{-1}$, while that in the first outer layer of the product was reaching 206.5 $ng \cdot g^{-1}$. Layer 1 and layer 2 contained about 90% of the total amount of DEHP that had migrated from the packaging material. A significant level of DEHP in the inner layers of sausages was seen only after six months of storage.

Human exposure to phthalates can occur orally (from food/water), by inhalation (from air), by dermal absorption or parenteral application [11]. Due to their lipophilic nature, phthalates may also accumulate in animal tissues, muscle and fat by being transferred from feedstuffs and the environment, which leads to further potential threat of the chain, thus humans [12].

Conclusion

Currently, there is a lack of information about contamination of food by phthalates and their content in food packaging materials. Due to the presence of phthalates in the environment and in the food chain and their negative impact on human health, it is desirable to accept all of the necessary legislative measures to reduce migration of PAE into the environment and foodstuffs.

One of the ways of the progressive reduction of risks of phthalates is to promote the substitution of toxic phthalates by other health-safe substances, e.g. citrates, phenol alkylsulphonate, benzoates, particularly in the production of materials used in agriculture, food and health care industries.

The found concentrations of PAE in the packages of meat products are in compliance with the limit of the aggregate migration per unit of area, thus do not exceed 10 $mg \cdot dm^{-2}$ of the surface of the material or product. The limit, however, includes other phthalates and many other substances which are able to release from the material and migrate into the food. The highest DBP + DEHP concentration found in this study was 127.28 $\mu g \cdot dm^{-2}$. When converting the contents of DBP and DEHP per $mg \cdot kg^{-1}$, five packages would be exceeding the specific migration limit in the event of PAE migration into the packed food, one set by the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact. This analysis is the subject of another study.

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Antimicrobial Activity of Aqueous Herbal Extracts

LENKA DOSTALOVA, LENKA DETVANOVA, LIBOR KALHOTKA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

lenka.dostalova@mendelu.cz

Abstract: Some plants contain various compounds, which can influence microbial growth, reproduction or basic cell functions. Phenols, polyphenols, micronutrients, essential oils and other compounds belong to them. These compounds are primarily present in various herbs. Extracts from these plants could be used as natural food preservatives, which could inhibit growth of undesirable microorganisms. In this work, antimicrobial activity of aqueous extracts of *Thymus vulgaris*, *Lavandula angustifolia*, *Melissa officinalis*, O*cimum basilicum*, *Allium schoenoprasum* and *Petroselinum crispum* was tested on five gram negative bacteria – *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Hafnia alvei* and *Raoultella terrigena*, which were isolated from dairy products except *Escherichia coli* (CCM 7929). Antimicrobial activity was tested by disc diffusion method. It was found out the less effective extracts were from thyme and lavender. Basil, chive and parsley extracts showed higher potential to inhibit bacterial growth.

Key-Words: antimicrobial activity, herbal aqueous extracts, gram negative bacteria

Introduction

Natural botanical sources contain a diverse array of compounds such as phenolic acids, flavonoids, tannins, vitamins and terpenoids that account for their biological properties. It was suggested that the antibacterial activity of a total of 46 extracts from spices and herbs was closely associated with the presence of their phenolic constituents. The authors have reported that all the tested spices have a strong antibacterial effect against *B. cereus*, *L. monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella Anatum* [1]. Microbial cells are negatively affected by plant-derived substances via various mechanisms of actions that attack the phospholipid bilayer of the cell membrane and disrupt enzyme systems [2]. They have an influence on enzyme activity associated with energy production or they can cause denaturation of proteins, modify cell wall permeability, causing the loss of macromolecules [3]. Therefore, it is difficult for microorganisms to create resistance against these [4, 5]. Herbal extracts are used as natural preservatives for prolonging shelf-life of food and maintaining of food safety [6, 7]. Antimicrobial activity depends on herb species, concentration of active substances, species and concentration of microorganisms, substrate composition and conditions of treatment and storage of food [8, 9].

Material and Methods

In this work, antimicrobial activity of herbal aqueous extracts was tested by disc diffusion method on gram negative bacteria. *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Hafnia alvei* and *Raoultella terrigena* were isolated from dairy products by a sequence of cross smears. *Escherichia coli* was used as pure culture from Czech Collection of Microorganisms (CCM 7929). Suspensions of density 0.1 and 1 McF were prepared from 24 hours culture of each bacterium. Herbal aqueous extracts were prepared from fresh *Thymus vulgaris, Lavandula angustifolia*, *Melissa officinalis*, O*cimum basilicum*, *Allium schoenoprasum* and *Petroselinum crispum* in two different concentrations – 1:5 (1 part of herbs, 5 parts of distilled water) and 1:10 (1 part of herbs, 10 parts of distilled water). Herbs were weighed out into distilled water and leaved to infuse for one hour at 95°C. Suspension of bacterium was inoculated on Petri dishes with nutrient medium (Plate Count Agar with Skimmed Milk, Biokar diagnostics, France). Sterile paper discs of diameter 9 mm were impregnated with 30 µl of aqueous extract and placed onto nutrient medium with inoculated bacterium. On each Petri dish, 3 discs were placed. All variants with bacteria and extracts were performed in duplicate. Prepared Petri dishes were placed in thermostat at 37°C. After 24 hours cultivation, zones of inhibition were evaluated by a ruler.

Results and Discussion

Diameters of zones of inhibition are stated in the Table 1 and Table 2. Extracts did not show any antimicrobial activity since the diameter was 9.00 mm. It is the diameter of paper disc. In the Table 1, there are stated results of testing antimicrobial activity of basil, chive and parsley, in the Table 2, there are shown inhibitory zones resulting from testing of thyme, lavender and lemon balm.

Table 1 Diameters of inhibitory zones – basil, chive and parsley

MO	Concentration of MO [McF]	Diameters of inhibitory zones [mm]					
		Basil		Chive		Parsley	
		1:10	1:5	1:10	1:5	1:10	1:5
***E. coli* CCM 7929**	**1**	10.00	9.33	9.00	9.00	9.17	10.00
	0.1	9.00	9.67	9.50	9.67	9.33	9.00
Hafnia alvei	**1**	9.67	10.17	10.17	10.20	10.50	11.80
	0.1	9.17	9.00	10.17	10.20	9.00	9.50
Raoultella terrigena	**1**	10.00	10.17	9.83	10.00	10.00	9.83
	0.1	9.00	9.00	9.33	9.67	9.00	9.00
Klebsiella pneumoniae	**1**	9.17	9.67	9.00	9.00	9.17	9.00
	0.1	9.00	9.00	9.00	9.00	9.00	9.00
Klebsiella oxytoca	**1**	10.20	10.20	9.33	9.67	10.33	10.83
	0.1	9.50	9.00	9.17	9.50	10.00	9.00

MO – Microorganism
McF – McFarland Units

Parsley showed relatively strong antimicrobial activity. Basil and chive showed some antimicrobial activity as well. In some cases, the less concentrated extract was more effective. *Klebsiella pneumoniae* was less susceptible to effect of these extracts.

Table 2 Diameters of inhibitory zones – thyme, lavender and lemon balm

MO	Concentration of MO [McF]	Diameters of inhibitory zones [mm]					
		Thyme		Lavender		Lemon balm	
		1:10	1:5	1:10	1:5	1:10	1:5
***E. coli* CCM 7929**	**1**	9.17	9.83	9.83	9.17	9.00	9.33
	0.1	9.17	9.00	9.00	9.00	9.67	9.67
Hafnia alvei	**1**	9.00	9.00	9.00	9.00	9.00	9.00
	0.1	9.17	9.00	9.00	9.00	9.00	9.00
Raoultella terrigena	**1**	9.00	9.17	9.00	9.17	9.00	9.00
	0.1	9.00	9.17	9.00	9.00	9.00	9.33
Klebsiella pneumoniae	**1**	9.00	9.00	9.00	9.17	9.33	9.50
	0.1	9.00	9.00	9.00	9.00	9.33	9.00
Klebsiella oxytoca	**1**	9.00	9.33	9.00	9.00	9.17	10.33
	0.1	9.00	9.00	9.00	9.17	9.00	9.00

These three herbs did not show any significant antimicrobial activity. Lemon balm was the most effective. Aqueous extracts from thyme and lavender influenced growth of microbes a little.

Parsley extract proved to be the most effective. It inhibited all of bacteria; most susceptible were *Hafnia alvei* and *Klebsiella oxytoca*. On the other side, *Raoultella terrigena* and *Klebsiella pneumoniae* were the least susceptible. Basil inhibited all of bacteria also; chive did not suppress growth of *Klebsiella pneumoniae*. *K. pneumoniae* was very resistant to impact of all tested extracts. Gyawali et Ibrahim [10] present that crude chive

extract containing sulfur compounds can be effective against the growth of *Salmonella* and could be used in food products to prevent the growth of this pathogen. The antimicrobial effect of chive against *E. coli* and yeast (*Pichia membranaefaciens* CCRC 20859) has been also reported [9]. Wahba et al. [11] proved antibacterial activity of different types of pepper, parsley and dill against natural microflora, coliforms, yeasts and moulds and *S. aureus* in Kareish cheese. The most susceptible bacterium to thyme was *E. coli*, contrarily growth of *Klebsiella pneumoniae* was not suppressed; *Hafnia alvei*, *Raoultella terrigena* and *Klebsiella oxytoca* were inhibited only by one concentration of thyme extract. Oregano and thyme possess significant in vitro colicidal and colistatic properties, and this bactericidal concentration irreversibly damaged *E. coli* O157:H7 cells within 1 minute [12]. Lavender and lemon balm did not suppress the growth of *Hafnia alvei*; remaining bacteria were inhibited only in limited way at least by one concentration of extracts. Jianu et al. [13] tested antibacterial properties of lavender, which showed significant bactericidal effects against microorganisms such as *Shigella flexneri*, *Staphylococcus aureus* and *E. coli*.

Conclusion

Some herbs tested in this work could be used as natural preservatives for prolonging shelf-life of food. Some of them showed moderate antimicrobial effect, thus they rather could be used in mixture with other herbs. Susceptibility of individual bacteria to extracts was very dissimilar. *H. alvei*, *R. terrigena* and *E. coli* 7929 were the most susceptible in the most of cases. It is important to research antimicrobial effects of herbs and other plants in many various forms, like different extracts or essential oils, as a natural source of antimicrobial compounds.

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The effect of late application of foliar nutrition and supporting materials on the yield and the quality of barley grain

YVONA DOSTALOVA, LUDEK HRIVNA
Department of Food Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

yvona.dostalova@mendelu.cz

Abstract: Within the small plot field trials, the effect was tested in 2014 of late application of K-gel, Yara Vita Kombiphos and Sunagreen on the yield and the quality of Bojos, the spring barley variety. Barley was grown at two levels of N-nutrition N1 = 84 and N2 = 104 kg N per hectare. The highest grain yield (9.268 t per hectare) was obtained after application of K-gel, the largest grain and one of the high mass density was harvested after fertilisation with Yara Vita Kombiphos at N1 nutrition and the highest starch content was supported at both of the N-nutrition levels by the application of Sunagreen (66.14%/65.93%). The higher dose of N generally decreased the yield and even worsened the grain quality.

Key-Words: barley, yield, foliar nutrition, Bojos, grain quality

Introduction

Malting barley is a crop that has high economic risk for farmers because of its high sensitivity to external growth factors. These are responsible for two of the physiological properties - the growth rate and the capability of nitrogen management [1]. In cereals, nitrogen is a key element for achieving consistently high yields and is involved in all the metabolic processes of plants. Since the nitrogen uptake rate and distribution in plants is largely determined by the needs and requirements of different stages of plant growth [2], nitrogen-based fertilisers are applied in a way to be able to meet the plant nitrogen requirements throughout the growing season, thereby maintaining the high yield while not worsening malting quality of the grain [3]. Uneven application of nitrogen causes stand irregularity during harvesting, increasing the share of green grain and pass-through rate [4]. Consequently, lack of nitrogen from the beginning of vegetation has the effect of reduced formation of structural and functional proteins, which negatively reflects in the growth and formation of all principal plant organs (roots, stalks and leaves) [5]. Achieving adequate grain yield in the desired malting quality often presents considerable challenges. A yield is dependent on the production potential and the capability of accumulation of assimilates in interaction with soil and climatic conditions [6]. Phosphorus and potassium play an important part in the energy metabolism of plants. They are important particularly for the young plants that need adequate amounts of the elements for offshoots and spikelets to develop [7], as well as in the second half of vegetation where they are involved in the grain-forming process. To achieve high yield and quality of spring barley, growth stimulants are used as auxiliary agents. They can reduce the negative effect of stressful periods during the growing season [8]. Of these, Sunagreen seems to be the most appropriate product [9]. It has gained popularity in recent years, particularly for its capability of stand balancing in a highly efficient manner and strengthening weaker fertile offshoots of barley, accompanied with effective but sensitive selection of weed offshoots. Applications of auxin stimulators are equal competitors, particularly in spring barley, with greater sensitivity to the crop, chiefly at higher temperatures at the time of application. During tillering, Sunagreen has a positive effect on the crude protein content of the grain [10]. The study aimed at identifying the effects of application of nitrogen, phosphorus and potassium foliar nutrition, and application of Sunagreen on the yield and quality of barley grains.

Materials and methods

The experiment was set up on the land owned by AGROSPOL Velká Bystřice Ltd. The Bojos variety of spring barley was sown on 12 March 2014 on plot size of 20.6 m^2, the seed rate being 3.7 millions of germinating seeds. Nitrogen was applied as part of pre-sowing treatment, the dosage being 54 kg of N

per ha (CAN, 27-27% of N). On 5 May 2014, when the stand was at the early stage of stem elongation (BBCH 30), there was the second application of N fertilisers. Amounts of N applied per hectare as urea involved 30 kg as part of N1 and 50 kg as part of N2.

On 21 June 2014, at the stage of late flowering, there was the application of products as indicated in Table 1, with each variant repeated four times. The products comprised K-gel, a foliar fertiliser to support the processes of photosynthesis, particularly in the late vegetation stages, with a prolonged effect due to the gelling component; Yara Vita Kombiphos, a foliar fertiliser formulated for complementary phosphorus, potassium and magnesium nutrition; and Sunagreen, an agent stimulating the growth and development of plants and used to optimise the productive offshoots of cereals. The aim was to verify whether or not the products listed above influence the development, yield and the quality of grain.

Table 1 Products applied as part of fertilising operations N1 and N2: application rates

N batch	Variant	Application rate
N1	Control	0
N1	K-gel	3 l per ha
N1	Yara Vita Kombiphos	3 l per ha
N1	Sunagreen	0.5 l per ha
N2	Control	0
N2	K-gel	3 l per ha
N2	Yara Vita Kombiphos	3 l per ha
N2	Sunagreen	0.5 l per ha

Note: Water supplied for spraying: 300 litres per hectare

Harvest as part of the trial was underway on 3 August 2014 using Wintersteiger - a small-scale plot harvester with an automatic scaling and sampling device. Grain yield was determined for each variant; in addition, grain sample analysis was carried out and involved identifying mass density (grain meter), the starch content (by Ewers), nitrogen substances (by Kjeldahl) and grain size fractions (Steinecker sifter).

The measured results were analysed statistically using STATISTICA 10 based on 2-factor analysis of variance.

Results and discussion

The effect of nitrogen batch considerably reflected in the barley grain yield (Table 2). The lower, all N1 variants average nitrogen dosage contributed to the increased grain yield (8.941 tonnes per hectare). Here, the yield was on average 0.591 tonnes per hectare higher than with the N2 variants, the relatively high level of lodging seen with the variants fertilised more intensely with nitrogen playing also its part. Should the N2 variant vegetation have been successfully retained in a non-lodged condition, the differences could well have been reverse. The higher grain yield seen in the N1 variants did not contribute to the reduced content of N-substances, which was on average higher in this case (12.33%) than with the N2 variants. The grain nitrogen level was not satisfactory in all the trial variants since currently, 10.8% is considered to be the optimal content of N-substances, while to ensure the production of quality malts, the limit of 12% should not be exceeded for barley. If the level is higher, the technological processes need to be modified by increasing the water content in steeping or germination time is extended [11]. The lower nitrogen supply and smaller level of lodging were favourable manifest in the grain formation and the final size. The N1 variants average grain above the 2.8 mm screen was c. 15% higher than with N2 application variants. Contrary to this, the percentage of grain above the 2.5 mm screen was higher with N2 variants, with however the level being only 9.63%, which was reflected in a higher pass-through rate, i.e. the quantity of grain useless for malting increased. Some authors [12] report that large grains generally provide higher starch content and lower protein content. For both of the N fertilisation levels, the starch content was balanced, with that of N1 being about 1% higher. Likewise, nitrogen dosage had not a significant influence on the difference in mass density. Nonetheless, it can be concluded that the lower nitrogen dosage not only promoted the increased yield, but also enhanced the grain technological quality.

Table 2 Nitrogen dosage effect on the technological parameters

Parameter	N1	N2
Yield (tonnes per ha)	8.94	8.35
N-substances (%)	12.33	12.10
2.8 mm	73.25	57.95
2.5 mm	2.,47	31.10
Pass-through rate %	5.11	10.91
Starch (%)	65.98	64.82
Mass density (kg per hl)	68.74	67.52

The yield and grain quality was influenced by not only nitrogen supply, but also the application of the tested products. The starch content is an important parameter, which is closely related to grain extract. The more starch in the grain, the better the beer production economy. The highest starch level was recorded after applying Sunagreen, within both of the N fertilisation level, confirming that the product has a positive effect on the deposition of storage

substances in the grain (Fig. 1). Conversely, K-gel contributed to increased yield, particularly for the fertilisation intensity of N1 (9.268 tonnes per ha). This is in line with the findings of [13] who report increased yield of barley as a result of K-gel application as well (Fig. 2).

Fig. 1 The influence of N supply and fertilisation variant on starch content

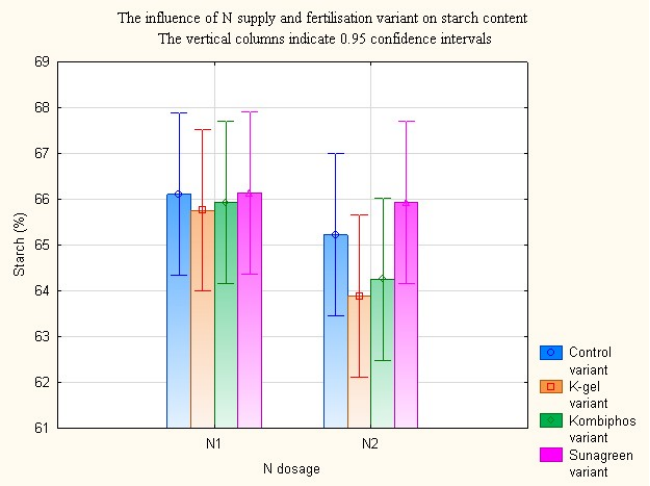
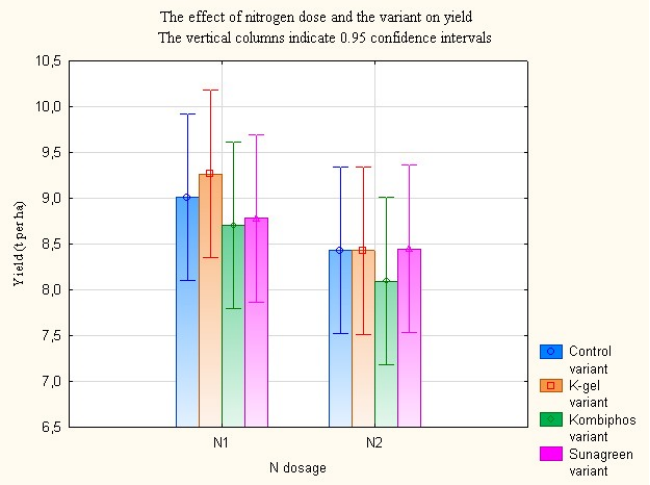
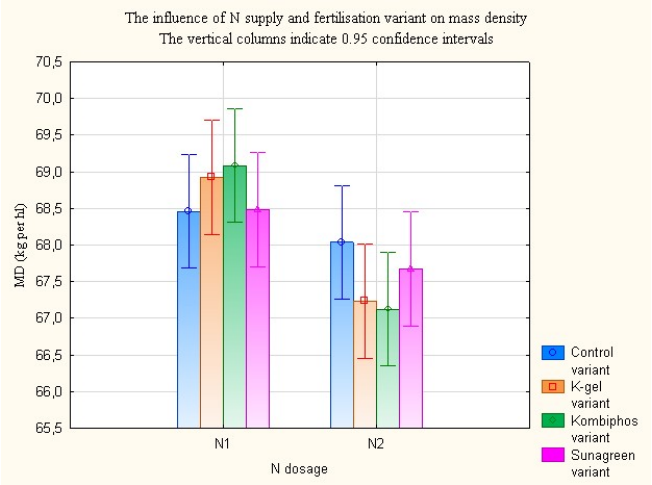


Fig. 2 The effect of nitrogen dose and the variant on yield



As is evident from Fig. 3, the lower nitrogen supply has a favourable effect on the grain mass density, with the best results recorded following the application of Yara Vita Kombiphos (69.09 kg per hl) and K-gel (68.93 kg per hl). The higher nitrogen fertilisation intensity along with lodging did not enable the products to show a positive effect. As a result, the highest levels were observed with the control variant. Mass density, as reported by [11], is directly linked to the malt extractability. From this aspect, the results found for nitrogen fertilisation intensity of N1 can be considered valuable.

Fig. 3 The influence of N supply and fertilisation variant on mass density



In terms of production and malting process, the percentage of grain that can be processed by malting industry out of the total yield, i.e. Σ 2.8 mm + 2.5 mm, is an important factor. The percentage of grain above the 2.8 mm/2.5 mm screen characterises the regularity and fullness of grain in the barley part since only size-uniform and regular grain of the given variety accepts water evenly in steeping, germinates uniformly and achieves the desired degree of cracking [11]. The lower nitrogen supply did increase the percentage of grain above the 2.8 mm screen after the application of each of the products, with however greatest increase being observed in Yara Vita Kombiphos (74.84%) and K-gel (74.21%). As with the evaluation of mass density, no effects were seen with the application of products at higher nitrogen fertilisation intensity (Fig. 4 and 5).

Fig. 4 The effect of N supply and variants on grain above the 2.8 mm screen

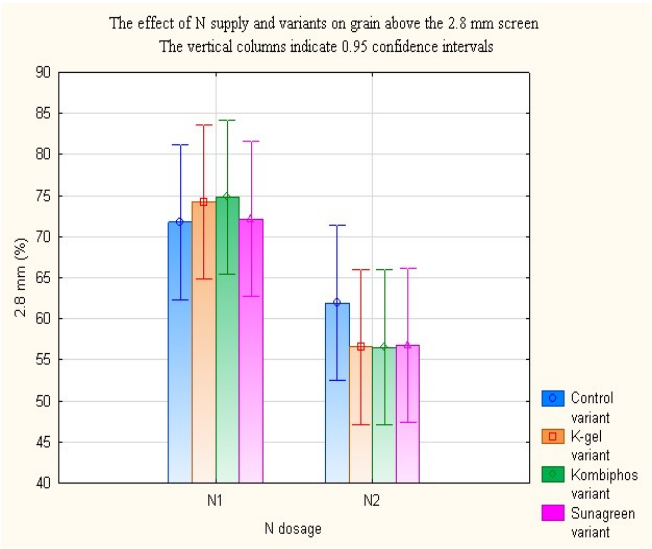
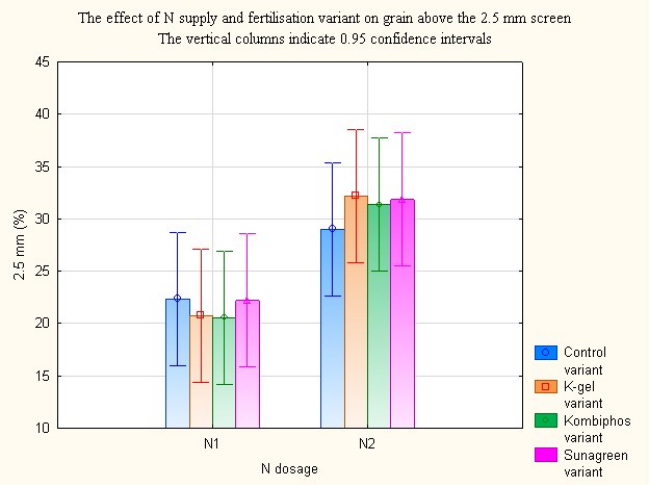
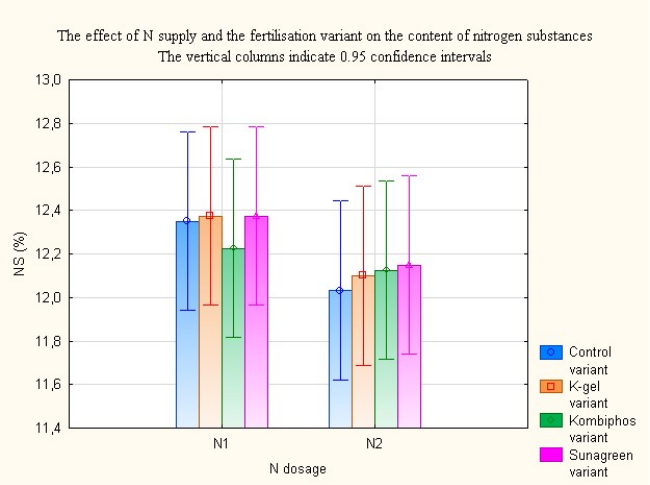


Fig. 5 The effect of N supply and fertilisation variant on grain above the 2.5 mm screen



As already mentioned, the lower nitrogen supply contributed to the higher level of N-substances in barley grain as a result of non-lodging vegetation. Here, the application of Yara Vita Kombiphos had the greatest effect – one that contributed to a decreased level of N-substances unlike with other variants (Fig. 6).

Fig. 6 The effect of N supply and the fertilisation variant on the content of nitrogen substances



Conclusion

As it results from the outcome of the single-year small-plot field trial, the nitrogen dosage plays a very important part in barley grain yield and quality, with the condition in which the vegetation is maintained until harvest being a critical factor. If lodging occurs, then there is a considerable decrease in the grain yield and quality. One should also take into account the fact that application of products beyond the framework of routine nutrition interventions will not pay off. If, however, a good condition of the crop is ensured until harvested, then the application can reflect to a considerable extent, which was reconfirmed in this study. The largest yield was seen after application of K-gel. In the event of Yara Vita Kombiphos, grain of the largest size and with the best mass density was harvested at the level of N1 nutrition. For Sunagreen, it increased the starch content at both of the N nutrition levels. A conclusion is thus possible that lower nitrogen doses stimulate the effects of products in the event of application of K-gel, Yara Vita Kombiphos and Sunagreen, thus providing better technological parameters, while higher doses of nitrogen inhibit the effect of these compounds.

Acknowledgment

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Possibilities of using purple wheat in producing bakery products

MARIE JANECKOVA[1], LUDEK HRIVNA[1], MIROSLAV JUZL[1], SARKA NEDOMOVA[1], TOMAS VYHNANEK[2], VACLAV TROJAN[2], EVA MRKVICOVA[3]
[1]Department of Food Technology
[2]Department of Plant Biology
[3]Department of Animal Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xjaneck5@mendelu.cz

Abstract: The aim of the study was to assess the possibilities of using grain mill products (flour, semolina and bran) obtained by milling purple grain wheat (variety Konini) in bakery products. The attention was focused on the use of bran with high levels of anthocyanins characterised by high antioxidant capacity. Mixtures were prepared with the mixture of bran making 10 – 30% of the total quantity of mill products. The addition of bran particles caused a negative impact on some sensory properties such as curvature of bread, crumb texture or taste. The product crust colour was significantly influenced as determined using the Konica Minolta Spectrophotometer CM-3500D unit. After adding bran, changes in texture were not only visual but also reflected in the physical analysis made using TIRAtest. It was shown that the addition of bran should not exceed more than 10% of the total mixture.

Key-Words: - wheat, purple pericarp, anthocyanins, bran, baking quality

Introduction

Currently gaining importance are the genotypes of wheat with caryopses of different colours, such as purple, blue, yellow and white. The colouring is due to the presence of colour pigments included in xanthophylls, carotenoids, anthocyans and anthocyanins. As regards the purple-coloured grain, this is mainly due to anthocyans. Found chiefly as cyanidin-3-glucoside and peonidin-3-glucoside (Knievel *et al* 2009), the substances possess a high antioxidant effect. In addition to preventing oxidative damage, they are capable of binding heavy metals (zinc, iron and copper) and acting pre-emptively against cardiovascular disease, cancer, rheumatoid arthritis, neurodegenerative disease, and diabetes mellitus, type 2 (Lutsey *et al* 2007, Fang *et al* 2002). With the content and the effect of the substances, coloured wheat grains find applications in the production of functional foods. In purple-coloured caryopses, pigments are stored in the pericarp. Due to the location of the colour pigment in the caryopsis, the addition of bran into the dough is necessary to increase the content of anthocyanins in the product which however may lead to a negative impact on certain properties of the bakery product. Other health-beneficial substances contained in purple wheat include phenolic compounds. These are usually found in the wheat grain as ferulic acid and vanillic acid. Found in wheat bran is also *p*-coumaric acid along with other free phenolic acids such as caffeic acid, chlorogenic acid, gentisic acid, syringic acid and *p*-hydroxybenzoic acid (Liu, 2007). Kequan *et al* (2005) indicate that purple wheat contains higher levels of ferulic acid in comparison with other types. Liu *et al* (2010) report a higher content of sinapinic acid. The content of phenolic acids is influenced by genetics, environmental factors and other stressors (Mpofu *et al* 2006). This study aimed at assessing the potential of mill products obtained by processing purple wheat grain (the Konini variety) for the production of bakery products.

Material and Methods

The evaluation was based on Konini, the variety of purple wheat grown in Brno-Chrlice and harvested in 2013. Konini has a purple pericarp and is a winter variety. The grains were ground using Chopin, the laboratory mill. A baking experiment was made (Rapid mix test; Tab. 1). The trial used various proportions of milling fractions sourced from the milled purple wheat grains (Tab. 2). The variant 3 differed from variant 4 in that the bran employed

for variant 3 was pulverised before use. In all the variants, bran was soaked the day before. Afterwards, it was sorted out via a sifter and excess water removed by squeezing. The water absorbed by the bran was deducted from that specified in the formulation.

Table 1 Baking experiment formulation

Wheat flour	500 g
Salt	7.5 g
Sugar	5 g
Yeast	25 g
Oil	5 g
Water	300 ml

The dough was prepared from all ingredients using a straight dough process, kneaded for about 1 minute using a mixer and allowed to rise in a proofer for 20 minutes at 32 ± 1 °C and humidity of 80 % ± 5 %. After removal from the proofer, the dough was allowed to rest for 10 minutes and then it was weighed down. Loaves were shaped, the weight of each being 80 g, and allowed to rise once again at 32 ± 1 °C and the humidity of 80 % ± 5 %, which this time took 25 minutes. Before loading into the oven, the loaves were moistened with water and then baked at 230 - 240 °C. At the beginning of the baking process, the oven was steamed with 50 ml of water. The baking time was 20 minutes.

Table 2. Trial variants

Variant	% bran	% semolina	% flour
1	0	0	100
2	10	10	80
3	20	10	70
4	20	10	70
5	30	10	60

Subsequently, the baked products were sensorially evaluated by experienced specialists (n = 10). The sensory analysis involved evaluating the following descriptors: volume, shape, colour, integrity, gloss, porosity, aroma and taste. The sensory evaluation of the baking trial was making use of unstructured graphic scales. The bread colour was measured using Konica Minolta Spectrophotometer CM-3500D. Modes chosen for the colorimetric determination of colour within the baking experiment were as follows: reflectance, d/8 geometry (*the instrument measures the reflected light at an angle of 8°*), SCE (specular component excluded - eliminating gloss), D 65 (illumination mode; 6.500 Kelvin), 30 mm slit. Statistical evaluation of colour was done by means of the UNISTAT 5.1 software and used analysis of variance (ANOVA) followed by testing for the significance level of $P < 0.05$ (Tukey test). Evaluation of ΔE^*_{ab} (the rate of the size of the colour difference; CIE 1976) was done using MS Excel 2010 under the equations listed below; the total difference in colour was commented by Třešňák (1999).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

$$\Delta L^* = L^*_{sample} - L^*_{\mathrm{mod}\,el}$$

$$\Delta a^* = a^*_{sample} - a^*_{\mathrm{mod}\,el}$$

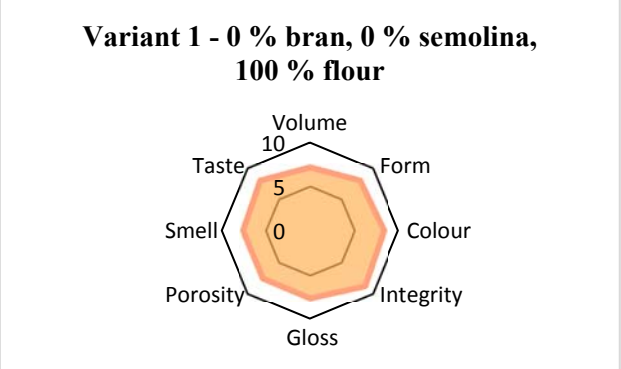
$$\Delta b^* = b^*_{sample} - b^*_{\mathrm{mod}\,el}$$

A penetration test was done using the TIRAtest 27025 device. The penetration test used a probe was with a diameter of 3 mm, and a force sensor of 200 N. The test speed v_1 corresponded to 50 mm·min^{-1} and the pathway was 10 mm. A record was obtained of the force required to push the plunger to the desired depth of the baked product.

Results and Discussion

Variant 1 was used as a control for this baking experiment. The baking products possessed a large volume and regular shape. The crust was integral and of typically colour (golden-brown). The glaziness of the surface was rather low. The crumb had uniform, thin-walled pores. The aroma and taste was indicated as being characteristic, pleasant (Fig. 1).

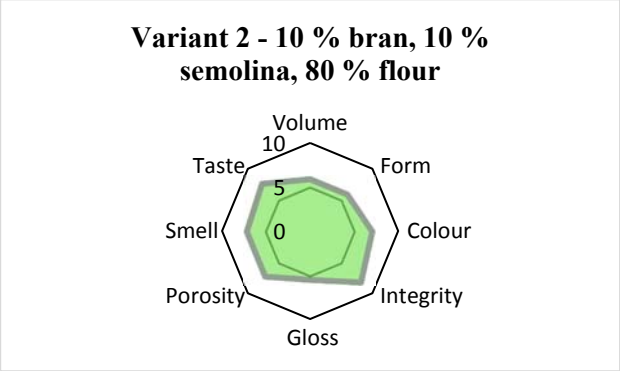
Fig. 1. Variant 1 of the baking experiment (bran 0 %, semolina 0 %, flour 100 %)



Variant 2 (Fig. 2) showed a slight decrease in the volume and a change in the shape against the control; the shape turned from regular, arched to slightly arched. Despite the above, these parameters were evaluated as the most acceptable of all the variants

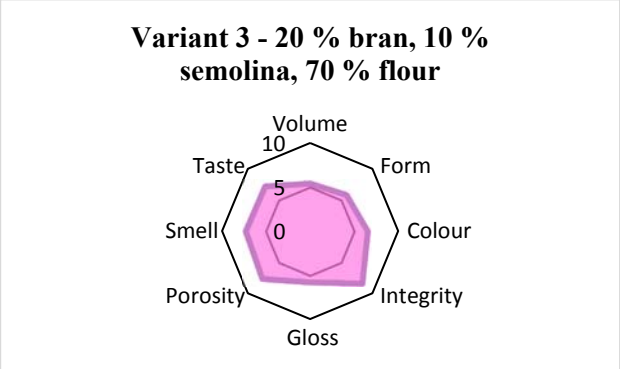
where bran was added. The same applied to the evaluation of the colour and taste. The crust was integral and rather dull. The crumb porosity was comparable to the control.

Fig. 2 Variant 2 of the baking experiment (bran 10%, semolina 10%, flour 80%)



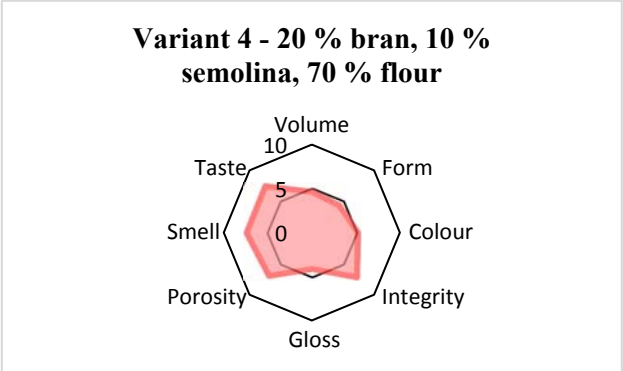
For baking products which contained 20 % of fine-crushed bran in their formulations, the crust integrity and crumb porosity were the properties evaluated the best of all experiment variants. The volume was less than with the control and variant 2, the shape was less arched. The aroma and colour of the products were comparable to variant 2. The taste was perceived by the evaluators as the second worst of all the presented samples (Fig. 3).

Fig. 3 Variant 3 of the baking experiment (fine-crushed bran 20%, semolina 10%, flour 70%)



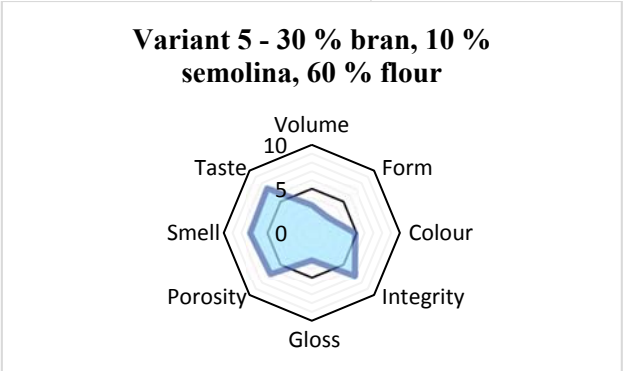
For variant 4 (Fig. 4), the same amount of bran was added as for variant 3. The exception was that the bran was not modified any further, the bran treatment before use causing the evaluators to have different perception of sensory indicators. Compared to the previous variant, the product height was low with less volume. The larger particle size of bran disrupts the gluten structure to the more extent, consequently reducing the product volume (Lai *et. al*, 1989). Sensory descriptors such as colour, integrity, product gloss and crumb porosity influenced the evaluators better in the previous variant. In contrast, the aroma and the taste were sweeter, cleaner and more distinctive.

Fig. 4 Variant 4 of the baking experiment (bran 20%, semolina 10%, flour 70%)



The last variant contained the highest proportion of purple wheat bran of all the test variants. All the descriptors reached the lowest values of total sensory evaluation, with the volume, shape, gloss and product colour showing the greatest difference of the evaluated parameters compared with the control (Fig. 5).

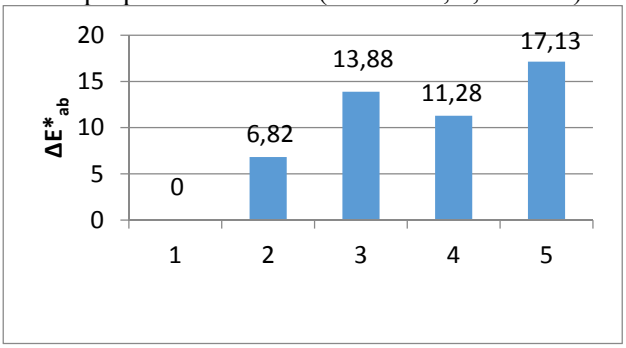
Fig. 5 Variant 5 of the baking experiment (bran 30%, semolina 10%, flour 60%)



To objectively assess colour, analysis was done using the Konica Minolta spectrophotometer, with a discordant or very prominent difference found among the samples compared with the control (Tab. 3). Fig. 6 shows that there was a very prominent difference between the control and variants 3 and 5 with these two variants being significantly darker than the control, which for variant 5 was due to the largest addition of bran into the dough, while bran treatment before use was involved with variant 3. Even though variants 3 and 4 contained the same proportion of bran, for variant 4 the colour difference against the control was not so great in comparison with variant 3 (ΔE^*_{ab}= 11.3). The difference ΔE^*_{ab} between variant 3 and variant 4 was 4.2, which indicates a medium degree of colour variation. The

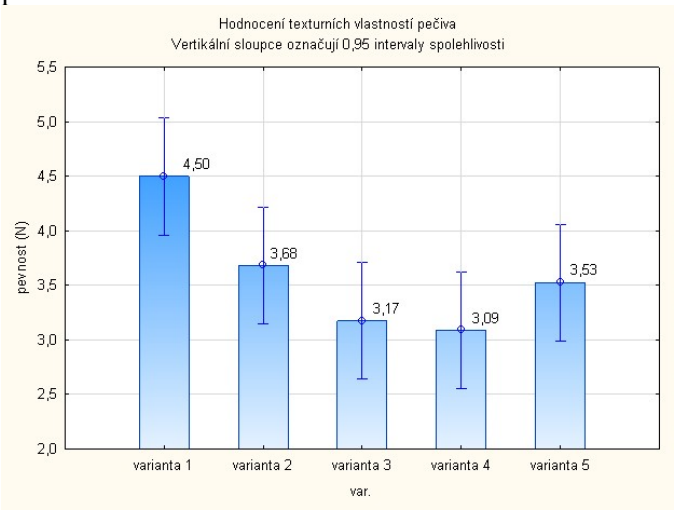
smallest, but moderately discordant difference was found between the control and variant 2 (ΔE^*_{ab}= 6.8).

Fig. 6. The rate of the extent of the colour difference between the control (variant 1) and samples with varied proportions of bran (variants 2, 3, 4 and 5)



The TIRAtest 27025 unit was used to determine the force required to push the plunger to the desired depth of the baking product. As can be seen on Fig. 7, the greatest force required to break the crust was necessary to exert for variant 1 (control; 4.5 N). As the bran addition into the products increased, the firmness decreased. The increasing proportion of bran reduced that of flour in the dough, thus the quantity of gluten that forms the skeleton of the baking product. Bran disrupts the gluten structure. Glutenins, as components of gluten, are substances that provide strength and stability to the dough (Příhoda *et al* 2003; Noort *et. al* 2010). The lowest strength was measured for variant 4 (3.09 N). For variant 5, where the measured strength was 3.53 N, it is possible that there was an error in measurements.

Fig. 7 Evaluation of textural properties of baking products



Tab. 3. The evaluation of colour differences per baking experiment depending on the formulation

	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5
L* (D65)	58.86 ± 1.37^a	55.25 ± 0.82^b	51.11 ± 0.33^c	50.73 ± 1.36^c	48.69 ± 0.21^d
a* (D65)	17.39 ± 0.12^a	14.03 ± 0.34^c	13.07 ± 0.74^c	16.01 ± 0.30^b	13.24 ± 0.21^c
b* (D65)	38.69 ± 1.23^a	33.98 ± 1.46^b	28.01 ± 0.74^c	30.99 ± 0.86^c	25.54 ± 0.27^d
ΔE^*_{ab}	-	6.8*	13.9**	11.3**	17.1***

$^{a, b, c, d}$ *- The letters with different indices in the rows denote statistically significant differences (P < 0.05)*

** - Indicates an already moderately discordant difference compared with the control*

*** - Indicates a very prominent difference compared with the control*

**** - Indicates a disturbing difference compared with the control*

Conclusion

Based on the results of the sensory analysis and the strength test it can be argued that the bran addition into the dough should not exceed 10 % of the total mill fractions in the bakery production making use of purple wheat. Products made of purple wheat are a source of health-beneficial compounds. Quantifying and evaluating the effect of these after baking should be the subject of further studies.

Acknowledgment

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The effect of storage temperature and production method of chocolate confectionery on changes in its quality

LENKA MACHALKOVA, LUDEK HRIVNA, SARKA NEDOMOVA, MIROSLAV JUZL
Department of Food Technology
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

lenka.machalkova@mendelu.cz

Abstract: The aim of the study was to assess the dynamics of changes in the quality of certain types of chocolate confectionary over the storage period, the main attention being paid to the formation and development of fat bloom in chocolate products, whether or not treated by retempering. Six products were chosen from the product range of and in cooperation with Zora Olomouc, a chocolate factory controlled by Nestlé Czech Ltd. The samples were stored at four types of temperature modes: 6°C, 12°C, 20°C and 30°C, and periodically sampled for analysis. Each sampling was followed by sensory assessment by trained assessment specialists, identification of changes in colour using Konica Minolta Spectrophotometer CM-3500d and physical analysis of the product texture using the TIRAtest unit. The results showed a significant effect of the storage temperature on the intensity of changes. There was a product-specific intensity of positive effects of retempering observed in the products.

Key-Words: chocolate, storage temperature, fat bloom, sensory assessment, texture, colour

Introduction

The taste and aroma of chocolate should be pleasant, aromatic and should resemble the raw materials used. The surface of the chocolate should be glossy; there should be no stains or coatings. Failure to follow production practices, improper storage or transportation may cause defects of chocolate.

Typically, there are two basic types of defects - fat bloom and sugar bloom, with the material losing its sheen and is covered with a fine whitish layer [1]. Since the fat bloom is a much more feared phenomenon, highly undesirable in the chocolate industry, its prevention is the subject of increased attention within the industry [2]. The bloom looks similar to the blooms of certain fruit species such as plum or common grapevine; it is of a greasy nature when touched [3]. The bloom usually covers the entire surface, thus making the product unacceptable for sale or consumption. Although grey chocolate does not constitute a threat to the public or consumer health, the process converts the product to being unattractive and therefore inedible [1].

Causes for the fat bloom to occur can be technological – poor chocolate tempering, incorrect cooling methods, the presence of soft fat in the fillings of chocolate products or addition of fats incompatible with cocoa butter; the bloom may also occur through improper storage at higher or fluctuating temperatures [3]. Currently, essential for chocolate to be of high quality are particularly a crystalline form of cocoa butter and the percentage of solid fat during production [1]. Cocoa butter exhibits polymorphism, which involves the capability of crystallising (conditions-dependent) to create several crystalline forms based on the temperature and the cooling rate of the melt. Of these, the lipid crystalline form V (β_2) is desirable for the production and prevails in a well-tempered chocolate [4, 5]. Cocoa butter is the major factor for the stability at room temperature and is responsible for the characteristics of melting and hardness in the mouth. The crystalline structure of fat thus determines the macroscopic properties of chocolate and sensory perception [6, 7].

Incomplete tempering can produce a large amount of unstable modifications with a low melting point, these dissolving under warm storage conditions and then rise to the surface where the molten fraction of cocoa butter solidifies in the form of fatty coating. The same defect can also be caused by the fat filling used in chocolate confectionery [8].

To consumers, the appearance and texture form the main attributes for the selection and acceptability of chocolate; the taste is considered to be important when identifying the product. The final texture (hardness) of chocolate is influenced by several factors including formulations, production

techniques, tempering, polymorphism (fat crystal stability) and cooling temperatures.

Visual information describing objects, starting with sheen, colour, shape, roughness, texture, reflections and translucency are summarised in the attributes of appearance. These attributes result from complex interactions of incident light, the optical properties and human perception. Relevant information can be obtained from modern technology, such as computer imaging analysis with colour calibration, HunterLab and CIELAB models. Given that chocolate should be meeting consumer expectations previously acquired, the appearance traits may possess significant commercial implications [1].

Texture and appearance also play an important role in the sensory assessment of chocolate. Sensory analysis is an objective method of analysis which employs trained staff instead of devices. The method is important in that it covers such quantitative indicators which cannot be directly characterised by instrument methods, establishing a set of factors that determine the final impression of the consumer [9].

Therefore, this study was carried out to determine the effect of fat migration on texture, bloom formation and sensory attributes of chocolate at different storage temperatures.

Materials and methods

Six brands in two variants were chosen from the product range of and in cooperation with Zora Olomouc, a chocolate factory controlled by Nestlé Czech Ltd. These involved *Kaštany ledové*, *Milena*, *Margot Artemis*, *Orion Krémová oříšková*, *BOCI fekete erdö* (a dark chocolate with cherry filling) and dessert candies called *Black magic - Orange Sensation* (a dark chocolate filled with orange fondant).

Each of the products was made in two variants of R and N, where R refers to retempered and N to not retempered products. The difference in this regard consists in the last production step where the process of retempering refers to exposing the packed, normally produced articles to an ideal temperature for a certain period to create a perfect structure of cocoa butter crystalline grid.

Sample storage and analysis

The prepared products were taken to stock at Mendel University in Brno and an assessment was carried out, including determining the colour using Konica Minolta Spectrophotometer CM-3500d and making a physical analysis of the product texture using the TIRAtest unit. Simultaneously, a part of the samples was deep-frozen to (-18°C) for later use as "standards" in the sensory analysis, while the remainder was split into controlled temperature regimes - warehouses with cooling temperatures of 6°C and 12°C, a laboratory room with constant temperature of 20°C and the thermostat set to 30°C and then stored under such settings without any temperature fluctuations. The second set of samples was taken two weeks after the production date, now from the various storage temperature settings, and again subjected to sensory assessment and identifying the colour changes and changes in texture. The same was done for the third sampling after six weeks and the fourth sampling after ten weeks of production date. Before each analysis, the samples were equilibrated to room temperature.

Sensory assessment

The sensory profile method was used to determine the sensory attributes of chocolate products with respect to storage time and temperature. Each of the assessments was carried out by at least eight trained assessment specialists. The sensory protocols for each product contained the same descriptors. To measure the perceptions, unstructured graphical scales were used with the verbal description of the endpoints, the scale length being 10 cm. All of the sensory assessments were underway in a specialised laboratory under standard conditions.

Measuring colour

Konica Minolta Spectrophotometer CM 3500d was used for determining the colour and its changes during the storage time of each sample. The unit is connected to a computer with installed software (CMS-100W SpectraMagic NX) in which different modes can be set for data processing and export, e.g. selecting desired values (L*a*b*, L*C*h, Hunter Lab). The modes selected for the colorimetric determination of colour for chocolate products were as follows:

- Reflectance
- Geometry d/8 (the instrument measures the reflected light at an angle of 8°)
- SCE (specular component excluded - eliminating sheen)
- D 65 (illumination mode; 6,500 Kelvin)
- 8 mm slot

Measurements were done each time three times on the cavity and three times on the coating in two samples per group.

Analysing texture
Texture measurements used a universal instrument for the measurement of physical characteristics - Tira test (type 27025). It is a desktop single-column unit with a maximum load of 2.5 kN. The high-quality sensors of values along with the microprocessor-controlled equipment achieve the maximum precision.

The penetration test used for assessing the chocolate products involved a probe of a stick shape which penetrated into the sample, so obtaining a record of the force necessary to push the punch to a selected depth (in Newton). The selected criteria for the penetration test of chocolate products:
Type of test: pressure testing
Type of attachment: straight ending
Probe diameter: 3 mm
Force sensor: 200 N
Test speed: v_1 = 100 mm per min
End of test: 7 mm track

Results and Discussion
The acquired data were analysed using MS Excel. The statistical analysis of all the sourced data was carried out using STATISTICA version 12 - analysis of variance (ANOVA).

Sensory assessment
The results of the graphical scales were obtained by measuring the distance of the mark from the right scale end (in cm) and are graphically rendered in the form of radar charts as an average rating of all assessment specialists (n = 8). "10" refers to the highest/best quality at the scale left end, while "0" is the least favourable / lowest quality at the right end. The radar charts thus graphically express the sensory profiles of the products during storage, clearly illustrating the differences between each of the production variants as well as between the temperature modes.

The results make it apparent that all the products and their variants stored at 6°C and 12°C did not show any deterioration in the descriptors even after ten weeks of the production date. Their attributes (both visual and taste characteristics) were comparable to the standards, achieving the overall sample rating of the same extent.

During the storage period at 20°C, the not retempered *Margot* products started to show signs of fat bloom already on sampling 3. On sampling 4, the fat bloom was found in the retempered products as well. This means that the influence of technology in these products is evident - retempered products resist longer to the development of fat bloom.

On sampling 4, the fat bloom was found even on the surface of the cavity in the not retempered *Kaštany* products. Significant presence of the same was found in the filling as well. This reconfirms the benefit of retempering. Deterioration was generally found in multiple sensory descriptors at these storage temperatures in chocolate bars, which specially involved sheen or even aroma for the *Margot* product. Table chocolates (*Boci*, *Orion Krémová oříšková*) did not show any deficiencies in this type of storage; the same applies to fondants. The worst changes during such storage temperature were observed for the *Margot* and *Milena* products as early as on sampling 1, the samples being repulsive in terms of both appearance and taste. In addition, *Milena* was found to have a dry filling. Observed in both of the products was also overall hardening, manifest in loose and crumbly consistency.

Results for individual products as well as their variants stored at 30°C show a clear deterioration of all the sensory descriptors. The same results were achieved by Ali et al [10] who state that the migration of fat under these temperatures adversely affects the product integrity and appearance. In chocolate products, the typical deterioration associated with fat migration is manifest in softening, fat bloom and unacceptable textural changes within the product due to the leakage of liquid glycerides from the filling onto the surface of the product. All of this reduces the product acceptability for the consumer. As a conclusion of studying the sensory changes in colour and texture of chocolate products, the authors report that the storage temperature at 30°C is significantly ($P < 0.05$) less advisable than that of 18°C.

The results were also confirmed in their study by Bui et Coad [11] when storing temperature above 30°C caused the chocolates to significantly decrease their sensory quality. The largest losses in terms of quality occurred with respect to appearance and the overall acceptance of the products. Although milk chocolate is usually considered resistant to fat bloom with the inhibitory effects of milk fat, the fact that milk fat content is negligible in the current production as a result of using dry skimmed milk resulted in rapid blooming due to storage conditions.

Sample sensory profile - *Kaštany*
The results of sensory rating in the *Kaštany* chocolate product after the end of the experiment (10 weeks of the production date) is presented on Fig. 1 and Fig. 2. The charts show the differences among the storage temperatures as well as between the retempered and the not retempered products.

Fig. 1 Sensory profile – *Kaštany retempered*

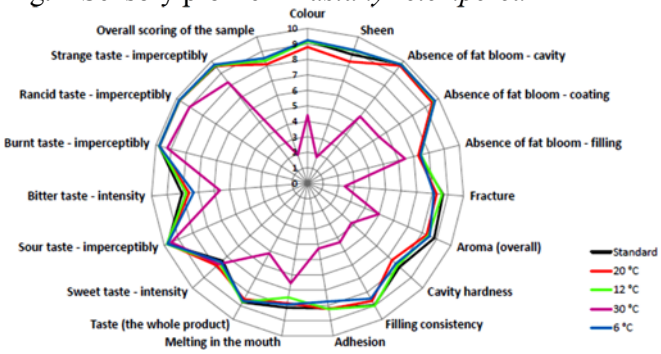
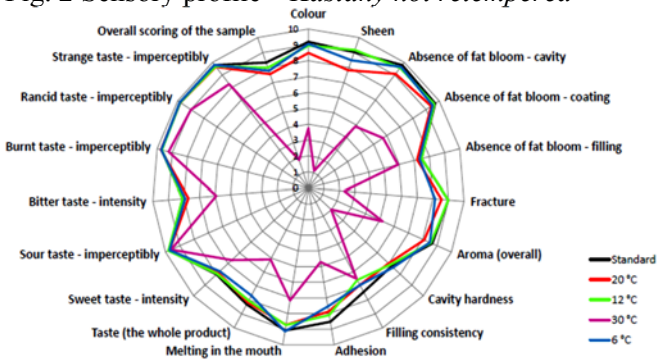


Fig. 2 Sensory profile – *Kaštany not retempered*

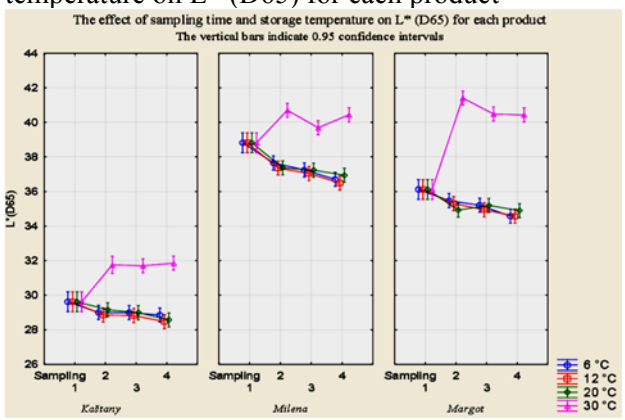


Measuring colour

The CM-S100w program enables expression of colour in a colour space CIELAB (balls) according to the International Commission on Illumination. The values of L* (lightness) represent the range from "0" (black) to "100" (white). The colour coordinates a* and b* take positive or negative values depending on the location within the three-dimensional system.

Since the effect of the temperatures during the storage period on the change in colour was significant chiefly in chocolate bars, results are provided for this very type of product.

Fig. 3 The effect of sampling time and storage temperature on L* (D65) for each product



L* (lightness) was the value selected for the statistical evaluation as it best characterises the colour changes per sample during the analysis. All of the results were statistically significant.

Significant changes in lightness were observed as part of the measurement of colour during the storage time, particularly for 30 °C temperatures. The lightness L* (D65) was significantly increased in all of the samples, mostly for *Margot* products. A noteworthy colour difference was observed in the other temperature modes when linearly, depending on the time, darkening occurred for all of the products. Between the modes of storage (6, 12 and 20°C), however, apparent difference in L* values were not observed. The effect of technology on the lightness L* was not significant.

Changes in the colour of the surface of the chocolate at the storage temperature of 18°C and 28°C over 52 days in milk chocolates were also studied by Briones et Aguilera [12], where significant changes in colour occurred after 33 days of storing. Initially, white spots appeared on the surface probably due to the rapid migration of the liquid fat through defects or pores in the surface layer. Adenier et al [13] report that initially the fat bloom occurs at the edges or along cracks on the surface layer of the chocolate to gradually replace the original brown surface of the chocolate table. Thus, fat blooming as a result of product exposure to high temperatures causes the chocolate to change its colour with time, as well as lose sheen and turn grey along the surface [12].

Changes in colour between the experimental and control samples during storage at 30°C were noted in their study by Bui et Coad [11] as well. The authors found that storage time mostly influenced the L* value. For a* value, the change was not demonstrated, i.e., there was no shift from red to green. The upward shift occurred for the b* value, indicating movement along the axis from the blue towards the yellow, meaning that the products became brighter over time. The shift of the axis to the yellow colour characterises the development of fat bloom, with however the changes being still not noticeable to the naked eye. These findings are reconfirmed in our results.

Bui et Coad [12] also compared the relationships between the sensory attributes and instrumental measurements of colour. All of the differences between the sensory and colour changes they found were statistically significant ($p < 0.001$). The correlation was the strongest for colour (L* and b*) and for texture. Other variables strongly correlated with colour (L* and b*) involved taste (0.96 to 0.97), overall acceptance (0.96 with b*) and percentage of fat bloom (0.96 with b*). These results conclude that

as the fat blooming evolved, the products became brighter, their taste and texture turned worse and the product became generally less acceptable.

Analysing texture
All of the results were statistically significant.

Fig. 4 The effect of storing temperature on the hardness of each product

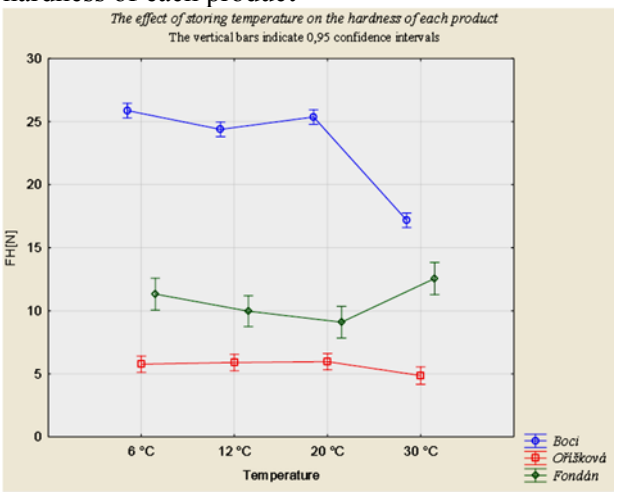
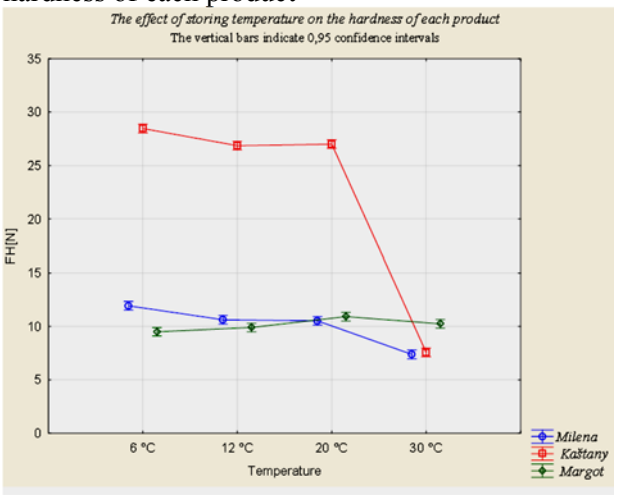


Fig. 5 The effect of storing temperature on the hardness of each product



The chart shows that the hardness of *Boci* – the filled chocolate - was significantly lower when stored at 30°C than that of other temperature modes (Fig. 4). For the *Orion Krémová oříšková* chocolate, the decrease in hardness was not so significant. The liquid filling in *Boci* thus may be causing softening in this type of chocolate. Ali et al [10] also report in their results that filled chocolates stored at 30°C were significantly softer than those stored at 18°C. This is explained by a significant migration of fat from fillings into the chocolate layer. An even more significant softening occurred in the *Kaštany* products (Fig. 5), which is probably due to the significantly different composition of fatty acids in the filling. According to Ali et al [10], in filled chocolate products where the filling contains lipids with a low melting point, these lipids tend over time to migrate to the surface of products, which initially most likely involves lipids with the lowest melting point and the highest fluidity. This migration may cause the chocolate to become sticky and soft, while the filling becomes stiffer; the migration has even an impact on the structure of the surface. Fat migration can largely occur already at room temperature (17-23°C); it accelerates as the temperature increases and becomes reduced as the solid fraction of lipids increases.

On the contrary, increase in hardness was seen in fondant products at storage temperatures of 30°C. Drying and subsequent hardening thus probably occurs in the products.

Storage temperatures of 6, 12 and 20°C were not clearly affecting the hardness of the products, with differences varying only in units of Newton. A significant process of softening thus does not occur under such conditions. A conclusion is also possible based on the study of Ali et al [10] that the migration of fats with rather lower melting points was very slow at storage temperatures of 18°C and the changes were minimal with respect to chemical composition, hardness, sheen and polymorphic stability.

Miquel et Hall [14] studied the migration of fat from chocolate product fillings using magnetic resonance imaging, with the samples stored at 20°C, 23°C and 28°C. Here the importance of storage temperature was clear with a distinct migration of fats occurring at 28°C as early as within 25 days, while at 23°C, the migration was in equilibrium even after two months. The slowest fat transition was observed for storage temperatures of 20°C. The authors also report that the products are sensitive not only to the presence of foreign fats in the chocolate, but also to the change in structure caused by their presence. Consequently, eutectic mixtures are usually formed by dissolving cocoa butter as foreign lipids migrate into the chocolate.

The results of this experiment therefore permit a conclusion that chocolate product storage temperature to 20°C is advisable with respect to hardness. Stortz et Maragoni [15] state that generally, finished chocolate products should be stored at 18°C to 20°C and under a relative humidity lower than 50%, without the access of light.

Conclusion

Advisable and not recommended storage conditions were assessed through each of the analysis as part of

monitoring the effect of storage temperature and method of manufacture of chocolate confectionery on changes in the product quality. Clear results were achieved for the temperature mode of 30°C, with the sensory rating, measurement of colour and texture analysis confirming that this temperature is not appropriate, even in the short term, for storing the chocolate products analysed. Products were observed to worsen their sensory attributes and turn to abnormally bright colours. Their strength and hardness was also changing regardless of having been subjected to retempering. Storage temperatures of 20°C generally achieved good results in the measurement of both colour and texture, with however visible shortcomings being seen during sensory rating. Reflected here was also the impact of technology when the retempered products achieved better resistance to fat blooming. Temperature modes of 6°C and 12°C were comparable in all the analyses performed. For texture measurements of products stored at 6°C, the results were even more favourable. A comprehensive assessment is therefore possible that when stored at 6°C, the products retain their initial attributes even after ten weeks of the production date. As a result, the storage temperature to 12°C can be referred to as appropriate storage conditions for chocolate products.

Acknowledgment

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Effect of dietary fatty acid composition on plasma lipid levels in rats

BARBORA NEMCOVA[1], VERONIKA ROZIKOVA[1], TOMAS KOMPRDA[1], BRANISLAV RUTTKAY-NEDECKY[2], ZBYSEK SLADEK[3]
[1]Department of Food Technology,
[2]Department of Chemistry and Biochemistry
[3]Department of Morphology, Physiology and Genetics of Animals
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

barbora.nemcova@mendelu.cz

Abstract: The aim of the research was to evaluate in a model organism an effect of different fatty acids on plasma level of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerols (TAG). The findings have been trying to be applied to human nutrition. Forty adult male rats (Wistar Albino) were divided into four groups with ten animals each and were examined for the effect of fatty acids contained in food on animal tissues. All groups were fed the first seven weeks standard feed for mice and rats with the addition of 20% of beef tallow. Fodder was adjusted in the next seven weeks. Basic feed mixture with 5% of safflower oil (n-6 group, SF), fish oil (n-3 group, F), *Schizochytrium* alga oil (n-3 group, DHA), and 20% of beef tallow (T-group). Beef tallow is representative of "atherogenic" saturated fatty acids, DHA oil and fish oil have high representation in proportion of n-3 fatty acids and safflower oil has high representation in proportion of n-6 fatty acids. At the end of the fattening period, the plasma concentrations of lipids and fatty acid content in the liver tissues was determined. DHA-diet had the most positive (decreasing) effect on TAG levels ($P<0.05$). It was concluded that dietary *Schizochytrium* microalga oil (with high DHA content) may have the positive potential for decreasing the risk of cardiovascular diseases, but the results obtained in rats should be applied to humans cautiously.

Key-Words: plasma lipids level; liver; dietary fatty acids; beef tallow; fish oil; safflower oil; *Schizochytrium* microalga

Introduction

Fatty acids are considered as the most important component of the lipid in the nutrition. Human nutrition is focused not only on the total lipid content, but also on representation of fatty acids groups (SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids) in foods. Polyunsaturated fatty acids are labelled a fatty acid containing from 2 to 6 double bonds.

Polyunsaturated fatty acids are divided into two groups, n-3 and n-6, characterized by different physiological effects. The group n-3 always has the first double bond on the third carbon, taken from the methyl residue. The same applies to the group n-6 having the first double bond on the sixth carbon [1].

The starting compounds of the series n-3 and n-6 are (essential for humans) α-linolenic acid (ALA; 18:3n-3) and linoleic acid (LA, 18:2n-6). These two essential fatty acids are metabolized by the same set of enzymes (elongase and desaturase) to long-chain polyunsaturated fatty acids, LC-PUFA.

Physiologically, the most important metabolites of group n-6 and n-3 are arachidonic acid (AA; 20:4n-6), respectively eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [2]. The above-mentioned PUFA families, n-6 and n-3, are essential components of the metabolically active tissues such as liver. The final metabolites of n-6 and n-3 PUFAs are eicosanoids (biologically active substances – prostaglandins, leukotrienes and tromboxanes), playing important roles in regulating different aspects of the inflammatory response [3].

McDaniel et al. [4] describe that diets with high n-6:n-3 ratios, in conjunction with genetic factors, have been associated with the increasing prevalence of chronic inflammatory diseases such as cardiovascular disease. It is generally considered that eicosanoids generated from arachidonic acid, have pro-inflammatory effect, causing platelet aggregation and shrinks the vascular wall. While their

counterparts generated from EPA/DHA are believed to elicit anti-inflammatory effect and ultimately also cardioprotective effect.

Process of atherogenesis is the principle of cardiovascular diseases. One of the risk factors for atherosclerosis (chronic inflammatory process of the vessel wall) is dyslipidemia (high total plasma cholesterol [TC] level, increased low-density lipoprotein cholesterol [LDL-C], decreased high-density lipoprotein cholesterol [HDL-C], high concentration of plasma triacylglycerols [TAG]) [5].

Cholesterol is transported in the blood plasma as part of lipoproteins mostly in the fraction of LDL, HDL less. Changes in the concentration of cholesterol from the perspective of cardiovascular risk are always evaluated in the context of concentrations of total cholesterol, HDL cholesterol, LDL cholesterol and triacylglycerols. The liver and other organs synthesize cholesterol. HDL lipoproteins ensure the removal of excess cholesterol to the liver. LDL lipoproteins ensures transport of cholesterol to the cells. Excess cholesterol contained in LDL particles is deposited in the vessel wall, leads to the formation of atherosclerotic plaques [6].

Material and Methods

Animals and dietary interventions

Forty adult male rats of the laboratory strain Wistar Albino (produced by Bio Test Ltd., Konárovice, Czech Republic) at the age of 10 weeks were used. Animals were reared in the plastic boxes (53.5 x 32.5 x 30.5 cm) of five animals in a room maintained at 23 ± 1°C, humidity 60% and 12/12 h light/dark cycle. The experiment was performed in compliance with the Czech National Council Act No. 246/1992 Coll. to protect animals against cruelty, the amended Act No. 162/1993 Coll., and was approved by the "Commission to protect animals against cruelty" of the Mendel University in Brno.

The basic feed mixture, pelletized complete chow for mice and rats (Biokron, Blučina, Czech Republic), composed of wheat, oat, wheat sprouts, soybean meal, extruded soybean, maize, dried milk, dried whey, dried yeast, grounded limestone, monocalcium phosphate, salt, L-lysine hydrochloride and premix of vitamins + minerals, was fed to all animals the first week of the experiment (acclimatization). The animals were fed daily *ad libidum* and had free access to water bottles.

All 40 rats (four groups per 10 animals) were fed (an atherogenic) diet 7 weeks. This diet contained basic feed mixture with beef tallow + evaporated sweetened milk + extra vitamins/minerals premix (200 + 400 + 20 g.kg-1; T). The next 7 weeks were the second phase of fattening. The rats were randomly divided with following dietary interventions. The first group was fed the (atherogenic) tallow diet continued (T-group). Other groups were divided into groups with 5% of safflower oil (SF-group), fish oil (F- group), *Schizochytrium* alga oil (DHA-group). The content of quantitatively and physiologically important fatty acids in the used feed demonstrates table 1.

At the end of the experiment was carried out taking samples for analysis. Blood samples were collected from all animals into the heparin-coated test tubes (DISPOLAB) and centrifuged at 200 x g for 10 min at 4 °C to obtain plasma. Liver samples were removed for analysis of fatty acids. The liver were stored at -20°C after lyophilization; the main drying at -45°C for 24 hours, the finish drying at -50°C for 3 hours (Freeze Christ Alpha 1-2 LD).

Plasma lipids and fatty acids determination

Total plasma cholesterol and its fractions (LDL cholesterol, HDL cholesterol and triacylglycerols) were determined by the enzymatic-colorimetric method using an automated chemical analyser BS-200 (Mindray, China) and commercial kits (Greiner Diagnostic GmbH, Germany). The determination was carried out at the Department of Chemistry and Biochemistry, Mendel University in Brno.

Fatty acids methyl ester were determined by gas chromatography after fatty acids derivatization referred to in article by Komprda et al. (2013) [7]. The analysis of liver tissue was performed on a gas chromatograph Fisons GC 8000 series with a flame ionization detector, capillary column DB-23 (60 m x 0.25 mm x 0.25 μm, Agilent J & W Scientific, USA) and temperature program 140˚C/ 1 min, gradient 5˚C/ min to 200˚C/ 1 min, gradient 3˚C/ min to 240˚C held for 15 min. The carrier gas was used nitrogen. Total lipids were extracted (hexane/isopropanol solvent) from the liver tissue by the method based on the extraction method of Hara and Radin (1987) [8]. For each sample, the measurement was carried out concurrently.

The measured data were statistically evaluated by one-way analysis of the variance ratio test, including Tukey's post-hoc test. For the evaluation of the data was used programme Statistica (StatSoft Inc., Tulsa, USA).

Table 1 Content of quantitatively and physiologically important fatty acids in the used feed

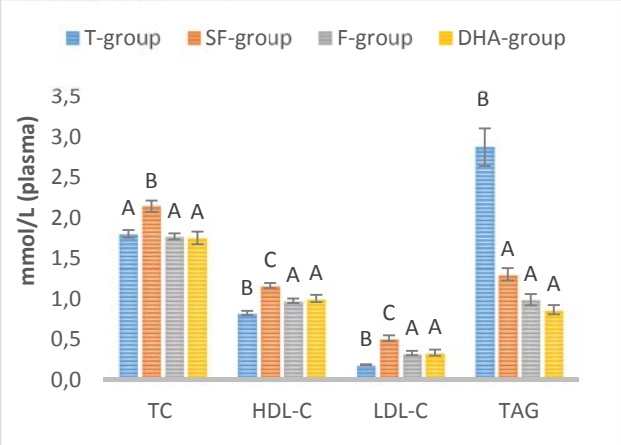
Fatty acid		Content in the diet (% of the sum of fatty acids)			
Name	Designation	T-group	SF-group	F-group	DHA-group
Myristic	14:0	4.7	0.2	3.2	3.1
Palmitic	16:0	17.2	10.5	15.2	16.4
Stearic	18:0	21.0	2.5	2.9	1.7
Oleic	18:1n-9	83.1	19.7	24.,6	21.8
Linoleic	18:2n-6	12.5	65.8	26.4	22.8
α-Linolenic	18:3n-3	0.8	0.1	5.2	4.1
Arachidonic	20:4n-6	0.1	0.1	0.4	0.4
EPA	20:5n-3	0.0	0.1	6.6	1.0
DPA	22:5n-3	0.0	0.2	1.1	0.7
DHA	22:6n-3	0.0	0.2	7.7	26.6

Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil (commercial oleum jecoris aselli); DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga

Results and Discussion

The experiment focused on the analysis of fatty acids in liver tissue and plasma lipid fractions determination having regard to the variability of dietary inputs. Indicators of plasma lipid levels are total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerols (TAG).

Fig. 1 The content of plasma lipids level of rats from all fed groups in mmol/L



Legend: TC - total cholesterol, HDL-C - high-density lipoprotein cholesterol, LDL-C - low-density lipoprotein cholesterol, TAG – triacylglycerols; T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga; a – c: means labelled with different letters differ significantly (P<0.05); one-way analysis of the variance ratio test, including Tukey's post-hoc test, n=20

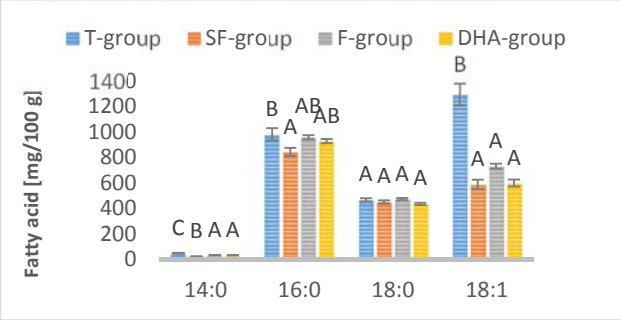
The content of fatty acids measured in liver tissue was converted to mg/100g of fresh weight of liver. The content of plasma lipid fractions was converted to mmol.l^{-1} of plasma.

As it is apparent from Figure 1, the highest (P<0.05) total cholesterol concentration was established in plasma of rats fed the diet with safflower oil compared with control (T-group). However, it is necessary to add that this cholesterol increasing effect of safflower oil was from the greater part due to the increase in the favourable HDL fraction. The most positive (i.e. decreasing) effect on the plasma levels of TAG rats (P <0.05) was DHA-oil compared with T-group fed the atherogenic diet, which plasma levels of TAG as expected substantially increased (P<0.05). As far as the LDL fraction, the lowering effect was established surprisingly in the T-group with tallow/evaporated milk diet.

It is generally known that a high content of saturated fatty acids and ratio of PUFA n-6/n-3 for the benefit n-6 in the diet increases the risk of cardio-vascular disease, while PUFAn-3 reduces the risk of cardio-vascular disease due to favorable physiological effects. Therefore, the concentrations of fatty acids in the liver tissue are divided into various groups in the charts (Figure 2-5).

Regarding saturated fatty acids, the T-group was significantly higher (P <0.05) deposition of myristic acid (14:0) and palmitic acid (16:0) compared with other SF-, F-, DHA-groups. Significant increase (P <0.05) of oleic acid in the feed for the control group (T-group) was expected because, oleic acid has the highest representation content (83.1%) in this compared with other SF-, F-, DHA-group.

Fig. 2 The content of saturated fatty acids and monounsaturated fatty acid in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)

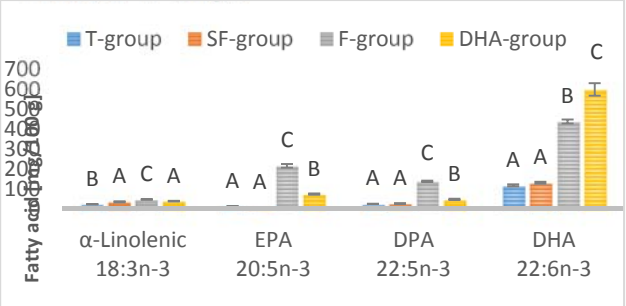


Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga; a – c: means labelled with different letters differ significantly (P<0.05); one-way analysis of the variance ratio test, including Tukey's post-hoc test, n=20

As far as the polyunsaturated fatty acid n-3 series are concerned, higher (P <0.05) deposition of EPA and DHA in animals fed a diet of fish oil (F-groups) and *Schizochytrium* oil (DHA-groups) is interesting in comparison with control (T-group). A significant increase of α-linolenic acid was measured in the group fed fish oil.

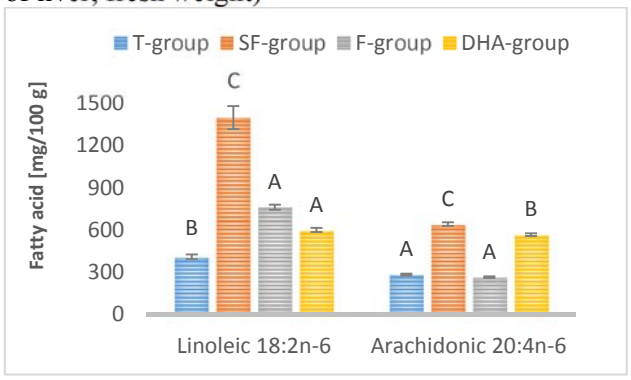
Regarding polyunsaturated fatty acids n-6 series, expected increase (P <0.05) of linoleic acid and arachidonic acid was confirmed in a group of diets with safflower oil (SF-group) compared with control (T-group).

Fig. 3 The content of polyunsaturated fatty acid n-3 series in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)



Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga; a – c: means labelled with different letters differ significantly (P<0.05); one-way analysis of the variance ratio test, including Tukey's post-hoc test,n=20

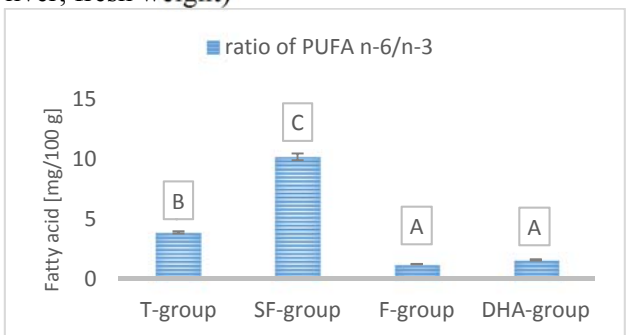
Fig. 4 The content of polyunsaturated fatty acid n-6 series in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)



Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga; a – c: means labelled with different letters differ significantly (P<0.05); one-way analysis of the variance ratio test, including Tukey's post-hoc test, n=20

The highest value of the PUFA n-6/n-3 ratio was observed in the group with a safflower oil diet, which was expected due to the high content of linoleic acid in safflower oil. The ratio of PUFA n-6/n-3 in the diet (rich source of PUFAn-3) with fish oil and *Schizochytrium* microalga was measured a significant reduction (P <0.05) compared to control (T-group), while the ratio of PUFA n-6/n-3 in the diet safflower oil is significantly increased (P <0.05) compared with other T-, F-, DHA-groups.

Fig. 5 The ratio of n-6/n-3 polyunsaturated fatty acids in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)



Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga; a – c: means labelled with different letters differ significantly (P<0.05); one-way analysis of the variance ratio test, including Tukey's post-hoc test, n=20

Figures 1 shows, that the addition of safflower oil in the feed tends to increase (P <0.05) serum total cholesterol as compared to control (T-group), which was found also in the experiment Poudyal et al. (2013) [9]. Chen et al. (2011) reported a significant decrease of plasma total cholesterol in hamsters fed a diet containing *Schizochytrium* lipid extract compared to the control, but this fact is not confirmed in our case regarding DHA diet (Figure1) [10].

HDL fraction increased after the fish oil diet in comparison with control (T-group), which was confirmed also in experimental Popović et al. (2011) [11] .

Plasma levels of TAG rats was significantly reduced after DHA-diet containing a high proportion of LC-PUFAn-3 compared to control (T-group) in our experiment. This finding can be explained by the fact that DHA and EPA inhibit the synthesis of diacylglycerol O-acyltransferase (DGAT), fatty acid synthase and acetyl CoA carboxylase enzymes and increase fatty acid β-oxidation via PPARα-mediated pathway resulting in decreased substrate availability for TAG formation [12].

The results of this experiment obtained in rats have to be interpreted cautiously regarding dietary recommendations for humans, because a better model for humans is a hamster or, better still, a pig than rats or mice [13].

Conclusion

It can be concluded based on our results that, DHA-diet (rich sours of DHA) significantly decrease (P <0.05) the plasma level of TAG, which may have a positive effect on reducing the risk of cardio vascular diseases. It is interesting that the safflower oil (containing about 65% of linoleic from the sum of total fatty acids) in the diet significantly increased the fraction of favorable HDL cholesterol in the rat plasma compared to the control (T-group), wherein said the fact should be related to F- and DHA-groups.

The different composition of fatty acids in the diet has the influence on their deposition in liver tissues. Deposition of linoleic acid in the liver increased (P <0.05) significantly under the diet rich in linoleic acid (SF-group), compared with the control (T-group). Deposition of EPA, DPA, DHA in the liver increased (P <0.05) significantly under the fish oil and *Schizochytrium* oil diet rich in PUFAn-3 compared with control (T-group).

Increased amounts of PUFAn-3 in the diet (F-group, DHA group) affecting desirable increase in the levels of these fatty acids in liver tissues, therefore the ratio of PUFA n-6/n-3 approached almost to an optimum, which is associated with reduced risk of disease occurrence heart and blood vessels. The ratio of PUFA n-6/n-3 1:1 is considered ideal, but such a relationship can´t be achieved in the diet of economically developed countries. Therefore, the World Health Organization recommends a ratio of 5:1-2:1. It is reported that populations with a high ratio of PUFA n-6/n-3 in the diet, such as for example the population of Indian subcontinent has extremely high incidence of cardiovascular diseases. Therefore, it is desirable to increase the consumption of n-3PUFA diet. In this context it is useful to mention that the higher consumption of PUFA, whose double bond is very susceptible to oxidation, it is recommended to consumption of the lipophilic antioxidant vitamin E higher than the recommended daily dose (10 mg/day), which prevents undesirable oxidation in molecules [14,15].

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Baking quality of genetics resources of hulled wheat species, grown in organic farming

STOLICKOVA MARTINA, KONVALINA PETR
Department of Agroecosystems
Faculty of Agriculture
University of South Bohemia in Ceske Budejovice
Studentska 13, 370 05 Ceske Budejovice
CZECH REPUBLIC

mrackova.martina@email.cz

Abstract: As organic farmers are searching for new market and sale opportunities, the organic farming system may grow and process a wider range of species than the conventional farming system. Concerning wheat, there are so called hulled wheat species (einkorn, emmer wheat, spelt wheat) comprising parts of collections of the world gene banks. Our paper aims at presenting the results of the study and the assessment of spring wheat forms, four einkorn cultivars, eight emmer wheat cultivars, seven spelt wheat cultivars in particular, as compared to modern bread wheat variety. Small-plot trials were established at organic farming research area of University of South Bohemia in Ceske Budejovice in 2009 and 2012. The results of the trials show that the grains are characterised by a high proportion of protein in grain (up to 18.1%). Higher content is in einkorn and emmer – but this species have bad baking quality (low sedimentation and gluten index values). However, they may be difficult to use for common baking. Different situation is in spelt wheat – some varieties had good yield level, lower protein content but suitable sedimentation and gluten index. Moreover, new food products demonstrating a different technological quality of the hulled wheat species have to be launched on the market. The food products will be suitable for regional marketing.

Key words: organic farming, hulled wheat, einkorn, emmer wheat, spelt wheat

Introduction

In 2011, organic cereals were grown on the area of 1,800,000.00 hectares in Europe. Bread wheat (*T. aestivum* L.) is one of the most significant crops, being grown in accordance with the organic farming principles. Spelt wheat (*T. spelta* L.) is the second most significant cereal species [17]. Einkorn (*Triticum monococcum* L.) and emmer wheat (*T. dicoccon* Schrank) are also important. The area of einkorn and emmer wheat is, however, smaller. Hulled wheat species used to be widespread. They used to be elementary and essential parts of everyday human diet. They have become significant again recently, as they have returned back to human diet [14].

Nowadays, einkorn is only grown in montane areas [19]. As Wieser et al.[16] state, it is mostly grown in Western Turkey, on the Balkan Peninsula, in Italy, Spain, Switzerland, Germany and Austria. It is popular among farmers and consumers because its seeds have favourable nutritional values. It can adapt to low-input farming conditions very well. It is highly disease and pest resistant [19]. Emmer wheat is a tetraploid species. It has been grown in Israel, Jordan, Lebanon, Syria, Turkey and Iran. As far as the European countries are concerned, the largest area can be found in Italy. Emmer wheat is a popular wheat species thanks to high protein content in grain and a suitable protein composition [8].

It is not clear how spelt wheat was born [13]. It is a young hexaploid species, younger than bread wheat. Nowadays, it is mostly grown in Central and Western Europe, in Germany, Switzerland, Austria, in the Czech Republic and Hungary in particular [15]. It is partly grown in the USA and Canada as well. Spelt wheat contains more proteins and more soluble fibres than bread wheat. It can be grown in less fertile soil than bread wheat. Spelt wheat contains similar amino acids to bread wheat [1].

It is very important to breed more land races and cultivate their characteristics, as there are a few hulled wheat species. Organic farmers have

been searching for more stable species and varieties, greater genetic diversity and the varieties adaptable to various climatic conditions. Brand new varieties have to be more competitive, disease and pest resistant [4]. Important will be also reduction of negative impact on environment, because of growing more resistant varieties [10,11]. Hulled wheat species are considered valuable resources of genes which can improve and enhance wheat characteristics and features, and nutritional values as well. Low yield rate and fierce competition between spelt wheat and bread wheat are major big disadvantages of spelt wheat varieties [19].

Hulled wheat species have become more attractive to farmers and producers recently. Requirements for diversity and good quality have been increasing [18]. Therefore, organic farmers are seeking after the varieties with high nutritional value and the varieties suitable for product processing. They aim at making the range of regional foodstuff wider [12]. These are unique wheat species which gain and have a competitive advantage on the market [5]. Lachman et al. [7] also agree with the hulled wheat species having a considerable food potential. They have favourable nutritional characteristics. Lachman et al. [7] also state hulled species are a good healthy diet.

This article aims at evaluating particular significant characteristics and quality of hulled wheat species belonging to a genetic resources collection.

Material and methods

Used varieties

The varieties came from the Gene bank of the Crop Research Institute in Prague-Ruzyně. Genetic resources of einkorn (*Triticum monococcum* L.), emmer wheat [*Triticum dicoccum* (Schrank) Schuebl], spelt wheat (*Triticum spelta* L.) and one bread wheat variety (*Triticum aestivum* L.) were chosen (Table 1).

Field Trials

Varieties were sown in a randomized, complete block design on the organic certified research area in České Budějovice during 2009 and 2012. The seeding rate was adjusted for a density of 350 germinable grains per m^2. The crop stands were treated in compliance with the European legislation (the European Council Regulation (EC) No. 834/2007, the European Commission Regulation (EC) No. 889/2008.

Characteristics of the Trial Station

The University of South Bohemia in České Budějovice (USB): Mild warm climate, soil type – pseudogley cambisols, kind of soil – loamy sand soil, altitude of 388 m.

Analyse

A) Before harvest there were taken 30 plants from every repetition. After the harvest we measured grain yield and calculated protein yield per hectar. From the plants were measured harvest index and make spike productivity analysis.

B) Baking Quality: The following parameters were tested after the harvest and dehulling of the grains by The International Association for Cereal Chemistry (ICC) methods: crude protein content (ICC 105/2); index of sedimentation - SDS test (ICC 151); wet gluten content (ICC 106/2) and gluten index (ICC 155).

Statistical Data Processing

Data were processed by the Statistica 9.0 (StatSoft. Inc., USA) program. Regression and correlation analyses provided the evaluation of interdependence. The comparison of varieties and their division into statistically different categories were provided by the Tukey HSD test.

Results and Discussion

The Table No. 2 shows the lowest content of proteins and wet gluten in bread wheat grains, whereas the highest content of proteins in emmer and spelt wheat grains. As stated by Mondini et al. [9], the content of proteins varies from 13.7 to 15.9 percent in spelt wheat grains. The content of proteins exceeds 15.9 percent in grains of four particular varieties (for more details, see the Table No. 3). The content of proteins may rise up to 20 percent in emmer wheat grains. However, it usually gets to 18 percent [9]. We have found a variety containing more than 18 percent of proteins (*Triticum dicoccon* - Palestine). According to Mondini et al. [9], the wet gluten content varies from 37.0 to 56.6 percent. It is much higher in hulled wheat grains than in bread wheat ones. After Zeleny test we have found the lowest wet gluten content in einkorn and emmer wheat grains, whereas the highest wet gluten content in bread wheat ones. The wet gluten content

measurement results have been very similar to the gluten index measurement results. Bread wheat grains have the highest gluten index values and emmer wheat grains have the lowest ones. The lowest einkorn and emmer wheat values originate from their genetic codes. As stated by Corbellini at al. [2], einkorn dough is not ideal for baking, as einkorn gluten is not drawable enough. Einkorn bread does not rise very well. Moreover, consumers do not like how einkorn bread looks like. However, einkorn biscuits are thinner and bigger than bread wheat ones [2]. According to Konvalina et al. [6], emmer wheat grains are valuable materials. A lot of proteins are concentrated in them. Their characteristics are suitable for no-bake products, e.g. pasta, biscuits, muesli, puree, etc. Fares et al. [3] also agree with emmer wheat grains, being suitable for pasta production, as they contain a lot of proteins. Emmer wheat pasta may be consumed by anyone, without any restrictions.

Table 1 List of used varieties

Name of Variety/Accession	Identifier[1]	Origin[2]	Botanical Variety[2]
***Triticum monococcum* L.**			
Triticum monococcum 38	01C0204038	GEO	*hohensteinii* FLAKSB.
Triticum monococcum 44	01C0204044	ALB	*vulgare* KOERN.
No. 8910	01C0204542	DNK	*macedonicum* PAPAG.
Schwedisches Einkorn	01C0204053	SWE	*vulgare* KOERN.
***Triticum diccocum* (SCHRANK) SCHUEBL**			
Rudico	01C0200948	CZE	*rufum* SCHUEBL.
Weisser Sommer	01C0203993	DEU	*Dicoccum*
May-Emmer	01C0203990	CHE	*Dicoccum*
Triticum dicoccon (Brno)	01C0204022	CZE	*rufum* SCHUEBL.
Triticum dicoccon (Dagestan)	01C0204016	RUS	*serbicum* A. SCHULZ
Triticum dicoccon (Palestine)	01C0201261	ISR	*serbicum* A. SCHULZ
Triticum dicoccon (Tapioszele)	01C0201280	-	*semicanum* KOERN.
Triticum dicoccum (Tabor)	01C0204318	-	*rufum* SCHUEBL.
***Triticum spelta* L.**			
Triticum spelta (Ruzyne)	01C0201257	CZE	*arduini* (MAZZ.) KOERN.
Triticum spelta (Tabor 22)	01C0204322	-	*duhamelianum* KOERN.
Triticum spelta (Tabor 23)	01C0204323	-	*duhamelianum* KOERN.
Spalda bila jarni	01C0200982	CZE	*album* (ALEF.) KOERN.
VIR St. Petersburg	01C0204865	CZE	*album* (ALEF.) KOERN.
Triticum spelta (Kew)	01C0200984	-	*caeruleum* (ALEF.) KOERN.
Triticum spelta No. 8930	01C0204506	-	*album* (ALEF.) KOERN.
***Triticum aestivum* L. – control**			
SW Kadrilj	01C0104877	SWE	*lutescens* (ALEF.) MANSF.

[1] EVIGEZ (http://genbank.vurv.cz/genetic/resources/asp2/default_c.h); [2]Abbreviations of countries comply with ISO 3166-1 alpha-3; [2]Clasification according: Dorofeev VF, Filatenko AA, Migušova EF (1980). Opredelitel pšenicy, Leningrad, 105 p.

Table 2 Baking quality of evaluated species (2009-2012) – Means with the same letter are not significantly different at P<0.05 according to the Tukey´s HSD test

Species	Protein content (%)	Wet gluten content (%)	Zeleny test (ml)	Gluten index	Falling number (s)
Einkorn	15.29_{b}	37.03_{b}	12_{a}	19_{a}	333_{b}
Emmer	16.34_{c}	38.39_{b}	15_{a}	19_{a}	308_{b}
Spelt	16.13_{bc}	42.62_{c}	36_{b}	39_{b}	330_{b}
Control variety SW Kadrilj	12.82_{a}	29.02_{a}	50_{c}	74_{c}	245_{a}

Table 3 Baking quality of evaluated varieties (2009-2012) – Means with the same letter are not significantly different at P<0.05 according to the Tukey´s HSD test

Variety	Protein content (%)	Wet gluten content (%)	Zeleny test (ml)	Gluten index	Falling number (s)
Einkorn					
Triticum monococum 38 – J1	15.68_{abc}	37.70_{abcde}	14_{a}	22_{abcd}	314_{abc}
Triticum monococum 44 – J2	16.63_{bc}	35.50_{abc}	13_{a}	18_{a}	329_{abc}
No. 8910 - J4	14.65_{ab}	37.40_{abcde}	10_{a}	18_{ab}	343_{bc}
Schwedisches Einkorn – J6	14.20_{ab}	37.53_{abcde}	11_{a}	20_{abc}	349_{bc}
Emmer					
Rudico	16.22_{bc}	39.72_{cde}	18_{ab}	20_{abc}	321_{abc}
Weisser Sommer – D11	15.97_{bc}	41.34_{cde}	18_{ab}	21_{abc}	338_{abc}
May-Emmer – D12	16.52_{bc}	39.07_{bcde}	18_{ab}	26_{abcd}	337_{abc}
Triticum dicoccon (Brno) – D13	14.70_{ab}	30.39_{ab}	15_{a}	15_{a}	292_{abc}
Triticum dicoccon (Dagestan) – D14	16.05_{bc}	36.25_{abcd}	12_{a}	17_{a}	238_{a}
Triticum dicoccon (Palestine) – D17	18.41_{c}	44.36_{cde}	15_{a}	20_{abc}	301_{abc}
Triticum dicoccon (Tapioszele) – D18	16.89_{bc}	37.78_{abcde}	13_{a}	15_{a}	325_{abc}
Triticum dicoccum (Tabor) – D19	15.96_{bc}	38.18_{bcde}	15_{a}	14_{a}	319_{abc}
Spelt					
Triticum spelta (Ruzyne) – SP1	15.99_{bc}	42.45_{cde}	31_{bc}	42_{bcd}	318_{abc}
Triticum spelta (Tabor 22) – SP2	15.89_{bc}	41.63_{cde}	40_{d}	43_{cd}	356_{c}
Triticum spelta (Tabor 23) – SP3	16.30_{bc}	41.77_{cde}	37_{cd}	36_{abcd}	332_{abc}
Spalda bila jarni - SP7	15.58_{abc}	40.25_{cde}	31_{bc}	32_{abcd}	335_{abc}
VIR St. Petersburg – SP6	16.72_{bc}	45.41_{e}	37_{cd}	37_{abcd}	330_{abc}
Triticum spelta (Kew) – SP8	15.75_{abc}	42.00_{cde}	42_{cd}	45_{d}	304_{abc}
Triticum spelta No. 8930 – SP9	16.70_{bc}	44.80_{de}	33_{c}	36_{abcd}	338_{abc}
***Triticum aestivum* L. – control**					
SW Kadrilj	12.82_{a}	29.02_{a}	50_{d}	74_{e}	245_{ab}

Conclusions

We compared four wheat species experimentally. We have found out there is a considerable difference in the baking quality between bread wheat and hulled wheat varieties. Bread wheat varieties have better baking quality, they are more suitable for bread baking and leavened bakeries production (the gluten characteristics). Hulled wheat species are more suitable for unleavened products (they contain more proteins, wet gluten, they attain lower Zeleny test values and gluten index values as well). Products made of hulled wheat species may be considered interesting alternatives by small and mid-size producers. Thanks to them, they can respond to an increasing demand for nature-friendly regional products.

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The effect of rats´ diet to production pro-inflammatory and anti-inflammatory cytokines

TEREZA SUSTROVA[1], VERONIKA ROZIKOVA[2], TOMAS KOMPRDA[2], ZBYSEK SLADEK[1]
[1]Department of Morphology, Physiology and Animal Genetics
[2]Department of Food Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

tereza.sustrova@mendelu.cz

Abstract: The aim of this study was to proof supposed influence of long-term consumption of n-3 PUFA on pro-inflammatory and anti-inflammatory cytokine levels in rats´ plasma. The results will be used to look for consequences to human nutrition. The plasma of experimental rats was examined for levels of IL-10, IL-6, TNF-α, TGF-β and adiponectin. The sources of PUFA n-3 and n-6 were added to standard feed for rats. The experimental animals were divided into four groups. In the standard feed for the first group 6% of safflower oil was added (n-6). The feed ration of the second group contained 6% of fish oil (n-3, eicosapentaenoic acid - EPA). The last group was fed with addition of 6% *Schizochytrium microalgae* oil (n-3, dokosahexaenoic acid – DHA). Control group was fed without addition of oils. Addition of oils didn't change homeostasis of cytokine levels of rats´ plasma.

Key-Words: ELISA, n-3 fatty acids, n-6 fatty acids, IL-10, IL-6, TNF-alfa, TGF-beta 1, adiponectin

Introduction

Atherosclerotic disease cause cardiovascular events and is considered to be a multifactorial condition. Disrupted endothelial homeostasis and infiltration of the intima by activated T cells and monocytes is observed in earlier stages and local production of a variety of inflammatory mediators can modulate and the immunologic reaction within atherosclerotic lesions [7].

Polyunsaturated fatty acids (PUFA) have affected the activity and functional status of blood vessels and process of atherogenesis which caused cardiovascular disease. Eicosanoids (PG2, TA2) are metabolites of PUFA n-6 and they act pro-inflammatory, vasoconstrictor, causing platelet aggregation. On the other side, eicosanoids of PUFA n-3 (PG3, TA3) act anti-inflammatory, vasodilator and anti- platelet aggregation. PUFA n-3 ultimately reduces the risk of cardio- vascular disease, autoimmune diseases and cancer [3]. Cytokines are highly active substances which production is regulated temporally and locally. Its signals lead to change in expression in target cells. Pro-inflammatory cytokines are for example interleukin 6, tumor necrosis factor-alpha, etc. On the contrary anti-inflammatory cytokines are interleukin 10, transforming growth factor-beta, adiponectin, etc. [9]. Interleukin 6 (IL-6) is a cytokine that can facilitate autoimmune phenomena, amplify acute inflammation and promote the evolution into a chronic inflammatory state [1]. IL-6 is produced by smooth muscle cells in the *tunica media* and occurs to the proliferation of these same cells. Persistent inflammation stimulates artery wall remodelling and foam-cell formation, the hallmark of early atherosclerotic lesion. Systemic inflammation may also contribute to atherosclerosis. Indeed serum levels of pro-inflammatory markers positively correlate with the risk of myocardial infarction and cardiovascular death [6] and particularly serum IL-6 levels are directly related to intima-media thickness [5]. Interleukin-10 (IL-10), a cytokine with anti-inflammatory properties, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host [8]. Transforming growth factor-beta 1 (TGF-β1) belongs to transforming growth factor beta family of cytokines which have got a lot of isoforms. TGF-β1 is an anti-inflammatory cytokine. TGF β-1 is a chemoattractant of macrophages and it applies in induction of inflammatory and later in its inhibition. TGF-β 1 is significant for tissue regeneration, induction of IgA and creation of extracellular matrix [9]. Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine and it has an antagonistic activity against TGF-β1. TNF-α is capable of

regulating the activity of cardiac fibroblasts by decreasing collagen synthesis and increasing matrix metalloproteinase activity [4]. Adiponectin is the most abundant adipokine secreted by adipocytes that may couple regulation of insulin sensitivity with energy metabolism. Decreased plasma adiponectin levels are observed in patients with obesity, type 2 diabetes, hypertension, metabolic syndrome and coronary artery disease. Low plasma adiponectin levels are significantly correlated with endothelial dysfunction. These results suggest that low adiponectin levels may be a useful marker for early-stage atherosclerosis [2].

In this study, we have dealt with the impact of income n-3 and n-6 fatty acids on production of pro-inflammatory and anti-inflammatory cytokines in plasma of rats. The aim is tested the hypothesis about effect of long-term consumation of n-3 PUFA on plasma cytokines levels in animal model and apply this knowledge in human nutrition.

Material and Methods

There were added sources of PUFA n-3 and n-6 to standard rat feed ration (Biokron, Czech Republic). Animals were divided into four groups and they were fed with standard diet and diet with 6% addition of safflower oil (n-6), group with 6% addition fish oil (n-3) and group with 6% addition *Schizochytrium microalgae* oil (n-3), which contain docosahexaenoic acid (DHA). The rats were fed for 40 days ad libitum and had ad libitum intake of water. Each group was composed of 10 animals. DHA oil and fish oil are rich in proportion of n-3 fatty acids, safflower oil is rich of n-6 fatty acids.

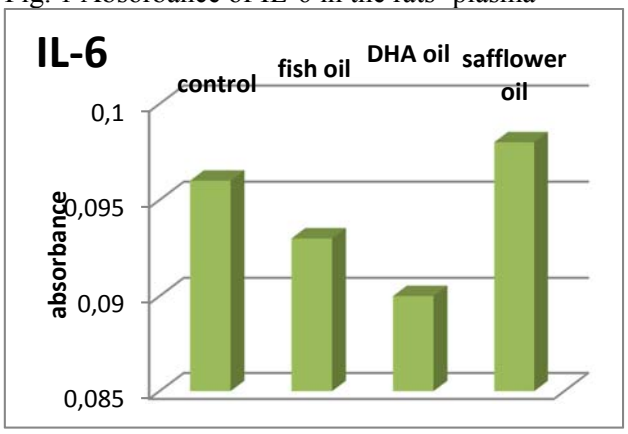
Blood samples were taken from all rats to heparin tubes (DISPOLAB, Czech Republic) at the end of the experiment. From this samples plasma was obtained and analyzed for the concentration of cytokines by ELISA (rat ELISA kits, Invitrogen, USA). Samples were evaluated by Hybrid Reader (Synergy H1, BioTek, USA) at wavelengths 450 nm.

Results and Discussion

Cytokine TGF-β 1 was the only with measurable concentration. Concentrations of other cytokines were so low that they were not detected by ELISA. Absorbance was the only parameter we gained for those cytokines.

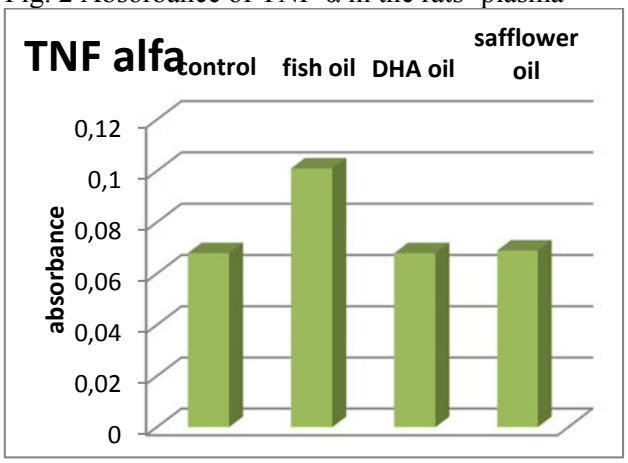
IL-6 is a pro-inflammatory cytokine. According to our hypothesis, its concentration should be highest in the group of rats which was fed a diet containing safflower oil and the lowest concentration in the groups of rats which were fed a diet with fish and DHA oil. The 0.098 average absorbance was determined in a group fed with safflower oil. In the fish oil group average absorbance of 0.093 was determined and the group of DHA oil had a 0.090 average absorbance. In the control group was the average absorbance 0.096. The tendency of absorbance corresponds to our hypothesis but the results are not significant. (Fig.1)

Fig. 1 Absorbance of IL-6 in the rats´ plasma



TNF-α is a pro-inlammatory cytokine. The average absorbance of TNF-α in the control group was 0.068. According to our hypohtesis when the fish and DHA oils have anti-infalmmatory effects, the level of TNF-α in plasma should be lowest in these two groups. It was determined an average absorbance 0.101 in the fish oil group and 0.068 in the DHA group of rats. Absorbance of TNF-α in the fish oil group is higher than in a control group which means that the fish oil induced disruption of homeostasis and increase of pro-inflammatory cytokine level in the rats´ plasma. In the safflower oil group a significant increase of this cytokine was expected. The average absorbance was 0.069 and this is not significant compared to control group. (Fig.2)

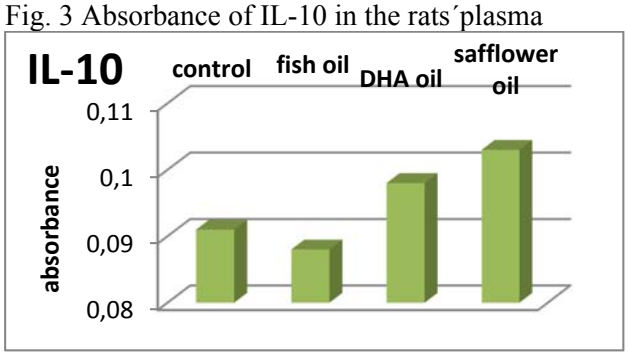
Fig. 2 Absorbance of TNF-α in the rats´ plasma



IL-10 is an anti-inflammatory cytokine. According to our hypothesis the lowest concentration of this

cytokine should be in groups fed with fish and DHA oils addition. In the control group the average absorbance of 0.091 was determined. In the group fed with fish oil the absorbance was 0.088. In the DHA group was determined on average absorbance of 0.098. This value is highert than control. In case of higher concentration IL-10 in the DHA oil group with anti-inflammatory effects means that this type of diet caused changes in homeostasis. There was a suppression of inflammation by IL-10. Average absorbance 0.103 in the group fed with addition of safflower oil corresponds with our hypothesis. Safflower oil with pro-inflammatory effects may induced production of IL-10. Differences of IL-10 levels among fat diets were not significant. (Fig.3)

Fig. 3 Absorbance of IL-10 in the rats´plasma



Average concentration of TGF-β 1 in rats´plasma was 5516.31 pg/mL in the control group. In the group which was fed with addition of fish oil was the average concentration 6380.61 pg/mL. In the group fed with addition of DHA oil average concentraion of the TGF-β 1 was 6453.86 pg/mL. In the group fed with addition of safflower oil the average concentration was 7210.81 pg/mL. These results show that all of these fat diets may caused disruption of homeostasis and production of TGF-β 1 with its pro-inflammatory effects. The results are not statistically significant. (Fig. 4 and Fig.5)

Fig. 4 Absorbance of TGF-β 1 in rats´ plasma

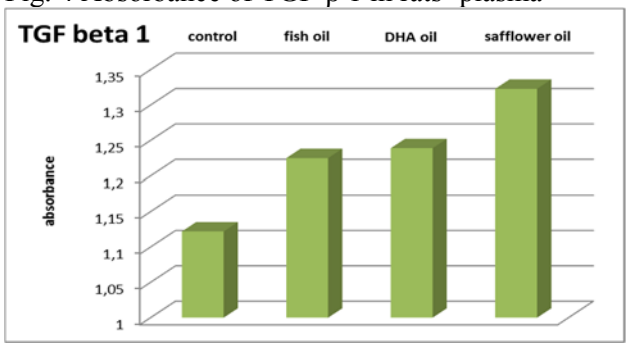
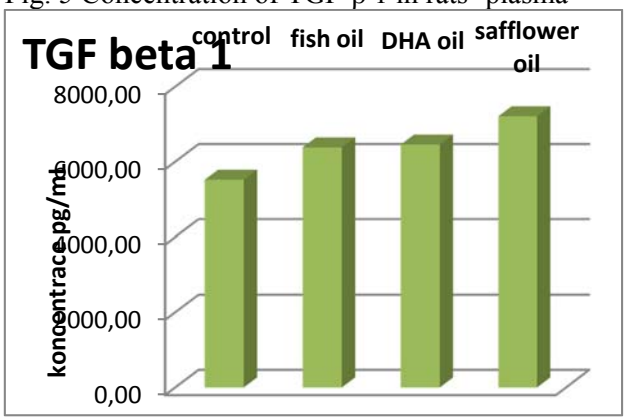
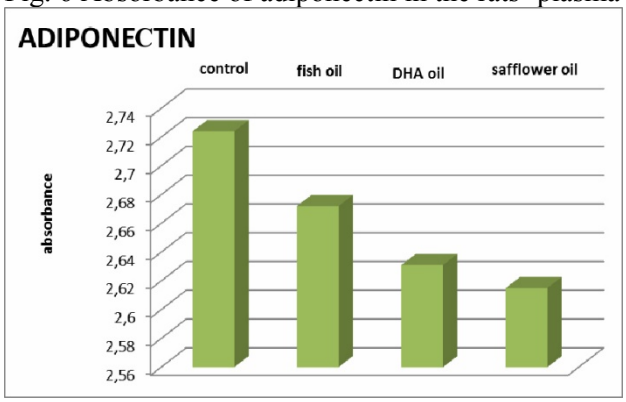


Fig. 5 Concentration of TGF-β 1 in rats´ plasma



The average absorbance of adiponectin in the control group was 2.724. According to our hypothesis the lowest values should be in groups fed with addition of fish and DHA oils. In the group fed with addition of fish oil the average absorbance of adiponectin was 2.672. The group fed with DHA oil had average absorbance 2.631. On the contrary, the highest concentration of adiponectin should be in the group fed with addition of safflower oil to supress the inflammation. This group showed average absorbance 2.651 and it means that fat diet did not disrupt the homeostasis. Results are not significant. (Fig.6)

Fig. 6 Absorbance of adiponectin in the rats´ plasma



Conclusion

No significant results were obtained in this study this supports our hypothesis that DHA and fish oil don´t have any influence of lowering levels of cytokines in rats´ plasma.

Acknowledgments

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Section – Plant Biology

Effect of exogenous application of growth regulators on the physiological parameters and the yield of winter wheat under drought stress

IRENA BARANYIOVA[1], KAREL KLEM[1,2], JAN KREN[1]
[1] Department of Agrosystems and Bioclimatology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2] Global Change Research Centre AS CR, v. v. i.
Belidla 986/4a, 603 00 Brno
CZECH REPUBLIC

irenka2308@azet.sk

Abstract: The field trial aimed to evaluate the effect of different growth regulators in winter wheat under growth stress was conducted in 2013/2014. Within this experiment following growth regulators and fungicide with growth regulation effect were used: Retacel extra R68 (chlormequat chloride 720 g/l), Moddus (trinexapac-ethyl 250 g/l), Cerone (ethephon 480 g/l), Amistar (strobulirin 250 g/l). These growth regulators were applied at growth stages between BBCH 31 and BBCH 59. The aim of the experiment was to determine the impact of these regulators on the growth, development and yield of winter wheat when simulating the drought stress using experimental rain-out shelters. The attention was paid to assess the effect of exogenous application of growth regulators on the physiology and the yield of selected varieties of winter wheat under drought stress. From our preliminary results it can be concluded that almost all growth regulators increase the rate of CO_2 assimilation and the stomatal conductance. Definitely positive effect on water use efficiency was found in fungicide treatment with growth regulation effect - azoxystrobin. Under drought stress the decrease of chlorophyll content in leaves was found. Growth regulators CCC and trinexapac mitigate the decline of chlorophyll content caused by drought in the upper leaves but rather increased the impact in older (lower) leaves. Fungicide azoxystrobin alleviates the decrease of chlorophyll caused by drought in all leaves. The results show that all regulators increased yield, which demonstrate a positive effect under dry conditions because the vegetation season was very dry and lodging did not occur. Furthermore, the most pronounced mitigation of drought stress was found for strobilurin and partly also trinexapac. Results of field experiments can contribute to mitigating the impact of drought on yield formation and quality of winter wheat production in the realization of biological potential of wheat genotypes.

Key-Words: plant growth regulators, winter wheat, drought stress

Introduction

Drought still belongs to the most significant environmental factors which negatively influences plant growth. Drought stress in winter wheat is evidently more significant in Central Europe than in other regions, it is also supposed that according to the model of climate prediction periods of drought are going to be more often [1].

Period of drought during the early phase of growing season can have serious consequences for crop production, excess of water in this period has negative influence as well on the production and quality of crops because it increases the risk of infection by certain diseases, it leads to root anoxia and tillage is more difficult. Drought decreases the growth of plants, influences various physiological and biochemical processes such as photosynthesis, respiration, metabolism of nutrients and growth stimulators [2, 3]. Partial elimination of environmental stress can be reached by application of growth regulators.

Growth regulators can improve water use efficiency by closing stomata. They also have effect on increase of ratio roots: above ground biomass and can also influence the accumulation of antioxidants that protect plants during stress conditions. For agricultural purposes the growth regulators are such substances which have effect on physiological processes in metabolism of plants and thereby they

positively influence the yield and the quality of production.

This mostly concerns increase of cold resistance, limitation of lodging, tiller levelling, reduction of apical dominance, higher deployment of generative organs, more effective use of nutrients, decrease of harvesting losses and facilitating harvest [4]. Drought – lack of water or water stress - is the most limiting stressor for plants, it decreases the activity of all enzymes in the plant, slows down the growth of the plant, leads to closing stomata and reduction of CO_2 assimilation [5, 6].

By applying growth regulators we can affect levelling of productive tillers and prolongation of leaf area activity. Greenhouse research proves that growth regulators can decrease evapotranspiration by 29% [7]. Some studies are pointing out that growth regulators can, in fact, increase rooting [8]. Previous studies proved that plants with slow growth can survive longer period of drought than fast growing plants [9]. By using phytohormones or synthetic regulators we might achieve a partial elimination of environmental stress effect or help plants with regeneration after active stress. Theoretically, it is possible to suppose that the regulator itself causes increase or decrease of yield because its activity is in correlation with the influence of all other parameters of the environment.

The aim of the experiment was to evaluate positive and negative impacts of growth regulators on physiology and winter wheat yield in conditions of drought, to choose suitable types of regulators and the time of applying in order to improve tolerance to drought.

Fig. 1 Sheds over the experimental area of winter wheat

Material and Methods

The experiment was carried out at the field experimental station in Žabčice with winter wheat variety Matylda.

The experimental station is situated in Southern Moravia (the Czech Republic). Moderate soils are dominant type in this region. The location is considered to be one of the hottest areas in the Czech Republic [18].

Sowing of the variety Matylda was carried out on October 15th, 2013 in three replications randomly distributed on selected plot with sowing rate of 4MGS/ha. Variety Matylda belongs to the set of early varieties. The variety has a medium plant length with an average resistance to lodging. Variety Matylda has a very high yield potential. During the growth phase by the end of stem elongation period BBCH 39 there were over the half of the experimental area built short-termed rain out shelters providing induction of drought stress. Measuring of physiological parameters (water use efficiency, CO_2 assimilation rate (Fig. 4) and the chlorophyll content in leaves (Fig. 5) was done in the middle of drought stress (May 26th, 2014), and at the end of drought stress effect.

After wheat ripening evaluation of yield (Fig. 6) and yield structure has been done. For evaluation of CO_2 assimilation rate, transpiration and stomatal conductivity gas exchange system LI 6400 XT with a assimilation chamber equipped with LED light source has been used. The measurement took place at constant temperature, relative air humidity, CO_2 concentration and at saturation light intensity. The observed parameters allowed calculation of water use efficiency (WUE) and indirect parameter of water use efficiency A/Gs.

As an additional parameter for evaluation of primary phase of photosynthesis measuring of chlorophyll fluorescence by the apparatus FluorPen has been done. Parameters of the maximal and actual quantum yield PS II were evaluated. The content of chlorophyll and flavonols was determined in vivo by the method of transmittance and UV screening of chlorophyll fluorescence by the instrument Dualex4 FLAV. The individual regulators were applied in the following phenophases and in amounts according the Table 1.

Table 1

Number of variants	Name of product	The planned dose		The term application
		product ha (l, kg)	water ha (l)	
1	KONTROLA			
2	CCC (Retacel)	1,88 l	300 l	3.4.2014 BBCH 31
3	TRINEXAPAX-ETHYL (Moddus)	0,4 l	300 l	23.4.2014 BBCH 32-35
4	ETHEPHON (Cerone 480SL)	0,6 l	300 l	7.5.2014 BBCH 45
5	AZOXYSTROBIN (AMSTAR XTRA)	0,8 l	300 l	13.5.2014 BBCH 59

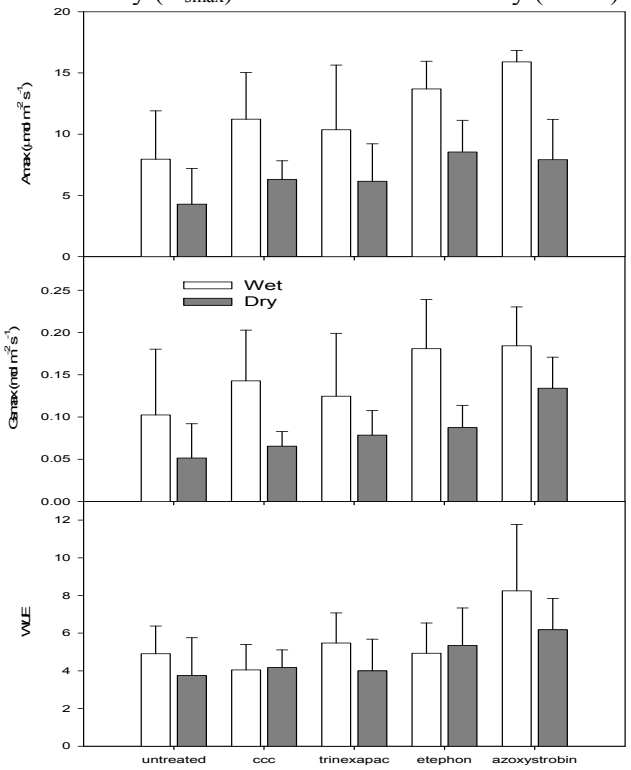
Fig. 2 Aplication of CCC (4.4.2014)

Fig. 3 Aplication of trinexapax-ethyl (23.4.2014)

Results and Discussion

The results indicate a negative effect of drought on the CO_2 assimilation rate (A_{max}) and stomatal conductance (G_s). In the untreated control were A_{max} and G_s reduced by almost 50%. The application of growth regulators increased CO_2 assimilation rate in both treatments with ambient rainfall and drought stress, with the highest increase recorded after application of azoxystrobin and etephon. Relative differences between dry and wet treatment, however, changed relatively little. The decrease of relative reduction of A_{max} due to drought stress occurred mainly after application of etephon and trinexapac-ethyl. In the case of G_s the decreased reduction was noted after the application of azoxystrobin and trinexapac-ethyl. The most significant effect on increasing water use efficiency (WUE), was found following the application of azoxystrobin both in dry and wet variants. The effect of drought was also reflected in the decrease of chlorophyll content in individual leaves within vertical profile. This decrease was mitigated mainly by application of azoxystrobin. In the case of applications of trinexapac-ethyl the effect of drought on the chlorophyll content was reduced in upper leaves but enhanced in bottom leaves. Yield results showed a positive effect of all applications of growth regulators, with the highest impact of the trinexapac-ethyl, etephon and azoxystrobin. All three treatments also reduced the negative impact of drought on yield.

Fig. 4 CO_2 assimilation rate (A_{max}), stomatal conductivity (G_{smax}) and water use efficiency (WUE)



Definitely positive influence on water use efficiency was caused only by fungicide with regulatory effects - azoxystrobin.

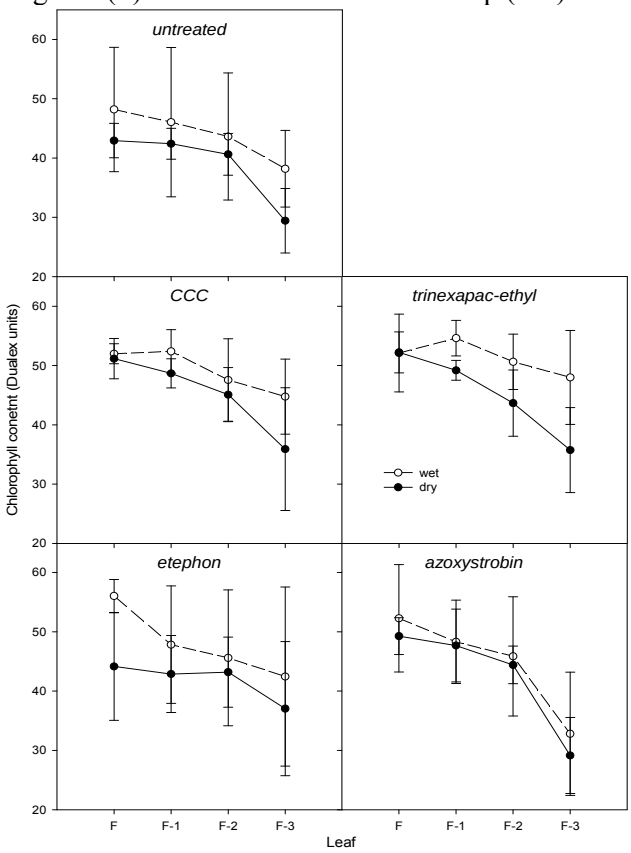
Conclusion

The use of growth regulators is accompanied with a number of positive effects, especially in the conditions of water deficit.

By applying growth regulators we can reach a partial elimination of environmental stress effect. Growth regulators can improve water use efficiency. They also have influence on increase of roots: above ground biomass ratio and can also influence the accumulation of antioxidants that protect plants

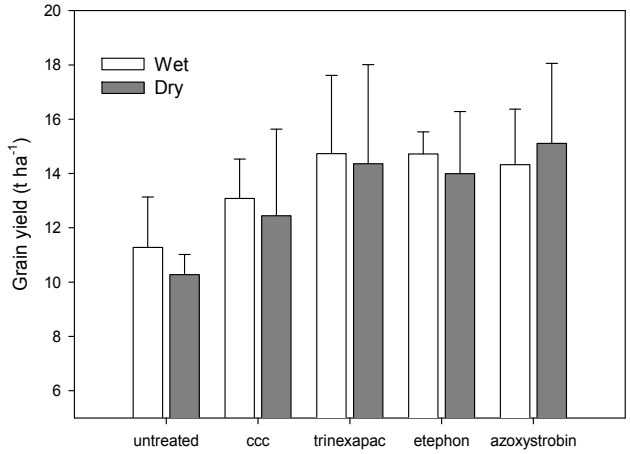
during stress conditions. According to our preliminary results it is possible to be stated that, practically, all growth regulators used increase the CO_2 assimilation rate and stomatal conductance.

Fig. 5 Chlorophyll content in leaves in order from flag leaf (F) to the fourth leaf from the top (F-3)



Regulators CCC and trinexapac reduce decrease of chlorophyll caused by drought at upper leaves but on the other hand they increase it at older lower leaves.

Fig. 6 Effect of drought and growth regulators application on grain yield



The biggest reduction of drought stress influence (shed) on yield was evident at strobilurin and partially at trinexapac as well.

- Unambiguously positive influence on water use efficiency had mostly fungicide with regulatory effects - azoxystrobin.
- Due to drought activity there is decrease of chlorophyll content in leaves. Regulators CCC and trinexapac reduce decrease of chlorophyll caused by drought at upper leaves but on the other hand they increase it at older lower leaves.
- Fungicide azoxystrobin reduces decrease of chlorophyll caused by drought at all the leaves.
- It is evident from the measurements that all the regulators increased the yield and which it itself proves the positive effect during the drought because it was a very dry year and the canopy did not lodge. Furthermore, the biggest reduction of drought stress effect on yield was evident in strobilurin and partially at trinexapac-ethyl application as well.

The results of field experiments can significantly contribute to reduction of drought impact on yield formation and quality of production of winter wheat in realization of biological potential of varieties.

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Interaction of Strigolactone with polar auxin transport in roots of *Arabidopsis thaliana*

NELA DANKOVA, ONDREJ MACOSZEK, VILEM REINOHL
CEITEC – Central European Institute of Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

nelladankova@email.cz

Abstract: One of the major regulators of root architecture is the plant hormone auxin. According to recent investigation strigolactone is also involved in this process and is supposed to act by regulation of polar auxin transport mediated by PIN efflux carriers. To choose an optimal concentration for the subsequent study the influence of 12 nM to 50 μM strigolactone GR24 on Arabidopsis root system was evaluated. 242 ecotypes of *Arabidopsis thaliana* were analyzed by genome-wide association study (GWAS) under the influence of 100 nM of strigolactone. Results of the study are analyzed for a search of candidate genes which participate in strigolactone signaling pathways. From obtained results we have chosen 5 candidate genes (AFB4, F-box, BEN2, F-box associated, MPK) for analysis of their expression levels dependent on strigolactone concentration in Arabidopsis roots and simultaneously auxin efflux (PIN1,2,3,4,7) and influx (AUX1) carriers were analyzed by Real-Time PCR.

Key-Words: - Strigolactone, root architecture, PINs, GWAS analysis, gene expression

Introduction

Intensive research activity on the new plant hormone strigolactone started with its description as a regulator of shoot branching [1, 2] and revealed much broader influence on regulation of development throughout the whole plant body [3]. The existence and signal molecule function was elucidated by the analysis of a series of shoot branching mutants, cloning and grafting experiments in *Arabidopsis*, pea and petunia. Strigolactone is transported acropetally from the root system into above ground plant body where it causes inhibition of axillary bud outgrowth [1, 2]. Strigolactone is further connected with various functions in plants like regulation of root architecture, inhibition of branching, control of cambium secondary growth, stimulation of parasitic seed germination and initiation of mycorrhiza

Strigolactone perception and signal transduction are poorly understood. An F-box leucine-rich repeat protein (referred to as AtMAX2 F-box) that is a component of a SCF complex [4] is involved in strigolactone signaling which has been identified from mutation studies in several species. It is related to the TIR1 and COI1 F-box receptors for auxin and jasmonic acid, and they are suggested to be involved in ubiquitin-mediated protein degradation [5].
Basipetal auxin transport in stem and acropetal in the root is realized in floem and parenchyma cells surrounding vascular strands. This characteristic polar auxin transport is facilitated by specific auxin carriers located at the plasma membrane of the cells – AUX and LAX (Like AUX1) ensuring influx into the cell and family of PIN proteins (PIN1-8) which facilitate auxin efflux from the cell [6, 7]. Initiation of primary root growth and branching is dependent on auxin transport and formation of its local maxima. Control of root architecture [8] and adventitious root formation are related to auxin-dependent processes, and strigolactone acts at least in part by regulation of auxin transport via the efflux carrier PIN1 [9].

The study of changes of root architecture caused by strigolactone application is an optimal system for elucidation of the influence of strigolactone on polar auxin transport.

Material and Methods

GWAS analysis

Preliminary experiment to assess strigolactone influence on root architecture was realized to obtain an optimal concentration for the GWAS experiment and included 6 different ecotypes of *Arabidopsis thaliana* [10].

For the Genome-wide association study (GWAS) seeds of 242 ecotypes of *Arabidopsis thaliana* were selected, sterilized by bleach and chloride gas,

stratified and grown on sterile agar medium for 3 weeks. Based on the preliminary experiment 100 nM concentration of GR24 was chosen and control variant with only DMSO were prepared. Individual Petri dishes with plants were scanned for 3 weeks and obtained images were evaluated by BRAT program – Busch Lab Analysis Toolchain [11] for required parameters of root system of individual ecotypes. GWAS analysis followed.

Genome-wide association study (GWAS) is a useful technique for identifying genetic loci responsible for natural variation in *A. thaliana*. Based on common genetic variability of individual ecotypes specific differences associated with investigated trait are studied. How significantly SNPs – single nucleotide polymorphisms – are associated with required traits like root length, root tortuosity, root growth rate, root angle etc. can be determined. Previously genotyped accessions (natural inbred lines) are grown in replicate under different conditions and phenotyped for different traits.

Experimental material

Seeds of *Arabidopsis thaliana* were sterilized by adding 70% ethanol to prevent possible contamination and stratification was carried out in sterile water in dark and cold conditions in a fridge for 3 days. After that the seeds were transferred by pipette onto Petri dishes with media. Plants were grown for 6 days in vertical position in a growth chamber Klimacel.

Four different variants of cultivation media were prepared – 10 nM, 100 nM, 1000 nM GR24 and control medium (5 µl of DMSO, the same amount in which GR24 was dissolved and added). Strigolactone was added into the medium directly after sterilization during the preparation process in sterile conditions. On the surface of the medium the sterile nylon sieve (Uhelon) was placed for better manipulation during harvesting of plants. Seeds were sown on the sieve in two horizontal lines and after 6 days were cut by scalpel in the height of hypocotyls, thus divided into above and below ground parts and collected separately in Eppendorf tubes.

Gene expression

For the assessment of expression of selected genes RNA was isolated from roots by RNAeasy Plant Mini Kit (Qiagen, Germany). Concentration of obtained RNA was measured on Picodrop Pico100 (Picodrop,UK). Reverse transcription was realized with the Enhanced Avian HS RT-PCR Kit (Sigma-Aldrich, USA). Enzyme Reverse transcriptase was used for generating cDNA from isolated RNA.

Transcription levels of selected genes by Real-Time PCR reaction with specific primers – *AtPIN1,2,3,4,7, AtAUX1, AtAFB4, AtFbox, AtBEN2, AtFbox associated, AtMitogenPK (MPK)* was used. Final volume of PCR reaction was 10 µl. For all reactions 5 fold diluted cDNA was used. At first all primers pairs were optimized, specific temperature conditions for efficient amplification process were defined and standard curves were created by evaluation of the selected genes against 2 constitutive genes – *UBQ10* and *UBC*. Two negative controls - sterile water and reverse transcribed water instead of cDNA which was used. For real-Time PCR thermal cycler C1000TM Thermal Cycler/CFX96TM Real-Time System (BIORAD, USA), Intercalation dye Syber Green included in Syber Green MasterMix (Light Cycler 480 SYBER Green, Roche, Diagnostics GmbH, Germany) were used. The experiment was done with 2 biological replicates and 3 technical replicates. Standard deviation of the mean was calculated.

Results and discussion

Influence of strigolactone on root architecture

Preliminary experiment has proven that the dependence of primary root length on concentration of GR24 is non-linear and strongly genotype dependent (Fig. 1).

Fig. 1 Primary root growth of ecotype Ren-11 after GR24 treatment

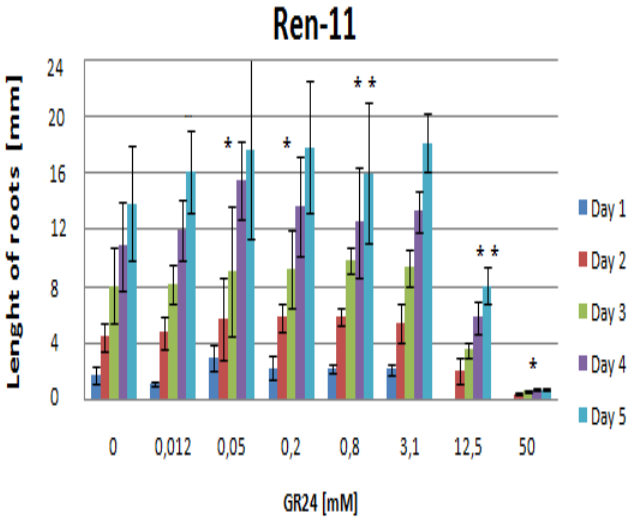


Table 1 Summary of primary root growth dependence on GR24 concentration for individual ecotypes

GR24 conc. (µM)	VOU-5	Wilcox-4	DraIII-1	Bå1-2	Ts-1	Ren-11
0.012	**	*		*		
0.05			*			*
0.2						*
0.8						**
3.1	**	*	**	**		
12.5	*	*	*	*	*	**
50	*	n/a	*	n/a	*	*

Legend	
	Variance less than 5%
	Shortening 5-10%
	Shortening more than 10%
	Elongation 5-10%
	Elongation more than 10%
*	*Statistically significant data*
**	*Statistically very significant data*

The randomly chosen 6 genotypes have shown a wide range of sensitivity caused by changes in the genotype. The data for ecotype Ren-11 (Tab. 1) represent an ecotype in which concentrations till 3.1 µM cause significant elongation of the primry root and only 2 highest conctentration of GR24 reverse the effect to signicicant inhibition of root growth. Ecotype VOU-5 represents another extreme situation where all used concentrations of GR24 caused primary root inhibition. The other analysed ecotypes form a continues transition between VOU-5 and Ren-11.

This results corespond with observed stimulation of primary root growth by 1 and 2.5 µM and inhibition by 5 and 10 µM [8] and by 27µM of GR24 [12]. Considering these results 100 nM concentration was chosen for GWAS study of GR24 influence on root architecture of *Arabidopsis thaliana* ecotypes.

GWAS analysis

For our experiment 242 ecotypes were selected for studying strigolactone influence on *Arabidopsis thaliana* root architecture.

Fig. 2 SNPs associated with primary root elongation by GR24 in selected ecotypes are marked in red area whereas in series of ecotypes under red line with root length shortening by GR24 SNPs are missing. Evaluated by Genome browser on 1001 Genomes site (http://signal.salk.edu/atg1001/3.0/gebrowser.php).

16 different traits in 9 time points were investigated and in the control-strigolactone ratio dataset 158 significant associations were found in 30 associated traits (Tab. 2). These 158 significant associated SNPs (P-value higher than 6) are located in promtor, coding or flanking regions (10 kb before and behind gene were considered) (Fig.2).

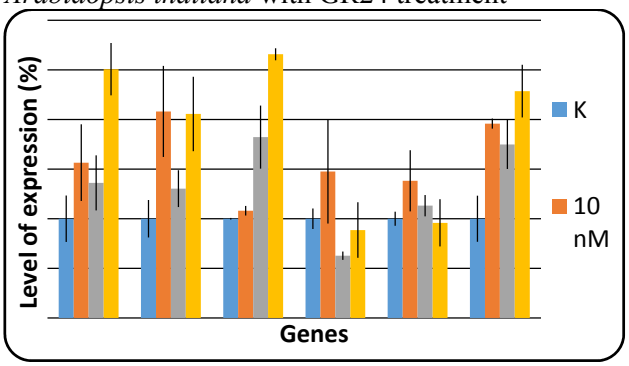
Table 2 Summary of associated traits found in the analysis

TRAIT	DAY
Total length	1, 2, 3, 4, 5
Euclidian length	1, 2, 3, 4, 5
Root tortuosity	4, average
Root growth rate	1-2, 4-5
Relative root growth rate	2-3, 4-5
Root angle	2, 3, 4, 5
Root directional equivalent	2
Root horizontal index	1
Root vertical index	1, 2, 3, 4, 5
Root linearity	1, 4
Average root width	-
Root width 20	-
Root width 40	-
Root width 60	-
Root width 80	1
Root width 100	-

Phenotypic variability of roots under strigolactone influence analyzed by GWAS revealed candidate genes involved in processes that caused the changes. The results were verified using comparison of SNPs associated with the traits by a genome browser on 1001 Genomes site (http://signal.salk.edu/atg1001/3.0/gebrowser.php) (Fig. 2). This analysis includes extreme phenotypes with associated trait and can be suggested as significant if this SNP is present only in one of the two extremes.

According to GWAS analysis results of 242 ecotypes of *Arabidopsis thaliana* and subsequent evaluation of significant associated SNPs 5 candidate genes were chosen for gene expression experiments. *AFB4* (AT4G24390) F-box protein involved in negative polar auxin transport regulation [13], *F-box* (AT5G27920), *F-box associated* (AT2G18780) and *Mitogen-activated Proteinkinase* (AT4G36450) may be considered to be involved in signaling pathway of strigolactone, and cooperate with auxin transport thereby regulating root growth. *BEN2* (AT1G77140) – vacuolar protein sorting 45 – is necessary for polar PIN localization [14].

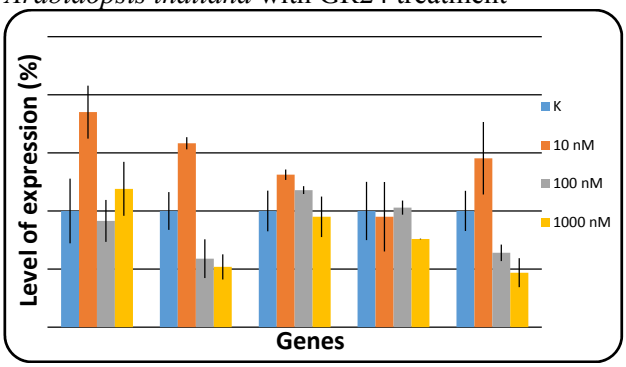
Fig. 3 Gene expresion of auxin transporters in roots *Arabidopsis thaliana* with GR24 treatment



Strigolactone effect on gene expression

The results demonstrate that strigolactone treatment led to non liner response of several genes as well as the primary root lenght response. In some cases the expression has typical bell-shaped response (*AFB4, PIN1, PIN2, PIN4, AUX1*) (Fig. 3,4). With the exception of *F-boxAs*.10 nM GR24 caused mostly significant increase in gene expression. Increase to 100 nM GR24 decreased the gene expression against the lower concentration and only in *PIN3* an increase was observed. Most differences were observed with the highest used concentration (1000 nM) where (*AFB4, PIN1, PIN2, PIN4, AUX1*) after increase of gene expression display the bell-shaped response. *PIN3* expression increases steadily. *PIN7 F-box, BEN2, MPK* and *F-boxAs* show a decrease at the highest concentration thus leading to the steadily decreasing character of response.

Fig. 4 Gene expression of GWAS genes in roots of *Arabidopsis thaliana* with GR24 treatment



Several data were published on influence of strigolactone on gene expression of genes directly involved in polar auxin transport but the conditions of the experiment diverge immensely.

Microarray analysis of gene expression after 90 minutes GR24 treatment [15] demonstrate that 76% of genes which prove increased expression due to GR24 influence are auxin-inducible genes [9]. Also they showed that cycloheximide (proteosynthesis

inhibitor) pretreatment doesn´t lead to changes of *PIN1:PIN1::GFP* fluorescence signal decline after GR24 which suggest that it is independent on the proteosynthesis. They assume that PIN1 decrease is transcription independent [9].

Similarly like our data for several *PINs* threefold induction in *PIN2* expression was detected in the WT but not in *max2-1* upon GR24 treatment, suggesting that the increase in *PIN2* signal under these conditions is at least partly a result of *PIN2* expression induced by the GR24 treatment in a MAX2-dependent fashion [16].
Other genes in our study were until now not analyzed.

Conclusion

Study of GR24 influence on polar auxin transport was realized throught analysis of changes of *Arabidopsis thaliana* root architecture.

According to genome-wide association study of 242 *Arabidpopsis thaliana* ecotypes 5 candidate genes that could be involved in strigolactone action were taken into gene expression analysis together with auxin tramsporters gene family (*PINs, AUX1*).

Taken together data have shown that expression of most of the auxin transporter genes show typical U-shaped response on strigolactone concentration and from the chosen genes only *AFB4* phenocopies this behavior. The other genes have steadily decreasing gene expression pattern which might be due to the different sensitivity to GR24 treatment and only further increase in GR24 could cause a U-shaped- response.

Acknowledgement

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Effects of plant growth regulators on proteome dynamics during seed germination

HANA HABANOVA
Department of Molecular Biology and Radiobiology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

habanova.h@seznam.cz

Abstract: Seed germination is a complex process during which an embryonic plant contained within a seed transforms into a seedling. The majority of species, including model plant *Arabidopsis thaliana* produce dry seeds that will fully re-establish metabolism after imbibition. This early phase of a plant's life is highly important for its survival and its progress depends on both internal and external stimuli. This thesis entitled „Effects of plant growth regulators on proteome dynamics during seed germination" is focused on effects of light and plant growth regulators on germination. To get an insight into molecular mechanism of germination, proteome dynamics in germinating *Arabidopsis* were investigated. Seeds were imbibed in presence of plant growth regulators in continuous light or dark and material for LC-MS proteome profiling was harvested after 24 and 48 hours. Altogether, abundances of more than 1100 proteins were followed. PCA analysis and a detailed pair-wise comparison of mock-treated seeds provided evidence that the modern proteome-wide analysis is a promising tool for plant germination research.

Key-Words: - seeds, growth regulators, proteomics, mass spectrometry

Introduction

Germination is a crucial phase of plant life which is influenced by many external and internal factors. These stimuli may affect a seedling's vitality and even have an impact on the adult plant yield. Among others, external environmental stimuli include the light intensity, water quantity, temperature or pH. The internal stimuli are more complex and include DNA integrity, damage to cellular structures or levels of individual plant hormones. Internal and external factors interact with each other during germination and create highly complicated signaling networks. It is crucial to understand particular processes and their links during germination not only for the basic research but also for the optimization of sustainable agricultural and horticultural practice [1].

At least part of the seed germination is regulated on protein level. For example, DELLA proteins must be ubiquitinated and degraded to promote the germination onset. There are many obstacles in seed proteome analysis. For example, seed contains a large portion of storage proteins that interfere with the detection of lower abundant proteins and limit the total number of detectable proteoforms. Still, proteome analysis is an important source of new data. Here, we show that the state-of-the-art modern proteomics offer new possibilities to comprehend the molecular mechanism of plant germination.

Material and Methods

Cultivation and hormonal treatment

Seeds of *Arabidopsis thaliana* ecotype Columbia (Col-0) were surface-sterilized (using 75% ethanol) and sown on a Whatman filter paper rinsed with distilled water supplemented with DMSO (0.01% v/v) or selected growth regulator (abscisic acid, ABA; gibberellin GA3; cytokinin trans-zeatin, tZ; auxin indole-3-acetic acid, IAA; karrikin KAR1) to the final concentration of 1 μM .

Table 1 Scheme of the experiment

	light	darkness
24h	IAA, tZ, ABA, GA3, KAR, DMSO	IAA, tZ, ABA, GA3, KAR, DMSO
48h	IAA, tZ, ABA, GA3, KAR, DMSO	IAA, tZ, ABA, GA3, KAR, DMSO
dry seeds	-	-

The seeds were cultivated at 20°C for 48 hours at continuous light (80 μmol m^{-2} s^{-1}) or covered with aluminium foil to simulate germination in the

absence of light. Germinating seeds were sampled in two aliquots after 24 and 48 hours. In total we prepared 26 samples including control aliquot of dry seeds (Table 1).

LC-MS proteome profiling

Proteomic analyses were performed using a gel-free shotgun protocol based on nano-HPLC and MS/MS [2]. Briefly, proteins were extracted by a combination of acetone/TCA and phenol extraction then digested in solution with endoproteinase Lys-C and immobilized trypsin beads (Promega). The resulting peptides were desalted, dried and dissolved in 0.5% (v/v) formic acid in 5% (v/v) acetonitrile, then analyzed online by nanoflow C18 reverse-phase liquid chromatography using a 15 cm Ascentis Express Column (0.1 mm inner diameter; Sigma-Aldrich) and a Dionex Ultimate 3000 RSLC nano UPLC system (Thermo) directly coupled to a nanoESI source CaptiveSpray (Bruker) and an UHR maXis impact q-TOF mass spectrometer (Bruker). Peptides were eluted with a 60-min, 4% to 35% acetonitrile gradient. Raw files obtained from the MS analysis were analyzed by Profile Analysis 2.1 (Bruker) and MS precursors with significant differences (absolute ratio ≥1.5, with t-test p-values <0.05) were targeted and identified in consecutive MS/MS analyses. Peptide spectra were searched against the TAIR10 Arabidopsis database using the Mascot algorithm and Bruker's ProteinScape inbuilt percolator algorithm (target FDR<1%). Only high confidence peptides (p<0.05) with better than 10 ppm precursor mass accuracy and at least one distinct proteotypic peptide per protein met identification criteria. Quantitative differences were further manually validated by comparing respective peptide ion signal peak areas in Skyline 1.4 (MacCossLab Software; available on https://skyline.gs.washington.edu).

Data analysis

Information about protein/metabolite function(s) was collected from the UniProt database, (http://www.uniprot.org/) UniGene database (http://www.ncbi.nlm.nih.gov/unigene), TAIR database (http://www.arabidopsis.org), a conserved domains search (http://www.ncbi.nlm.nih.gov/Structure/index.shtml), a homology search (http://blast.ncbi.nlm.nih.gov/Blast.cgi), Kyoto Encyclopedia of Genes and Genomes (http://www.kegg.jp/kegg/), GeneVestigator (NEBION AG, http://www.genevestigator.com; Hruz et al., 2008), and literature.

Results and Discussion

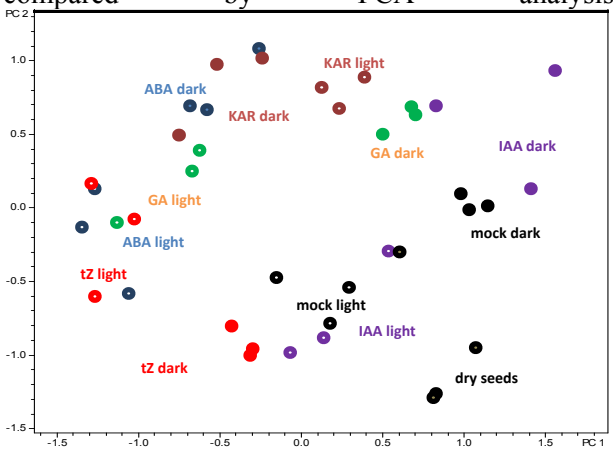
Plant material was prepared and harvested as described in Materials and Methods. Three time points were selected that correspond to dormant seed, phase I and phase II of germination [3, 7]. It is well known that plant growth and development is regulated by substances designated as plant hormones or plant growth regulators [4]. The interplay of these substances is also crucial for timing and progress of germination. Here, we followed effects of four major hormones and one recently discovered growth regulator originating from smoke of the burning vegetation.

Comparison of proteome-wide changes by Principal Component Analysis

Principal Component Analysis (PCA) is a statistical method that can be used to cluster samples according to the distribution of their characteristics. In this case, the raw data from LC-MS analyses are processed and intensity values for distinct m/z within a specific retention time window (buckets) are calculated. These values are than used for PCA analysis. Most of the buckets originate from protein digestion, but even those that are of non-protein origin can be used to characterize sample. The visualization of PCA results is presented in Figure 1.

The biplot in Figure 1 explains more than 40% of system variance and (with certain level of confidence) can be used to draw some preliminary conclusions about similarities and differences between individual treatments. For example, we can conclude that the sample treated with cytokinin and cultivated in dark has similar characteristics to the mock-treated sample cultivated in light. This would indicate that cytokinin treatment at least partially compensates the absence of light in germinating seeds.

Fig. 1 Proteome of germinating Arabidopsis seeds compared by PCA analysis

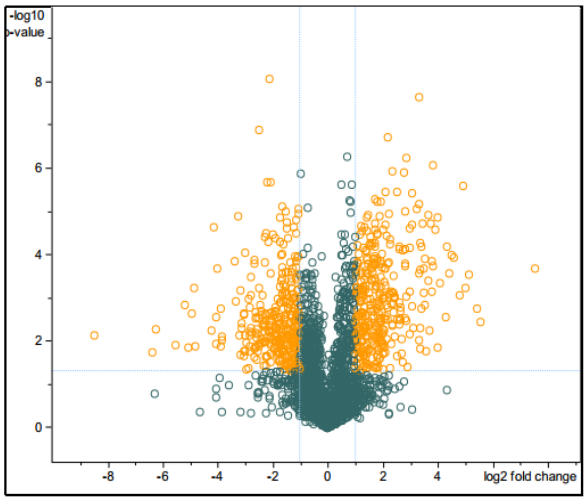


Even though the PCA analysis presented only a very simplified view of complex germination process, it outlines the benefits of proteome analysis and clearly demonstrates the power in proteomics approach to study seed germination.

Pair-wise comparison

To get more in-depth results, samples were compared in a pair-wise comparison. Here, mock treated samples will be discussed. T-test analysis

Fig. 2 Volcano plot representing differentially abundant buckets found in proteome of mock treated seeds germinating in light or dark.

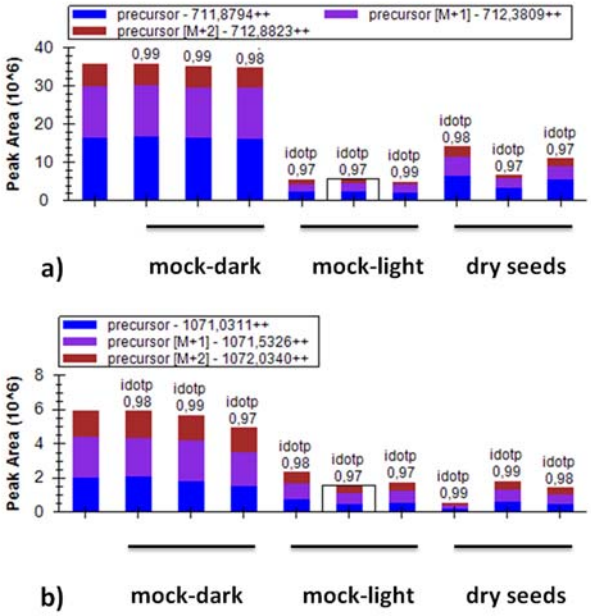


revealed that germination in the absence of light has a significant effect on a large number of buckets (Figure 2). In numbers, 1565 out of 2876 showed significant ($p<0.05$, absolute ratio <2.00) differences. The following automatic assignment of MS/MS spectra identified 2858 peptides that represented more than 1100 identified and quantified proteins.

Dark induced accumulation of Aspartate protease APA1 during seed germination

Proteome analysis revealed (among others) accumulation of protease APA1 in dark-grown *Arabidopsis seeds.* This protease is apparently involved in the breakdown of propeptides of storage proteins in protein-storage vacuoles and is supposedly activated during senescence and in response to light [5, 6]. In our experiments, APA1 levels in light grown seeds are similar to that of dry seed (Figure 3). Germination in the absence of light induces accumulation of APA1 and thus it is likely that this enzyme could be important in processes preserving seed viability prior the onset of autotrophic metabolism.

Fig. 3 Graphs shoving peak areas of two regulated peptides of APA1 protein. The first one (a) shows the intensity of peptide with the amino acid sequence K.VFDLAPEEYVLK. The second graph (b) shows the peak area intensity of peptide with the amino acid sequence K.NYLDAQYYGEIAIGTPPQK.F.



Conclusion

In conclusion, we have used LC-MS proteome profiling to analyze *Arabidopsis* germination. Our results illustrate that proteomics can provide insight into plant germination and indicate some novel aspects of hormonal regulation in this process.

Acknowledgement

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Growth response of *Lemna minor* L. to paracetamol

MARKETA HAJKOVA, MARIE KUMMEROVA
Department of Experimental biology
Masaryk University in Brno
Kamenice 753/5, 625 00 Brno
CZECH REPUBLIC

hajkova.marketa@email.cz

Abstract: Surface water and groundwater is contaminated with pseudoperistent xenobiotics present in the environment. The risk associated with an increase in the consumption of drugs and their permanent occurrence especially in the aquatic environment falls on non-target organisms. The aim of this thesis was to assess the effect of increasing concentrations of paracetamol (0.1; 10; 100 μg/l) on the growth and selected physiological parameters of the model organism *Lemna minor* (L.). Duckweed, as an important model plant for ecotoxicological research, has been subjected to a semichronic exposure to paracetamol.
The presence of a stressor – paracetamol – after ten days of cultivation significantly influenced some growth and physiological parameters. It has been shown to significantly reduce the content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids), increase the value of non-photochemical quenching of chlorophyll fluorescence (NPQ), and lower the relative decline of chlorophyll fluorescence (Rfd). The results show that the increased load of paracetamol in the environment may negatively affect the growth of duckweed.

Key-Words: -chlorophyll fluorescence, duckweed, growth, *Lemna minor*, paracetamol, pharmaceuticals, photosynthetic pigments, phytotoxicity tests

Introduction

Plants have the ability to accumulate in their tissues toxic metals and organic pollutants, which can cause changes in growth and morphology. At present, drugs or pharmaceuticals include among the substances hazardous to living organisms [1, 2]. It is primarily about environmental contamination and wastewater sludge. It was found that in addition to acidification and eutrophication of soils and water may also indirectly contribute to the loss of plant and animal species. Paracetamol, often used as a representative of analgesics and antipyretics, is among the candidates for an analysis of the effect on a model organism *Lemna minor* (L.).

Pharmaceutically active substances are detected in surface water, groundwater and soil, wherein their concentration ranges from ng/l in μg/l [3], to small flows to 1 mg/l. These mobile elements of pollution enter the environment continuously [4] and their effect depends on the time during which the organism operates [5]. Studies also demonstrate that due to the high polarity and low volatility drugs bioaccumulate and are persistent in the environment [6].

Paracetamol is in 75% excreted as conjugates and in the remaining percentage the original substance persists [7]. The active paracetamol in these environments is easily transformed from conjugates thanks to the activities of microbial metabolism. It is assumed that paracetamol pseudo-persistent in the environment [8]. The concentration of analgesics which include paracetamol in the aquatic environment varies between 0.0016 and 373 μg/l [9]. The average concentration of paracetamol was calculated in this study at 246 μg/l. Paracetamol and ibuprofen at the same time showed the highest mean values of the inflow to municipal wastewater treatment plants (38 and 37 μg/l respectively). Other researchers have found that the highest concentration reaches paracetamol in Europe compared with other continents (influent concentrations ranged from 59-220 ng/l, the concentration in rivers range from 12 to 777 ng/l) [10].

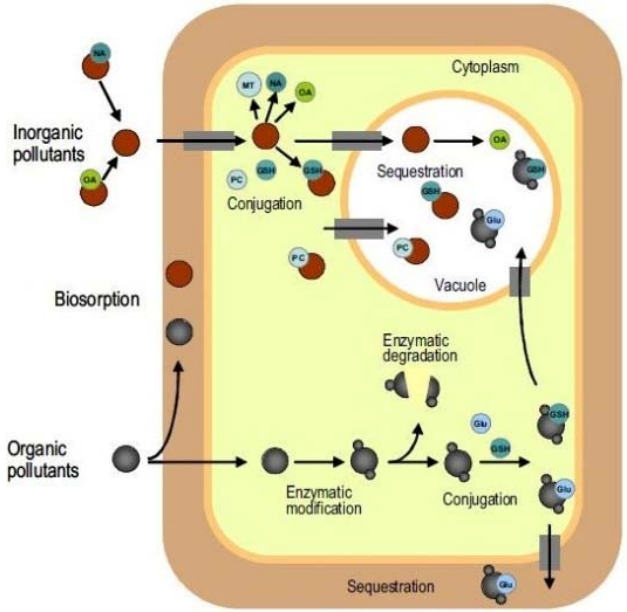
Removal of paracetamol in the WWTP is very effective in the order of 95% [11], yet the gate is paracetamol into the environment continuously. The concentration of paracetamol, which are normally determined in surface water can cause serious chronic effects on aquatic organisms [12]. Paracetamol has compared to ibuprofen a very low Kow value (0.40), is more accessible and therefore more toxic [13]. In particular, induction of oxidative stress may reduce the ability of effective degradation of paracetamol and other xenobiotics [14].

Hydrophyte communities - aquatic plants - form an important ecological element in the nutrient cycle.

They are part of the food chain of aquatic communities [15]. Interventions in the niches of aquatic plants may also have a negative impact on the surrounding biotes, including the quality of drinking water [16].

To protect water and aquatic ecosystems, which have far-reaching consequences for the entire biosphere, it is necessary to conduct toxicity testing of new and already used substances on aquatic plants. A preferred model organism for this purposewidely used due to its sensitivity and ability to accumulate is the higher aquatic vascular plant duckweed (*Lemna minor* L.)[17].

Fig. 1 Tolerance mechanism for inorganic and organic pollutants in *Lemna* cells.



Detoxification involves active sequestration in parts that can do least harm. The chelators involved are: glutathione (GSH); glucose (Glu); metallothioneins (MT); nicotinamines (NA); organic acids (OA) and polychelatines. This is the reason why Lemna species accumulate high concentration of contaminants. Taken from [24].

Duckweed is used for the assessment of the ecotoxicity of target compounds in aquatic communities, mainly due to easy cultivation in the laboratory, small size, rapid vegetative propagation and reduced organization [18]. Sensitivity ofcommon duckweed against often tested substances were compared with selected representatives of fauna and significantly clearer evidence of the presence of some toxic metals [19], pesticides [20] and pollutants, which are part of the wastewater [21] were obtained.

The conventional tests are carried out on the plant growth inhibition test by common duckweed growth curve. For ecotoxicological studies, this biomarker provides basic data and information on the toxicity of substances or mixtures in aquatic ecosystem. Provides accurate data for determining the ecotoxicity of drugs or other substances [22]. The main advantage of this test lies in the plasticity of use and relatively low cost [23].

The aim of this paper is to analyse the effect of increasing concentration of paracetamol in a nutrient medium for vegetative growth of common duckweed. Paracetamol concentrations were chosen to correspond with the inlet into the continuous natural environment, and that the highest chosen concentration simulates a moderate degree of loading.

Material and Methods

Characterization of the model organism *Lemna minor* (L.) and experimental design

Cultivation of duckweed (*Lemna minor* L.) was carried out under controlled conditions (temperature 22 ± 2˚C, relative air humidity 60%, photoperiod 12/12, irradiance 150 μmol m^{-2} s^{-1} provided by white fluorescent tubes, Osram, Germany). The experiment was run in transparent 6-well plates (well volume of 12 ml, well surface area 9.62 cm^2; NUNC A/S, Denmark). Duckweed plants were placed into the plates (6 per well, i.e. 36 plants per plate) and cultivated in SM (10 ml per well) without (control) or with an addition of paracetamol (0.1 – 10 – 100 μgl^{-1}). Each treatment was represented by 6 plates. At the beginning of the experiment (day 0) and after 4 and 10 days of the cultivation the number of plants per well, fresh weight per plant, dry weight per plant, leaf area size per plant, the content of photosynthetic pigments per biomass unit and selected parameters of chlorophyll fluorescence of plants per well were assessed in each treatment.

Growth analysis

The number of duckweed plats was counted in each single well in all treatments and repetitions. Plants were then dried at 85˚C for 6 h and their dry weight was determined and expressed as the mean per plant. Images captured during the chlorophyll fluorescence measurements (seeSection Chlorophyll fluorescence measurements) were used for evaluation of size of leaf area. Values (as given in Fig.) represent the average leaf area per plant.

Chlorophyll fluorescence measurement

A set of chlorophyll fluorescence parameters (F_0 – basal chlorophyll fluorescence, F_V/F_M – potential yield of photochemical reactions in photosystem II, Φ_{II} – effective quantum yield of photosystem II, NPQ

– non-photochemical quenching, Rfd - ratio of chlorophyll fluorescence decrease) was determined from an analysis of slow kinetics supplemented with saturation pulses (recorded by a fluorescence imaging system HandyFluorCam, PSI, Czech Republic). This tool measured whole plates from each treatment in only one step. Measuring setup and calculation of selected parameters were adopted from Kummerová *et al.* (2007) [23]. The measurements were done at ambient temperature 21 ± 2 ˚C.

Analysis of photosynthetic pigments

For an analysis of photosynthetic pigments (chlorophyll *a* and *b*, carotenoids) approx. 0.03 g fresh duckweed plants (equal to approx. 36 plants) per treatment were extracted with 100% acetone and the pigment content was measured spectrophotometrically (UV-VIS Spectrophotometer SPECORD 205, Jena, Germany) and calculated according to Lichtenthaler (1987) [25]. Pigment analysis was repeated three times for each treatment.

Statistics

For statistical evaluation of results, the software STATISTICA 10 (StatSoft Inc.®) was used. The results are means of at least three repetitions forall assessed parameters. The significance of the difference of the average values between the treatments was evaluated by one-way and multifactorial analysis of variance after verification of normality (Shapiro-Wilk test) and homogeneity (Cochran, Hartley, Bartlett test) of data variance (ANOVA, $P < 0.05$). The comparison of means was based on Scheffe test ($P \leq 0.05$).

Table 1 Results of selected parameters of chlorophyll fluorescence

Treatment (µg/l)	Day of cultivation	F_0	NPQ	Rfd
Control	0	70.56 ± 6.40	0.14 ± 0.01	0.97 ± 0.09
Control	10	69.16 ± 5.72^a	0.13 ± 0.01^a	0.91 ± 0.07^a
0.1	10	71.89 ± 6.59^a	0.26 ± 0.02^b	1.07 ± 0.09^b
10	10	79.37 ± 6.39^a	0.22 ± 0.02^c	0.52 ± 0.01^c
100	10	74.62 ± 6.43^a	0.22 ± 0.02^c	0.51 ± 0.04^c

Results and Discussion

In duckweed phytotoxicity tests usually the plant number and at least one more measurement variable, such as the total leaf area, fresh dry weight orthe content of photosynthetic pigments are assessed (OECD, 2006; ČSN EN ISO, 20079, 2007) [26]. In this study after 10 days of culture, duckweed did not demonstrate growth inhibition for any of the concentrations of paracetamol (0.1 to 100 µg/l). Number of common duckweed plants during the experiment in the presence of paracetamol in comparison with control did not change significantly.

It is known that lower concentrations of xenobiotics can stimulate plantsfor a short period, as evidenced by the significant increase in the fresh weight of duckweed plants after 10 days of culture at a load of 10 µg/l of paracetamol. However, in dry weight there were not detected significant differences in this nor in the other investigated variants.It can be assumed that the higher fresh weight related in this case only with higher water content in the tissues of duckweed. Also, a statistically significant increase in leaf area, which was shown at this load (10 µg/l paracetamol) compared to the control variant, may just be associated with increased water intake, which can lead to increased volume of cells.

In many studies, induction of oxidative stress by paracetamol with representatives of both vertebrates and invertebrates was observed [14]. Substance-induced oxidative stress can also contribute to inhibition of plant growth, as found in wheat plants or cucumber (*Cucumis sativus*). Zhang *et al.* [27] found that oxidative damage induced by cadmium was associated with decreased activity of antioxidant enzymes, which leads to increased production of oxygen free radicals which destabilize membranes. Paracetamol may, however, in the plant body behave differently. So far there are no relevant studies to confirm the results obtained in animal models.

Content of chlorophyll *a* and *b*, carotenoids

One of the negative consequences of the presence of xenobiotics is the reduction of content photosynthetic pigments [28]. Significant reduction of chlorophyll *a*, chlorophyll *b* and carotenoids when compared to control was observed after 10 days of duckweed culture, (Fig. 2, 3, 4) already at the lowest concentrations of paracetamol applied (0.1 µg/l).

Fig. 2 Content of chlorophyll *a* after 10 days cultivation.

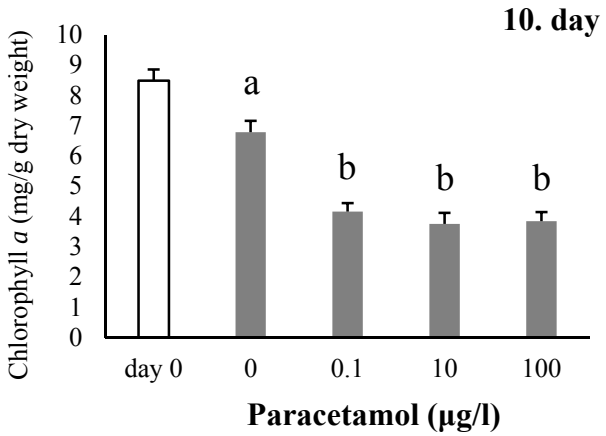
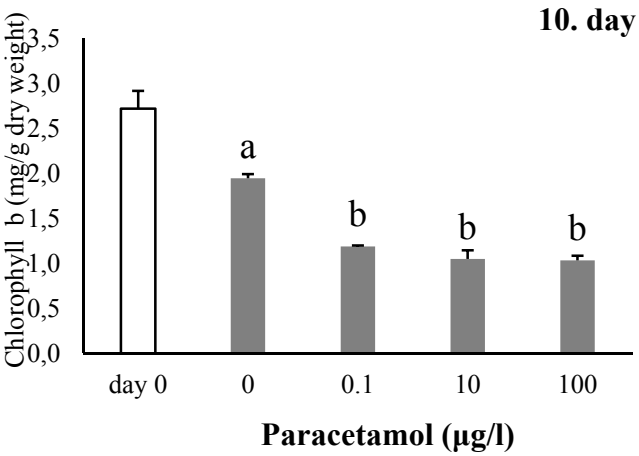
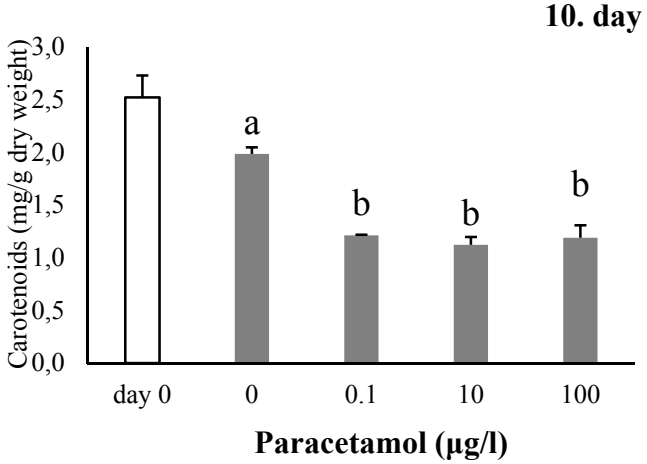


Fig. 3 Content of chlorophyll *b* after 10 days cultivation.



Azevedo *et al.* [29] reported that the reduction in chlorophyll content in leaves may occur both as a result of enzymatic degradation of these pigments (but none of the genes encoding the catabolic enzymes has yet been isolated), or by inhibiting their biosynthesis. This suggests that it may be one of the primary sites of xenobiotic metabolism toxicityin plants [30]. Carotenoids are according Hannoufa *et* Hossain [31] an important component of the photosynthetic systems. They are involved in both light absorption and protection of the chlorophyll molecule and plastid membranes from destruction by quenching of the triplet state of chlorophyll byremoving oxygen in an excited oxygen-chlorophyll complex as antioxidants [32]. In our study, a significant reduction in the content of carotenoidsof duckweed plants was observed, which thus cannot effectively protect the chlorophyll molecule. Reduced protective effect of carotenoids is also evident from the increase in the ratio of chl*a*+*b*/carotenoids, which was recorded in comparison with the control.

Fig. 4 Contend of carotenids after 10 days cultivation.



Parameters of chlorophyll fluorescence

The value of minimal chlorophyll fluorescence (F_0) is due to stress conditions increases, while the effective quantum yield (Φ_{II}), expressing the rate of ongoing primary processes of photosynthesis, usually due to a stressor decreases. In our study, the value of basic fluorescence after 10 days of cultivation of duckweed in the presence of paracetamol did not change significantly (Table 1). No significant changes were observed either in terms of the maximum quantum yield of PSII (F_V/F_M) and effective quantum yield of PSII (Φ_{II}). We believe that the values of fluorescence were not sensitive enough to ensure that they are reflected in a reduced content of photosynthetic pigments.

Many authors have pointed out that the increase of non-photochemical quenching (NPQ) correlates with the presence of various stressors [33]. In our study, the value of this parameter compared to control increased significantly after 4 and 10 days of culturealready at the lowest concentration (Table 1). These results demonstrate the possible stress effect of paracetamol.

Decrease of the relative chlorophyll fluorescence (Rfd) indicates higher levels of stress. This so-called vitality index is sensitive to the presence of a stressor more than the quantum yields. But it is not still clear, in what section of the photosynthetic apparatus stressor acts [34]. After ten days of culture, duckweed (Table 1) showed a significant increase in the value of the variant with 0.1 µg/l of paracetamol, which may be related to the mechanisms of acclimation to the stressor, and a significant reduction in the value of other loads (10 and 100 µg/l paracetamol).

The results presented in this paper contribute to the understanding of the impact of pharmaceuticals in plants, which are an important group of pollutants. This study evaluates the effect of paracetamol on growth and selected physiological parameters and their values however, reflect the changes occurring at the biochemical level. It can be assumed that the observed minor changes may be related to the low efficiency of selected concentrations of paracetamol on duckweed and the capacity of its defense mechanisms. It is clear, however, that due to the increasing environmental pollution by drugs and their unclear toxicological effects, special attention must be paid to this area.

Conclusion

Effect of increasing concentrations of paracetamol on the monitored physiological parameters of growth becamemore visible after ten days of cultivation. A significant increase in leaf area at all paracetamol concentrations (0.1 - 100 µg/l) was observed. Significant reduction of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids) has been shown to apply already at the lowest concentration (0.1 µg/l paracetamol). The value of non-photochemical quenching of chlorophyll fluorescence (NPQ) for all paracetamolconcentrations increased, and the relative decline of chlorophyll fluorescence (Rfd) with 10 µg/l and 100 µg/l of paracetamoldecreased, which indicates the presence and impact of stressors.

The results obtained suggest that the increased paracetamol load in the environmentcan negatively affect the growth of common duckweed. In the future, it would be appropriate to focus on study ofactivities and content of selected enzymes involved in antioxidative mechanisms.

Acknowledgement

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The impact of humic substances on oxidative stress and plant growth of spring barley exposed to NaCl

MARKETA JAROSOVA[1], BORIVOJ KLEJDUS[1,2], JOZEF KOVACIK[1,2], JOSEF HEDBAVNY[1]
[1]Department of Chemistry and Biochemistry
[2]CEITEC – Central European Institute of Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jarosoma@seznam.cz

Abstract: Humic acid is commercial product that contains many elements which improve the soil fertility and increase the biological availability of nutrients for plants. It consequently affects plant growth, yield and ameliorates the deleterious effects of salt stress. The impact of hydroponically applied humic acids on the growth and nutrient uptake of barley (*Hordeum vulgare*, Poaceae) plants grown at different salt concentrations was investigated. Sodium salts (chloride and sulphate) were added to distilled water to obtain 100 mM saline solutions. Humic acids were applied in different doses (0.01 and 0.1%) to the growing media with sodium salt or alone. Salinity negatively affected the plant growth and uptake of nutrient elements except Na and K. The interaction between applied salt and humic acids showed the increase of nutrient uptake. Humic acids represent the opportunity for the development of the agricultural productivity negatively affected by saline conditions.

Key-Words: salinity, humic substances, barley, plant growth parameter, nutrient content

Introduction

Salinity is the one of the major stressors limiting the agricultural production worldwide [1]. Salinity is caused by increased activity of soluble salts in the soil solution with electrical conductivity exceeding 4 $dS.m^{-1}$ [2]. Salinity is responsible for the plant cell dehydration, imbalance of water potential between cells and surrounding soil solution and turgor reduction. The intracellular osmotic potential can be reduced by the ability of plants to accumulate Na and Cl ions. High concentration of salt ions in cell leads to the inhibition of the mineral nutrient uptake [3], inactivation and degradation of cytoplasmatic enzymes and induction of low molecular highly hydrophilic organic substances. Ion concentration in cells can also affect a variety of enzymes involved in cell signaling, ion transport, energy metabolism (photosynthesis, ATP synthesis, respiration), carbohydrate and lipid metabolism, hormones (e. g. jasmonic acid, gibberelin, abscisic acid) and secondary metabolites synthesis [4, 5, 6, 7].

The opportunity for the improvement of agricultural production which can help to enhance the crop productivity and ensure the soil protection is the application of humic substances. This under-researched area of organic substances is a mixture of humin, humic acids, fulvic and hematomelanic acids. Humates are created by three different ways – from leonardite, peat or lake sapronel and lignosulphonate (so-called lignohumate) [8]. Lignohumates have a positive effect on photosynthesis, respiration intensity, root system formation, aboveground biomass development and seed germination [9, 10]. Further abilities of humic substances are the reduction of stress conditions induced by different abiotic stressors (e. g. unfavorable temperature, pH and salinity), the enhancement of nutrient uptake and the reduction of toxic metal intake from soil solution [9, 11, 12, 13].

The aim of the present study is to investigate the impact of humic acids to decrease the negative effects of sodium chloride on the growth and nutrient uptake of barley plants (*Hordeum vulgare* L., Poaceae) under salinity stress in controlled laboratory conditions.

Materials and Methods

Plant Material and Experimental Design

Barley seeds were germinated four days in water on Petri dishes with filter paper. Then, uniform plants were repotted to the small boxes (25 plants per box) with boiling beads and filter paper using different cultivation solutions under controlled laboratory conditions: 12 h day (8.00 am. to 8.00 pm.), photon flux density was 210 $\mu molm^{-2}$ s^{-1} at leaf level,

25/20°C day/night temperature and a relative humidity of ~60% [14].

For the experiments were used three different cultivars of spring barley – Bojos (B), Xanadu (X) and Radegast (R). Four-days old plants were exposed to five different treatments: (i) 100 mM NaCl, (ii) 0.01% humic acid (Sigma Aldrich, USA), (iii) 0.1% humic acid, (iv) 100 mM NaCl+0.01% humic acid and (v) 100 mM NaCl+0.1% humic acid. Simultaneously, three other experiments were performed: (i) with corrected pH to 4.0 or 7.0 under the same conditions as describes below, (ii) with using two different salts (100 mM NaCl and 100 mM Na_2SO_4) and pH (4.0 and 7.0) and (iii) cultivar Radegast exposed to 100 mM NaCl and humic acid (0.01 and 0.1%) originating from commercially available Lignohumate (Amagro, Czech Republic) and from Sigma Aldrich in technical quality. Results were compared to blank treatment where distilled water was used.

Plants were harvested after one week of exposure to these treatments. The fresh material was at first measured for root and shoots length, then dried on filter paper (in the case of roots) and considered. For the estimation of plant water content were samples dried at 75°C to constant weight (100-(dry mass x 100/fresh mass)). The quality of germination was monitored using germination energy ($n_{24}+n_{48}+n_{72}$), germination index $((10(n_{24}+n_{48}+n_{72}))/(n_{24}+2n_{48}+3n_{72}))$, mean germination time $((n_{24}+2n_{48}+3n_{72})/ n_{24}+n_{48}+n_{72}))$ and germination rate $((5n_{24}+3n_{48}+n_{72})/20)$.

Quantification of Mineral Nutrients

Dry material was mineralized by mixture of concentrated ultra-pure HNO_3 and water in microwave extractor (Ethos Sel Microwave Extraction Labstation, Milestone Inc.) at 200 °C over 1 h. The clear mineralization outcome was quantitatively placed to plastic flasks and diluted to a final volume of 10 ml. Measurements were performed using an atomic absorption spectrometer AA30 (Varian Ltd., Mulgrave, Australia) and the air-acetylene flame. The mixture of HNO_3 and water used as a blank was also checked to ensure correctness of mineral quantifications [15, 16].

Statistical analyses

For the evaluation of significance of differences in parameters was used ANOVA followed by a Tukey's test (MINITAB Release 11, Minitab Inc., State College, Pennsylvania). Number of replications (*n*) in tables/figures denotes individual plants (root and shoot) measured for each parameter. Two independent repetitions were performed to ensure the reproducibility.

Results and Discussion

Humic acids are technically not a fertilizer, but they can be used as a suitable complement to organic and synthetic fertilizers. The application of humic substances along with fertilizer can reduce its required doses. Plant growth is dependent on the essential and non-essential nutrient uptake. Humic acids are able to chelate micronutrients and thus ensure their biological availability. Negative consequences of NaCl were described above. According to Khaled and Fawy [17], after the application of doses higher than 60 mM to reduction of calcium and potassium contents in corn occurs. The same reduction of mineral elements uptake was observed by Asik et al. [12] in wheat. Our analyzed data have shown that applied 100 mM NaCl increases the content of sodium ions (in pH 7.0) (Fig. 1). The accumulation of Na and Cl ions was also observed by Cimrin et al. [18]. The impact of lower pH in salt solution was observed. Acidity induced the enhancement of K^+ content. The same action was induced in neutral pH, when sodium ions were applied as sulphates. (Fig. 1).

Salinity decreased the maximal height of barley leaves (leaves and stems), which was by application of 100 mM NaCl reduced to 40%.

Dudeck et al. [19] found that plant aboveground biomass is more affected by NaCl effect than the root system, whose growth is not almost reduced. The impact of sodium chloride in neutral pH had not so visible effect on the leaf growth (Tab. 1). Salinity stress leads to the reduction of leaf water content (leaves with stems) which was also observed by Reina-Sánchez et al. [20]. Differences between NaCl- and Na2SO4-exposed plants were observed not only in the case of leaf height and total water content, but also in grain germination quality, which was monitored by germination energy, germination index, mean germination time and germination rate during three days. Acidic and neutral pH reduced the germination energy and other parameters, which were caltulated from the same input data, compared to control.

The most significant differences were observed in cultivar Radegast whose germination energy was higher than 90 % in control plants (Table 2).

Table 1 The impact of Na^+ salts on the aboveground biomass production and tissue water content of different cultivars of *Hordeum vulgare*.

		Control		100 mM NaCl		100 mM Na_2SO_4	
		pH 4.0	pH 7.0	pH 4.0	pH 7.0	pH 4.0	pH 7.0
BOJOS	**height (cm, *n*=3)**	13.2 ± 0.36^a	15.2 ± 0.60^a	6.23 ± 0.15^b	8.97 ± 0.40^c	13.4 ± 0.25^a	11.7 ± 0.26^b
	water content (%, *n*=3)	88.1 ± 3.49^a	84.9 ± 6.65^a	81.3 ± 6.85^{ab}	81.6 ± 4.65^a	75.9 ± 5.59^b	86.1 ± 5.22^a
XANADU	**height (cm, *n*=3)**	14.8 ± 0.74^a	16.2 ± 1.21^a	1.60 ± 0.53^c	9.87 ± 0.31^c	6.5 ± 1.01^b	13.3 ± 0.46^b
	water content (%, *n*=3)	77.9 ± 2.59^a	87.3 ± 6.17^a	63.7 ± 5.57^b	79.3 ± 3.01^a	69.3 ± 9.10^{ab}	87.2 ± 9.35^a
RADEGAST	**height (cm, *n*=3)**	14.2 ± 0.25^a	16.1 ± 0.35^a	3.37 ± 0.29^c	11.4 ± 0.75^c	10.7 ± 0.76^b	14.1 ± 0.72^b
	water content (%, *n*=3)	93.1 ± 2.60^a	96.7 ± 1.84^a	58.9 ± 3.95^b	79.9 ± 1.50^b	88.2 ± 2.70^a	82.3 ± 1.98^b

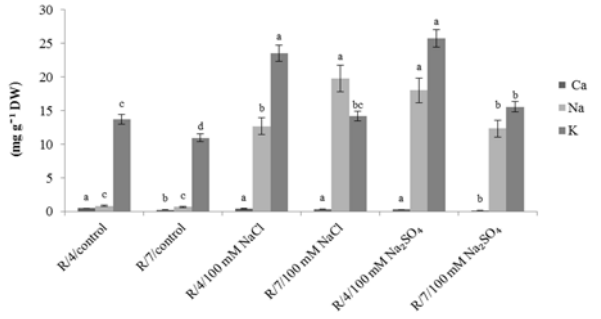
Data are means±SDs. Values within rows, followed by the same letter(s), are not significantly different according to Tukey's test ($P < 0.05$).

Table 2 Changes in germination quality of three barley cultivars exposed to different salt conditions.

	BOJOS						XANADU						RADEGAST					
	pH 4			pH 7			pH 4			pH 7			pH 4			pH 7		
	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4
GE (%)	90.6	60.3	35.7	90.2	65.9	35.3	85.9	45.7	50.2	85.9	50.1	40.3	90.1	40.7	20.8	95.1	45.7	55.3
GI	5.81	4.14	4.38	6.20	4.33	5.38	6.29	4.09	4.54	6.07	3.84	3.80	6.42	4.21	5.10	5.58	3.91	3.67
MGT (d)	1.72	2.42	2.28	1.61	2.30	1.85	1.58	2.44	2.2	1.64	2.6	2.62	1.55	2.37	2.35	1.78	2.55	2.72
GR (%)	2.56	1.04	0.68	2.72	1.24	0.92	2.6	0.76	1.04	2.52	0.72	0.56	2.8	0.72	0.48	2.6	0.68	0.68

Sodium salts were applied as 100 mM NaCl and 100 mM Na_2SO_4 into the distilled water and subsequently pH was corrected to 4.0 or 7.0. The germination was observed 72 h (*n*=3). Legend: GE - germination energy, GI - germination index, MGT - mean germination time and GR - germination rate

Fig. 1 The accumulation of selected mineral nutrients in *Hordeum vulgare* leaves exposed to different Na^+ salts for one week (*n*=3). Data are means±SDs. Values for each parameter, followed by the same letter(s), are not significantly different according to Tukey's test ($P < 0.05$).



The most significant differences were observed in cultivar Radegast whose germination energy was higher than 90 % in control plants (Table 2). The application of salts reduced the observed germination parameters, regardless of the used pH. The cultivar Radegast was chosen as the most suitable object to prove the positive effect of humic acids in agricultural production negatively affected by salinity to improve the germination, nutrient uptake ability and plant growth.

It was found that application of humic acids enhance the uptake of plant nutrients in 100 mM NaCl treatments. According Khan et al. [21] and Kurban et al. [22], small amounts of NaCl may cause a stimulative effect on the growth and nutrient uptake, but the increasing concentration have a toxic effects. In our study used concentration of NaCl caused the growth deceleration (Fig. 3). Many researchers studied the ability of NaCl to reduce the uptake of K^+ in plants due to the competetive process by Na^+ [23]. After the application of humic acids into the NaCl solution, the potassium content increased (Fig. 2). The content of Ca ions slightly decreased (Table 2) due to its antagonistic effect on the uptake of Na^+ [24]. In cultivars Bojos and Radegast are humic acids able to stimulate ion uptake. The ability of humic acids to enhance the nutrient uptake may be caused by fact that humic acids can interact with the phospholipid structures of the membranes and react as carriers of nutrients through them [17]. As well as Lee and Bartlett [25], with increasing concentration of humic acids the nutrient uptake does not increase (Fig. 2). We studied the impact of two types of humic acids. Humic acids in technical quality purchased from Sigma Aldrich induced the leaf growth in all treatments, but did not achieve the results set out in

control plants. The same was found in the determination of water content (Table 3). The nutrient accumulation can be dependent on used humic substances. Although, the content of Na ions was not affected by the origin of humic acids, K^+ content increased after the addition of Lignohumate (Fig. 4).

Fig. 2 The accumulation of selected mineral nutrients in *Hordeum vulgare* leaves exposed to different treatments for one week (*n=3*). Three cultivars were studied – Bojos (Fig. 2A), Xanadu (Fig. 2B) and Radegast (Fig. 2C). Other details as in Fig. 1.

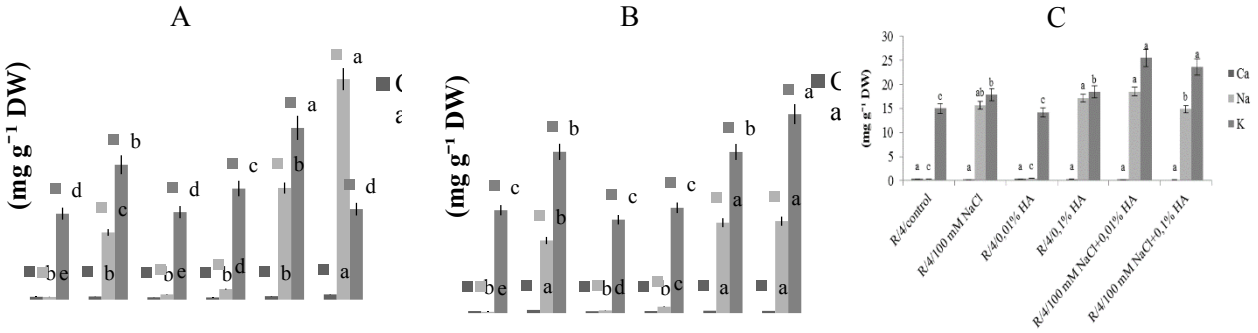
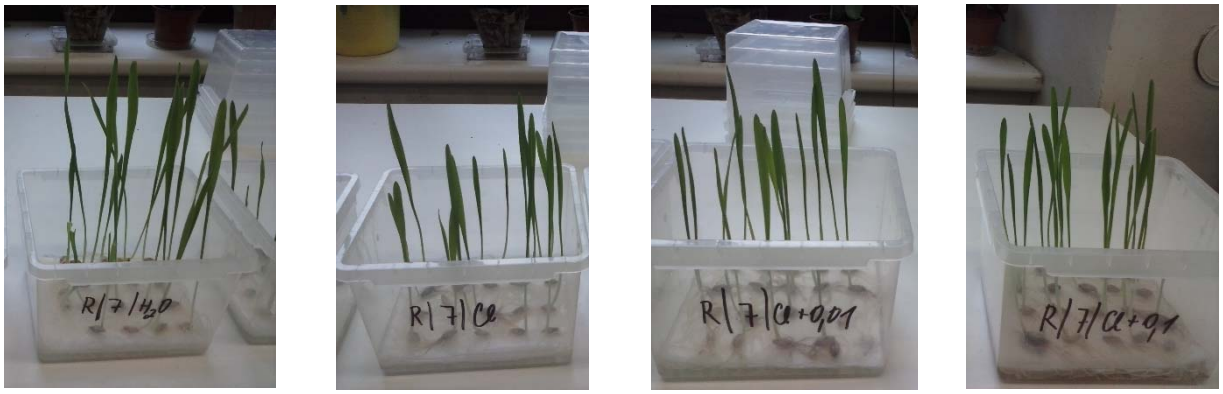


Table 3 The impact of using humic acid on the aboveground biomass production and the tissue water content in spring barley cv. Radegast.

		HA-L	HA-T
Control	**height (cm, *n=3*)**	16.4 ± 0.34[a]	18.2 ± 1.23[a]
	water content (%, *n=3*)	93.7 ± 2.27[a]	94.1 ± 4.87[a]
100 mM NaCl+0.01% HA	**height (cm, *n=3*)**	14.1 ± 0.19[b]	15.8 ± 0.78[b]
	water content (%, *n=3*)	89.2 ± 1.79[b]	93.2 ± 3.59[a]
100 mM NaCl+0.1% HA	**height (cm, *n=3*)**	14.3 ± 0.31[b]	16.2 ± 1.04[b]
	water content (%, *n=3*)	87.7 ± 3.01[b]	90.5 ± 3.38[a]

For the experiment were used two types of humic acids: (i) commercially available Lignohumate which contains >60 % humic acids from 10 % dry weight, (ii) commercially available humic acid in technical quality. Legend: HA-L – humic acid from Lignohumate, HA-T – humic acid in technical quality. Data are means±SDs. Values within rows, followed by the same letter(s), are not significantly different according to Tukey's test ($P < 0.05$).

Fig. 3 The comparison of one-week old barley plants exposed to 100 mM NaCl alone (Fig. 3B) or in combination with 0.01% (Fig. 3C) and 0.1% humic acid (Fig. 3D) against the control (in distilled water) plants (Fig. 3A).

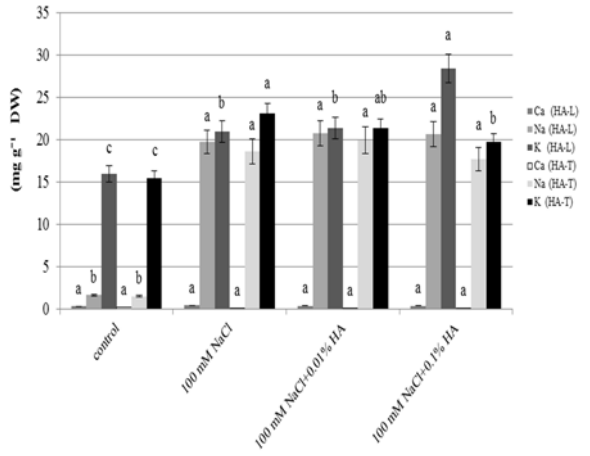


Conclusion

Humic substances can ameliorate negative soil properties; improve the plant growth and uptake of nutrients. It may be used in case of the negative effect of salt that would inhibit the plant growth and the uptake of nutrients. We found out that the applied doses and maybe also origin are important for taking benefits from humic acids under salt

conditions. The application of humic acids offers a solution for the improvement of the agricultural production of salt sensitive plants and gives us the opportunity for further research.

Fig. 4 The accumulation of selected mineral nutrients in barley (cv. Radegast) leaves exposed to different treatments for one week (n=3). Two types of humic acids were used: (i) commercially available Lignohumate which contains >60 % humic acids from 10 % dry weight, (ii) commercially available humic acid in technical quality. Legend: HA-L – humic acid from Lignohumate, HA-T – humic acid in technical quality. Other details as in Fig. 1.



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Primer efficiency determination for flavanone 3-hydroxylase gene in wheat (*Triticum aestivum* L.)

MARIA PRESINSZKA[1], KLARA STIASNA[1], TOMAS VYHNANEK[1], VACLAV TROJAN[1], WOLFGANG SCHWEIGER[2], JAN BARTOS[3], LADISLAV HAVEL[1]
[1]Department of Plant Biology
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Institute for Biotechnology in Plant Production
Department for Agrobiotechnology
BOKU/IFA-Tulln
Konrad-Lorenz-Str. 20, 3430 Tulln
AUSTRIA
[3]Centre of Plant Structural and Functional Genomics
Institute of Experimental Botany AS CR
Slechtitelu 31, 783 71 Olomouc-Holice
CZECH REPUBLIC

maria.presinszka@mendelu.cz

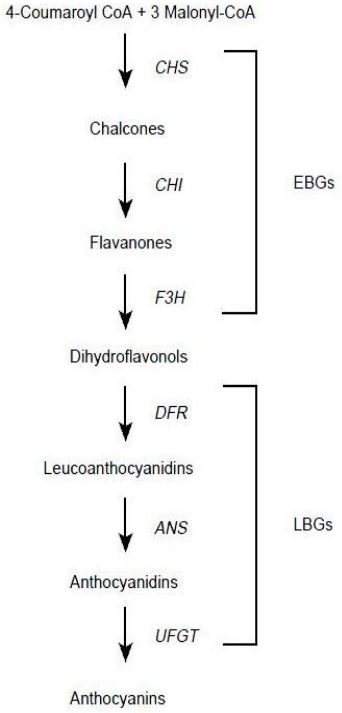
Abstract:
Anthocyanins are water-soluble pigments that may be red, purple, or blue. They belong to a parent class of molecules called flavonoids. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, blossoms, and fruits. Flavanon 3-hydroxylase (F3H) is one of the key enzymes of the flavanoid biosynthesis pathway. F3H converts flavanones to dihydroflavonols. The main aim was to study the *F3H* gene in wheat (*Triticum aestivum* L.). In our experiment 5 genotypes with nonstandard coloured caryopses were used (2 genotypes with blue aleurone, 2 genotypes with purple pericarp and 1 genotype with white caryopses- without anthocyanins). Total RNA was isolated by phenol-chloroform method and complementary DNA was obtained by reverse transcription. Specific primers for each of the *F3H* genes (*F3H_A*, *F3H_B* and *F3H_D*) that were found in the National Center for Biotechnology Information (NCBI) database were designed. The first results of quantitative PCR (qPCR) contained two or more products. New primers for *F3H-D* gene were found and qPCR was repeated. The only one PCR product of *F3H_D* amplification from all five genotypes was sent for sequence analysis. Obtained sequences of *F3H_D* have 99-100% conformity with the database NCBI.

Key Words: wheat, *Triticum aestivum* L., flavanone 3-hydroxylase, anthocyanins

Introduction

One of the major cereal crops, *Triticum aestivum* L. (genome 2n = 6x= 42, BBAADD), can also have anthocyanin pigmentation of different organs. High anthocyanin content in cereal plants has its role in plant defense, but it has also positive effects on human health [1]. Anthocyanins are phenolic phytochemicals classified within flavonoids together with flavonols, flavones, flavanols, flavanones, and isoflavonoids [2]. Flavonoids are a class of plants secondary metabolites which have an important role in pigmentation [3]. Biosynthesis of anthocyanins start with the formation of malonyl-CoA and p-coumaroyl-CoA derived from the phenylpropanoid biosynthetic pathway under the action of seven enzymes: chalconsynthase, chalconisomerase, flavanon 3-hydroxylase, flavonoid 3-hydroxylase, dihydroflavonol-4-reductase, anthocyanin synthase, and flavonoid-3-O-glucosyltransferase [4]. Anthocyanins are as pigments responsible for blue, purple, red, or orange coloration of plant tissues and organs [4]. There are known more than six hundreds natural anthocyanins. Six of the most frequented are cyanidins, delphinidins, malvidins, pelargonidins, petunidins, and peonidins [6].

Fig. 1 Scheme of the flavonoid biosynthetic pathway [5]



Legend: CHS- chalcone synthase, CHI- chalcone isomerase, F3H- flavanone 3-hydroxylase, DFR- dihydro-flavanone reductase, ANS- anthocyanidin synthase, UFGT- UDP glucose: flavonoid-3-O-glucosyltransferase, EBGs- early genes of biosynthesis pathway, LBGs- late genes of biosynthesis pathway

The purple color of wheat is caused by anthocyanins accumulated in the pericarp, while the blue color is in the aleurone layer of wheat grains [7]. The purple color of pericarp originated from purple Ethiopian tetraploid and hexaploid wheat (*Triticum aethiopicum Jakubz.*). The main anthocyanin of pericarp in purple wheat is cyanidine-3-glucoside and it is a predominant anthocyanin in these seeds [8]. The most frequent anthocyanins in blue wheat seeds are delphinidine-3-glucoside and delphinidine-3-rutinoside, cyanidine-3-glucoside and peonidine-3-glucoside [9].

F3H (*flavanone 3-hydroxylase*) is a member of a gene family involved in the biosynthesis of flavonoid compounds [10]. F3H catalyzes an early step in flavonoid metabolism - the formation of flavonols from dihydroflavonols, and therefore provides precursors for many classes of flavonoid compounds [11]. *F3H* belongs to the early genes of the biosynthesis pathway. The protein is classified as a soluble 2-oxoglutarate-dependent dioxygenase based on its requirements for 2-oxoglutarate, molecular oxygen, ferrous iron (Fe^{2+}) and ascorbate [12].

Material and Methods

The common wheat (*Triticum aestivum* L.) genotypes with anthocyanin pigmentation of grain were used: two genotypes with purple pericarp (ANK- ANK28B and AA- Abyssinskaya Arraseita), two genotypes with blue aleurone (UC- UC66049 and TBS- Tschermaks Blaukörniger Sommer-weizen) and one genotype with white caryopsis (N67- Novosibirskaya 67). Genotype Novosibirskaya 67 was used as a standard, because it does not contain anthocyanins.

All seed samples were sown in Botanical Garden and Arboretum of the Mendel University in Brno. The caryopses were collected 10th, 15th, 20th, 25th, 30th, 35th and 40th days after anthesis (days *post anthesis* - dpa) during maturation that is 7 samples for each genotype. For isolation of total RNA, RNA blue (Top Bio) was used. Reverse transcription from RNA to cDNA was performed using Enhanced Avian HS RT PCR kit (Sigma Aldrich). The allohexaploid genome of bread wheat carries four copies of the *F3H* gene, i.e. three homoeologs (orthologs) in A, B, and D genomes (labeled in NBCI: *F3H-A1* - AB223024.1, *F3H-B1* - AB223025.1, and *F3H-D1* - AB223026.1) and one paralog (labeled in NCBI *F3H-B2* - JN384122.1) in the B genome [13]. Specific primers for all *F3H* genes were designed using program Primer3. Sequence for *F3H* genes was found in the National Center for Biotechnology Information (NCBI) database. Quantitative polymerase chain reaction (qPCR, CFX96 Real Time Systems: Bio-Rad) was performed. One reaction generally run for 40 cycles and in Table 1 are the used temperatures.

Table 1 qPCR reaction conditions

Step	Temperature	Time
Initialization	94°C	5 min
Denaturation	94°C	30 sec
Annealing	60/62°C	30 sec
Extension	72°C	30 sec

For sequence analysis of *F3H_D* the PCR products were purified, ligated and cloned using p GEM®-T vector (Promega). After ligation recombinant p GEM®-T plasmid was transferred into cells of bacteria (electrocompetent cells - *E. coli*) using electroporation. SOC medium (Super-optimal broth with catabolite repression) was added to the samples and shaken 30 minutes. The samples were transferred under sterile conditions to Petri dishes with ampicillin (100 mg/ml) and components for blue/white selection (Xgal: 5-bromo-4-chloro-3-indolyl-β-D-galactosid and IPTG: Isopropyl-1-thio-

β-D-galactosid) to LB medium (Lysogeny broth). After blue-white screening, clones with recombinant plasmids were transferred into 1.5 ml of liquid LB medium and cultivated overnight at 37 °C [14]. PCR with these amplified plasmids was performed and PCR products were sent to Macrogen (Netherlands) for sequence analysis. After the analysis the sequences were evaluated using programme BLAST and Clustal W.

Results and Discussion

In the last years, studies of flavonoids attracted attention of many scientists [15]. Seeds of cereals are not typical sources of them; therefore relevant knowledge has been acquired also in maize, wheat, barley, oat, and rice as the most important world food sources. Colored-grain wheat varieties with good genetic stability, excellent stress resistance and high yield are still required [16].

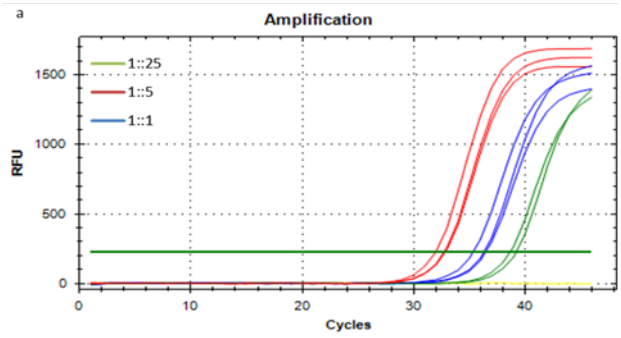
F3H genes were genetically mapped in wheat, barley and rye. Wheat contains three homoeologous copies of *F3H*: *F3H-A1*, *F3H-B1* and *F3H-D1* that are expressed in colored coleoptiles of different wheat genotypes [17]. Specific primers for sequences of the *F3H* genes (*F3H_A*, *F3H_B* and *F3H_D*) that were found in the National Center for Biotechnology Information (NCBI) database were designed. Primer efficiency test with primers for *F3H* genes and *GAPDH* (housekeeping gene: *glyceraldehyd-3-phosphate-dehydrogenase*) was performed. The cDNA samples were used in dilutions 1:1, 1:5 and 1:25. On the agarose gel we detected two or more products, because our *F3H* primers were not specific for studied genotypes. The best result was obtained for *F3H_D* gene with a PCR product approx. 240 bp and one longer. If for the qPCR reactions cDNA and genomic DNA were used, the same curves for all samples and the same size of the main PCR product (240 bp) were obtained. This is due to fact that designed primer for *F3H_D* gene was in the coding region that did not contain introns. For further work the *F3H_D* gene was chosen.

Himi et al. [3] designed *F3H-D* primers in their experiments that we tested on our genotypes (Table 2). The primer efficiency test with *F3H_D* and housekeeping gene (*GAPDH*) was repeated with the same cDNA dilutions (1:1, 1:5, 1:25). We obtained positive results. For all dilutions were done three repeats. In figure 2 the qPCR curves are highlighted. We detected for undiluted samples (1:1) and for dilution samples (1:5) three very similar curves. The size of PCR product was 229 bp. Only one run from three repeats was not successful with dilutions 1:25 with the same size of PCR product 229 bp. The flavonoids biosynthesis pathway was investigated and the most enzymes involved in the pathways of major flavonoids classes were determined, e.g. CHS, CHI, F3H, DFR, FLS, FNS and ANS [15].

Table 2 Sequences of F3H [3]

Name	sequences 5→3
F3H_F	CAA GAA GCA GGC CAA GGA C
F3HD_R	CTG CTA CAC ACG TAC GGA TAC C

Fig. 2 Primer efficiency test with F3H_D primer using cDNA mix



For the sequence analysis of the *F3H_D* gene suitable *F3H_D* sequences were obtained from PCR products by cloning. We cloned 5 genotypes with nonstandard colored caryopses (Abissinskaya arrasaita, ANK-28B, Novosibirskaya 67, UC66049 and Tschermaks Blaukörniger Sommerweizen). The sequences were 155 - 156 bp long and the variation in the number of bp was caused by insertions/deletions. The sequence analysis has shown that TBS has one, two, three and four nucleotide polymorphisms at the start of the sequence and ANK-28B has single nucleotide indel in the end of the sequence (Figure 3).

Comparing the sequences of putative proteins there were no differences among the genotypes AA, ANK-28B, N67 and UC66049 only the single nucleotide indel in sample ANK-28B caused protein shortening. One, two, three and four nucleotide polymorphisms in genotype TBS had different putative protein compared to the other genotypes. The similarity among all sequences of analyzed five genotypes and sequences obtained from NCBI was between 99 to 100%. The sequences of *F3H_D* gene were localized on the long arm of homoeologous chromosomes group 2 [18, 3]. *F3H* genes are critical for anthocyanin production in wheat coleoptiles. Within the *Triticeae* tribe it will be interesting to further investigate the extents to which are these

wheat regulatory genes specific in correlation with the resulting color [18].

Fig. 3 Comparison of the sequences of *F3H* genes with NBCI database using Clustal W

```
CLUSTAL 2.1 multiple sequence alignment


NCBI            GCGCCCATGCCCACCGCCACCAAGTCTCTCAACGAAATTCTTGCCTAGATCATTCCGGCG 60
ANK             GCGCCCATGCCCACCGCCACCAAGTCTCTCAACGAAATTCTTGCCTAGATCATTCCGGCG 60
AA              GCGCCCATGCCCACCGCCACCAAGTCTCTCAACGAAATTCTTGCCTAGATCATTCCGGCG 60
N67             GCGCCCATGCCCACCGCCACCAAGTCTCTCAACGAAATTCTTGCCTAGATCATTCCGGCG 60
UC              GCGCCCATGCCCACCGCCACCAAGTCTCTCAACGAAATTCTTGCCTAGATCATTCCGGCG 60
TBS             ATAGTAC GGTGATCGCCGTCAAGTCTCTCAACGAAATTCTTGCCTAGATCATTCCGGCG 60
                 **    **  ****** ****************************************


NCBI            GGGCGCGATTCAATATTTCAATTGATTAATGCGTGGGATTTGATTCTCCTAAGTACGAGA 120
ANK             GGGCGCGATTCAATATTTCAATTGATTAATGCGTGGGATTTGATTCTCCTAAGTACGAGA 120
AA              GGGCGCGATTCAATATTTCAATTGATTAATGCGTGGGATTTGATTCTCCTAAGTACGAGA 120
N67             GGGCGCGATTCAATATTTCAATTGATTAATGCGTGGGATTTGATTCTCCTAAGTACGAGA 120
UC              GGGCGCGATTCAATATTTCAATTGATTAATGCGTGGGATTTGATTCTCCTAAGTACGAGA 120
TBS             GGGCGCGATTCAATATTTCAATTGATTAATGCGTGGGATTTGATTCTCCTAAGTACGAGA 120
                ************************************************************


NCBI            TAAATTATGCATATGGTATCCGTACGTGTGTAGCAG 156
ANK             TAAATTATGCATATGGTATCCGTACGTGTGTAGC-- 155
AA              TAAATTATGCATATGGTATCCGTACGTGTGTAGCAG 156
N67             TAAATTATGCATATGGTATCCGTACGTGTGTAGCAG 156
UC              TAAATTATGCATATGGTATCCGTACGTGTGTAGCAG 156
TBS             TAAATTATGCATATGGTATCCGTACGTGTGTAGCAG 156
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Legend: NBCI- National Center for Biotechnology Information database, samples: ANK- ANK-28B, AA- Abissinskaya arrasaita, N67- Novosibirskaya 67, UC- UC66049, TBS- Tschermaks Blaukörniger Sommerweizen

Conclusion

The aim of the work was to test the primers for qPCR of *F3H* genes that will be used for studies of differently coloured wheat varieties and to obtaining the sequence of gene *F3H_D*. The first designed primer tests showed two or more products which was caused by non-specificity individual homoeologs of *F3H*. We obtained positive results from second qPCR with new *F3H_D* primers, where we obtained a single PCR product with the size 229 bp. We have used 5 genotypes of wheat with nonstandard coloured caryopses for qPCR and subsequently for sequence analysis the amplicons were cloned. The comparison of the *F3H_D* sequences with sequence from NCBI showed high degree of similarity (99-100%). The sequences have shown nucleotide polymorphisms in Tschermaks Blaukörniger Sommerweizen, which caused changes in the putative protein and one indel, which resulted in shortening of the protein.

Acknowledgement

I would like say thank our colleagues, Ing. P. Hanáček Ph.D. for his help with cloning and workers in Botanical Garden and Arboretum in Mendel University in Brno for area to support the growth of the material.

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Occurrence of the *Orchidaceae* Plant Species in the Territory of Železné Hory

ALOIS PROCHAZKA, MILADA STASTNA
Department of Applied and Landscape Ecology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

all.procha@seznam.cz

Abstract: The aim of this study is the comparison of current and historical occurrence of species of the *Orchidaceae* family in the territory of Železné hory. The thesis makes an assessment of 66 habitats within the territory of protected landscape area Železné hory and 20 other habitats in close vicinity of the protected landscape area, with total area of 7.35 km^2. The researched territory consist mostly of mezo-xerophyte meadow up to hygrophyte wetland phytocoenoses, forest eco systems from the vegetation degree of wild flower oak woods up to vertical degree of fir beech wood. The study is based on botanical field research on 86 habitats during vegetation seasons in the years 2004, 2011 and 2014. The occurrence of the *Orchidaceae* in the habitats was recorded by GPS in the environment of the program ArcPad 8.0 sp2. Based on recorded data and using the program Arc Gis Desktop 10 sp2 the grid maps and cartograms with occurrence of individual species of the *Orchidaceae* were set up. Moreover, vitality and sociability of individual identified species was assessed. The occurrence of the *Orchidaceae* was confirmed in 42 habitats. Most frequently, on 23 habitats occurred *Dactylorhiza majalis*. On the other hand, *Dactylorhiza sambucina* and *Epipactis purpurata* species were growing in one place only. Also the number of individuals was monitored. The highest number of individuals - 3233 pieces – reached the species *Dactylorhiza majalis.* On the other hand, the lowest number of individuals was recorded for the species *Dactylorhiza sambucina* with 3 pieces and *Epipactis albensis* with 2 pieces.

Key-Words: Protected landscape area Železné hory, orchidaceous family, nature protection and care management, endangered species, occurrence of the Orchidaceous.

Introduction

The plants from the *Orchidaceae* family are growing from tropical to temperate zones of both hemispheres. Several genera of the family grow even in the tundra behind the Arctic circle, respectively in the heights of around 4 000 m above the sea level.

Most species of the *Orchidaceae* family grow in tropical areas of South America, Africa and Asia, where some 30 000 species occur, but there are also hundreds inhabiting the temperate zones. There are about 70 known *Orchidaceae* species in the Czech Republic. Two of them are local endemites – *Dactylorhiza bohemica* and *Dactylorhiza carpatica* – and five species are considered to be extinct in the territory [15].

Most of the species of the *Orchidaceae* family growing in the nature of the Czech Republic are protected by Act No. 114/1992 Coll. on nature and landscape protection [16] and related regulations. For the efficiency of protection of the *Orchidaceae*, not only the plants themselves must be protected but also their whole natural eco systems.

This study deals with the occurrence of the *Orchidaceae* family in the territory of Železné hory. It maps the occurrence of the *Orchidaceae* family as well as it compares the results of botanical researches with historical literary data and it assesses the vitality and sociability of the exemplars found using the scale Braun and Blanquet [2].

It shows in a complex way the trend in occurrence of the *Orchidaceae* in Železné hory, which may support setting of the correct management of given territory for preservation of the *Orchidaceae* as well as other protected species in monitored locations for future generations.

Material and Methods

General information about the territory of Železné hory and extensive territorial relations were taken mainly from the Plan of care for the Protected

Landscape Area of Železné hory for the period 2011 – 2020 [12] .Main information on the historical incidence of the *Orchidaceae* family plants in the selected territory is based on botanical researches held in 1994 [6] and in 1995 [8]. Based on literature86 habitats with probable incidence of the *Orchidaceae* were chosen.

All habitats with strongly and critically endangered species of the *Orchidaceae,* than habitats with higher numbers of species and finally with probable incidence of plants from the *Orchidaceae* family were included.

Having defined 86 habitats with the total area of 7.35 km^2 in the territory of Železné hory, a detailed botanical field research aimed at vascular plants with emphasis on the *Orchidaceae* in the course of vegetation seasons in the years 2004, 2011, 2014 was performed.

An exact position of individual detected plants of the *Orchidaceae* family was recorded using the GPS Trimble Juno Sc with the program Arc Pad 8.0 sp2 in the co-ordinate system S-JTSK East North. After processing the digital data of the *Orchidaceae* incidence the topical cartograms of the *Orchidaceae* incidence in Železné hory in the environment of Arc Gis Desktop 10 sp2 software was set up.

The nomenclature of vascular plants including the *Orchidaceae* is taken from the Key to the Flora of the Czech Republic [9].

Two quantitative features were investigated. The first is the sociability, expressing the distribution of the species individuals in the phytocoenosis according to the scale Braun and Blanquet [2]. The other established qualitative feature was vitality, expressing the ability of plant development and successful reproduction. It is set using the four-level scale Braun and Blanquet [2].

In the course of field researched of the habitats data on the *Orchidaceae* species, other vascular plants were recorded and photo documentation of the habitat and the orchid species were taken

Mapping of the *Orchidaceae* species

Botanical research was performed in the vegetation season on 86 habitats of Železné hory in 2004, 2011, 2014.

The incidence was confirmed 2004 on 42 out of historically proved 86 habitats with occurrence of the *Orchidaceae* in Železné hory. In the course of the revision in 2014, the incidence of the *Orchidaceae* was confirmed in 36 habitats only.

The most frequent species *Dactylorhiza majalis* was observed on 23 (41 historical) and *Epipactis helleborine* on 8 habitats (17 historical) in 2004 and 2014. On the other hand, the species *Dactylorhiza sambucina*, *Dactylorhiza x braunii*, *Epipactis albensis*, *Epipactis purpurata* were growing in only one place in all monitored years.

Table 1 Comparison of Historical Habitats of the *Orchidaceae* with Habitats Confirmed in the Course of Botanical Researche in 2004, 2011 and 2014 and Numbers of Identified Individuals.

Latin name	Number of historical habitats	Number of confirmed habitats in 2004	Number of confirmed habitats in 2011	Number of confirmed habitats in 2014	Number of individuals in 2004	Number of individuals in 2011	Number of individuals in 2014
Cephalanthera damasonium	5	2	2	2	183	64	249
Cephalanthera longifolia	0	0	0	1	0	0	5
Coeloglossum viride	2	0	0	0	0	0	0
Corallorhiza trifida	2	0	0	0	0	0	0
Dactylorhiza fuchsii	16	3	1	0	20	4	0
Dactylorhiza majalis	41	23	20	23	1634	2560	3233
Dactylorhiza sambucina	4	1	1	1	7	4	3
Dactylorhiza x braunii	1	1	1	1	3	6	11
Epipactis albensis	0	1	1	1	2	2	4
Epipactis helleborine	17	8	9	8	202	63	137
Epipactis palustris	7	3	3	3	1036	1958	2619
Epipactis purpurata	2	1	1	1	29	28	33
Gymnadenia conopsea	8	0	0	0	0	0	0
Listera ovata	9	4	0	0	22	0	0
Neottia nidus - avis	10	1	0	1	3	0	2
Orchis morio	7	0	0	0	0	0	0
Orchis ustulata	1	0	0	0	0	0	0
Platanthera bifolia	17	3	1	1	18	11	11
Platanthera chlorantha	9	6	5	3	194	95	105
Spiranthes spiralis	1	0	0	0	0	0	0

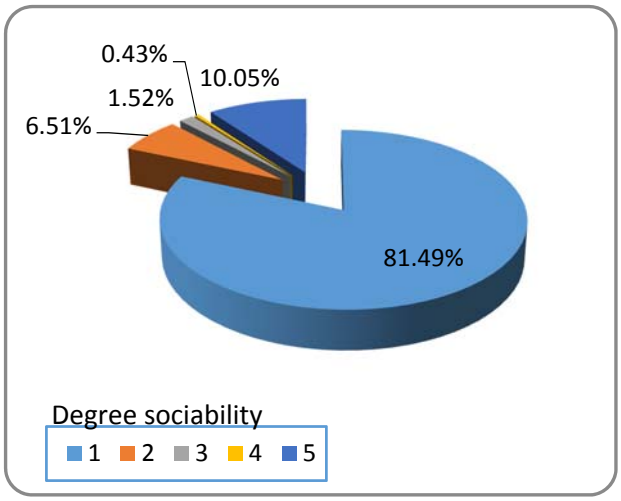
In the course of botanical researches also the numbers of individuals of orchids was monitored, the highest number was identified in the species *Dactylorhiza majalis* with 1634 pcs in 2004 and with 3 233 pcs in 2014.

An increasing trend was proven even in the case of *Epipactis palustris* with 1 036 pcs in 2004 and 2 619 in 2014. Just to the contrary, a low number of individuals and decreasing tendency of incidence was proven for *Platanthera bifolia* with 18 pcs in 2004 and 11 in 2014, *Dactylorhiza sambucina* with 7 pcs in 2004 and 3 in 2014, *Neottia nidus avis* with 3 pcs in 2004 and 2 in 2014.

Two quantitative features - sociability (distribution of individuals in the phytocoenosis) and vitality of plants according to the scale Braun and Blanquet [2] were assessed.

The sociability of the individuals found usually reached level 1, which means that the individuals most frequently grow individually or – less frequently – level 5, which means that the individuals grow in continuous stands.

Fig. 1 Sociability of all the detected orchids at all habitats in 2011.



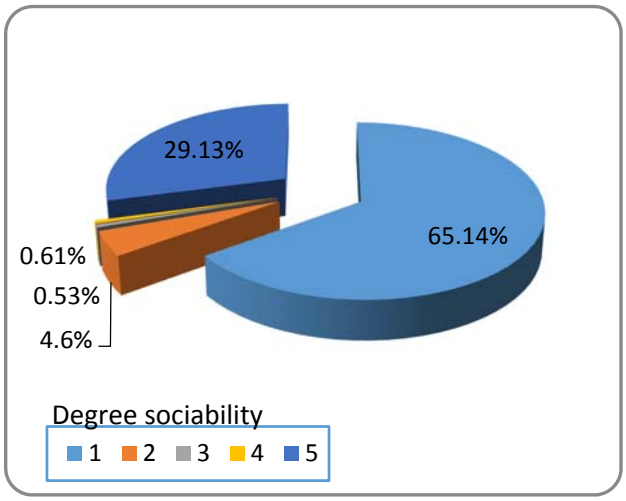
The second assessed quantitative feature was the vitality of orchid individuals found. The average vitality of all orchid species was 1.28 in 2004, 1.06 in 2011 and 1.07 in 2014. That means that most of the *Orchidaceae* were well developed according to the scale, with regular life cycle. In the course of the botanical field research in 2004, there were detected in total 299 species of vascular plants in monitored habitats. 17 species of them are protected according to Act No. 114/1992 Coll., on nature and landscape protection [16]. In 2011 and 2014 we succeeded to identify 346 and 387 vascular plants, 18 species of them being protected according to Act No. 114/1992 Coll. [16].

Discussion

In the territory of Železné hory 12 of the historically described 20 species of the *Orchidaceae* family were found. The incidence of the family on 42 out of original 86 historical habitats was confirmed in 2004 and only on 36 in 2014.

The highest number of plants of the *Orchidaceae* family were found in 2014, when 6 434 plants were successfully recorded in all habitats. Two aspects play the key role in the high number of observed plants. In three habitats of the small-area specially protected territory in Železné hory the endangered species *Epipactis palustris* occurs, which due to favourable conditions reached a total population of 2 619 pcs in 2014. Another reason for the high number of exemplars of the family is quite frequent incidence of the species *Dactylorhiza majalis*, which appeared in 23 habitats in Železné hory with the total number of 3 233 plants in 2014. Only slightly lower numbers of individuals of these both species were proven also in 2004 and 2011.

Fig. 2 Sociability of all the detected orchids at all habitats in 2014.



But originally, *Dactylorhiza majalis* appeared in 41 historical habitats and *Epipactis palustris* inhabited 7 places. Thus the populations must have been much more numerous in earlier times.

But other species of the family turned out much more badly. For example the *Platanthera bifolia* originally inhabited 17, while in 2014 only one place with a total number of 11 exemplars.

Even other detected species of the *Orchidaceae* family in the territory of Železné hory survive in just a small number of original habitats with lower numbers of exemplars. Moreover, the species *Coeloglossum viride*, *Corallorhiza trifida*, *Gymnadenia conopsea*, *Orchis morio*, *Orchis*

ustulata and *Spiranthes spiralis* were not found in the nature of Železné hory during botanical researches in 2004, 2011 and 2014 and the chances for recovery in that region are minimal for most of the species.

Two quantitative features of the found orchids were assessed - sociability (distribution of individuals in the phytocoenose) and vitality of plants according to the scale Braun and Blanquet [2]. The sociability of the individuals found usually reached level 1, which means that the individuals were growing most frequently individually or – less frequently – level 5, mainly *Epipactis palustris* and *Dactylorhiza majalis,* which means that the individuals were in continuous stands.

The second assessed quantitative feature was the vitality of orchid individuals. The average vitality of all species of the *Orchidaceae* was 1,28 in 2004, 1,06 in 2011 and 1,07 in 2014. That means that most of the orchid plants were well developed according to the scale, with regular life cycle.

The sociability and vitality set the momentary space and health parameters of the current researched population, but they do not assess any increase or decrease of populations or their extinction [5].

Pasture was stopped, respectively annual harvest of grass biomass by mowing was cancelled in many meadow location in the course of the recent twenty years. Succession change of species composition occurs in these habitats and it is not suitable any more for the species of the *Orchidaceae* family – the species quickly disappeared from those places.

Fortunately, at least some kind of well-preserved areas in Železné hory have recently been transferred by the Železné hory PLA Administration to the small-area specially protected area with fixed management of care respectively financial means from the Program of care for countryside were successfully used for arrangement of mowing of several other meadow habitats that will preserve current species variability including incidence of plants from the *Orchidaceae* family.

Conclusion

The study deepens the knowledge of the flora of Železné hory. It involves 86 habitats dispersed in the whole territory of Železné hory, with the total area of 7.35 km^2. The researched areas include mainly mezohygrophyte meadow phytocoenoses, wetland, fenny and peat meadows or forest communities from wildflower beech woods, oak woods up to fir and beech wood.

The main aim of the work was the performance of botanical field research of habitats, with focus on the *Orchidaceae* family. The botanical field researches were held in 2004, 2011 and 2014.

In the course of the study12 species of plants from the *Orchidaceae* family out of the 20 historically proven were confirmed. In the territory of Železné hory most frequently *Dactylorhiza majalis* was found, that currently inhabits 23 out of the original 41 historically proven places. Also for *Epipactis palustris*, growing in 3 natural reserves were recorded high number of exemplars.

Unfortunately, the situation with the other orchids is much worse. Their incidence persists in just a few original habitats and the numbers of exemplars are usually low.

The most valuable members of the *Orchidaceae* family in the territory of Železné hory include the seriously endangered *Dactylorhiza sambucina* with incidence in one place with 3 exemplars only and the seriously endangered *Epipactis albensis*, also growing in one habitat only, reaching the number of 4 exemplars found in 2014.

This work will make easier any future research in this field and it will serve to extend knowledge of the given territory.

Acknowledgement

Herewith, I would like to thank mainly Ing. Petr Jelínek, Ph.D. from the Faculty of Forestry, Mendel University, for many valuable advices and information and the employees of the Administration of Protected Landscape Area of Železné hory for provision of literature and facilitation of research of the *Orchidaceae* family in protected areas.

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Granule-bound starch synthase and endosperm mealiness correlation in wheat (*Triticum aestivum* L.) with nonstandard colored caryopses

KLARA STIASNA[1], MARIA PRESINSZKA[1], TOMAS VYHNANEK[1], VACLAV TROJAN[1], EVA MRKVICOVA[2], LUDEK HRIVNA[3], LADISLAV HAVEL[1]
[1]Department of Plant Biology
[2]Department of Animal Nutrition and Forage Production
[3]Department of Food Technology
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

klara.stiasna@mendelu.cz

Abstract: Granule-bound starch synthase (GBSS) is a key enzyme in starch biosynthesis pathway. It is responsible for production of amylose, therefore low amylose level has been associated with GBSS deficiency. This enzyme, also known as waxy protein, is encoded by three homologous genes – *Wx-A1*, *Wx-B1* and *Wx-D1* and their dysfunction of any origin causes decrease of amylose content. Starch content and, therefore amylose content, is closely linked with structure of the cereal endosperm. It is known, that mealy endosperm contains more starch than vitreous, which contains more proteins. To identify null alleles of waxy genes in 25 genotypes of wheat with nonstandard colored caryopses, PCR markers were used. To determine the endosperm structure of these genotypes, the non-destructive Light Transflectance Meter (LTm) was used. Null alleles for *Wx-B1* locus were observed in 5 tested genotypes, loci *Wx-A1* and *Wx-D1* were amplified in all genotypes. Percentage of mealy caryopses per sample ranged from 0 to 100 % among tested genotypes. No significant correlation between these two parameters was observed.

Key-Words: wheat, *Triticum aestivum* L., mealy endosperm, *waxy* protein, GBSS

Introduction

Starch, which accounts for 65 – 75% of wheat caryopsis dry weight, is one of the most significant elements of the cereal endosperm along with storage proteins [1]. It is a polymer composed of two glucose carbohydrates: amylose and amylopectin [2]. Amylose synthesis is regulated by enzyme granule-bound starch synthase (GBSS), also called waxy protein. In common wheat, because of its allohexaploidy (2n = 6x = BBAADD), this enzyme is encoded by three homologous *waxy* genes located on the short arm of the chromosomes 7A (*Wx-A1* locus), 7D (*Wx-D1*), and on the long arm of the chromosome 4A (*Wx-B1*) which was translocated from its original short arm of 7B [3, 4, 5]. Products of these genes, the waxy proteins, have slightly different molecular weight around 59 – 60 kDa [6]. When only one or two *GBSS* genes are functional, the wheat is called partially waxy, while genotypes with null allele at all three GBSS loci are called waxy (triple null), the starch has almost no amylose [7]. Waxy or partial waxy wheat types are desirable material for Asian noodles production [8] and has significant effect on bread making quality [9, 7]. Different null *Wx* alleles have different effect on amylose content in wheat.

Mealiness or vitreousness of wheat endosperm primary depends on starch content. Mealy endosperm contains more starch and less proteins and vice versa [10, 11]. This characteristic is essential for example in brewing industry in process of malting and other sectors of food industry and depends predominately on variety.

In this work, we wanted to verify whether there is a correlation between occurrence of waxy genes alleles and endosperm mealiness.

Table 1 Used genotypes overview, percentage of mealy grains and null allele incidence

Number	Genotype	Caryopsis color	Mealy grains [%]	Product size		
				Wx-D1 299 bp	*Wx-A1* 257 bp	*Wx-B1* 227 bp
1	Novosibirskaya 67	white	2	+	+	+
2	ANK-28A	purple	10	+	+	+
3	ANK-28B	purple	6	+	+	+
4	Abissinskaya Arraseita	purple	84	+	+	+
5	Konini	purple	30	+	+	-
6	Purple	purple	87	+	+	+
7	Purple Feed	purple	91	+	+	+
8	Indigo	purple	100	+	+	+
9	Rosso	purple	88	+	+	+
10	Citrus	yellow	0	+	+	+
11	Luteus	yellow	50	+	+	+
12	Bona Dea	yellow	89	+	+	+
13	TA 4024	yellow	3	+	+	-
14	UC66049	blue	99	+	+	+
15	Tschermaks Blaukörniger Sommerweizen	blue	98	+	+	-
16	Tschermaks Blaukörniger	blue	99	+	+	-
17	48M	blue	97	+	+	+
18	Skorpion (RU 440-6)	blue	76	+	+	+
19	RU 440-5	blue	96	+	+	+
20	Barevná 9	blue	23	+	+	-
21	Barevná 25	blue	74	+	+	+
22	Xiao Yian	blue	68	+	+	+
23	EF 02-54/9	blue	100	+	+	+
24	H 90-15-2	blue	85	+	+	+
25	Heroldo	white	34	+	+	+
Null allele incidence [%]				0	0	20

Material and Methods

Plant material

Total of 25 genotypes with nonstandard colored caryopses were analyzed. Sample collection was obtained from the Agricultural Research Institute Kroměříž, Ltd., Czech Republic and contained genotypes with white, purple, yellow and blue caryopses which are rich in natural pigments. The list of used genotypes is presented in Table 1.

Identification of null *GBSS* alleles

Genomic DNA was isolated using DNeasy Plant Mini Kit (Qiagen, Germany) from 5 to 7 days old wheat seedlings planted in controlled laboratory conditions. Primers #4F and #4R used in this study were developed by McLauchlan et al. [12]. Used primers were designed to amplify all three copies of *GBSS* genes on genome A, B and D. Primer details and PCR conditions are summarized in Table 2. The reaction took place in the volume of 25 µl. PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized by UV exposure. The largest fragment (299 bp) belonged to the D genome, the intermediate one (257-bp) to the A genome and the smallest one (227-bp) to the B genome.

Table 2 Primers sequence and PCR conditions

Name	Sequence	Amplified loci	Cycling condition
#4F #4R	AAGAGCAACTACCAGT TCGTACCCGTCGATGAAGTCGA	Wx-A1, Wx-B1 and Wx-D1	initial denaturation step of 2 min at 94 °C followed by 33 cycles of 94 °C 1 min, 54 °C 2 min, 72 °C 2 min

Mealiness determination

Mealiness or vitreousness of wheat endosperm was examined according to Chandra et al. [13] using Light Transflectance Meter (LTm) originally developed for barley testing. This non-destructive method is based on measurement of laser beam transfer through caryopses. For a single experiment, 97 caryopses are used from each genotype. Caryopses with mealy endosperm cause light scattering, LTm values are low. Vitreous caryopses

transmit more light thus LTm values are higher. Final number is an expression of mealy caryopses percentage in sample.

Results and Discussion

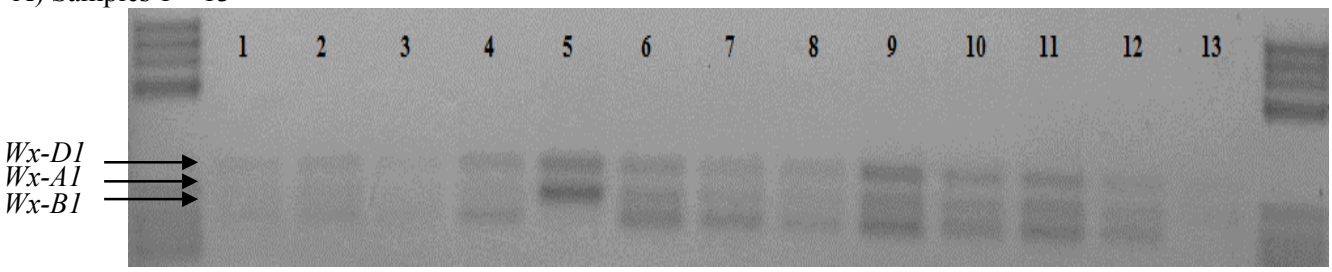
GBSS genes variability

In 25 tested genotypes variability occurred only in locus *Wx-B1*. Five genotypes (20%), namely Konini, TA 4024, Tschermaks Blaukörniger Sommerweizen, Tschermaks Blaukörniger and Barevná 9 were deficient in the 227 bp product. We can conclude that null allele is present in this locus. *Wx-A1* and *Wx-D1* alleles were present in the whole collection (Table 1, Figure 1). In a set of 103 Argentinian wheat cultivars [2], 24% samples lack the PCR fragment for *Wx-A1*. Results for *Wx-B1* and *Wx-D1* were the same as in this paper. In Iranian cultivars the incidence of *Wx-B1* null allele was lower, 4.2% had no PCR product in this locus [1].

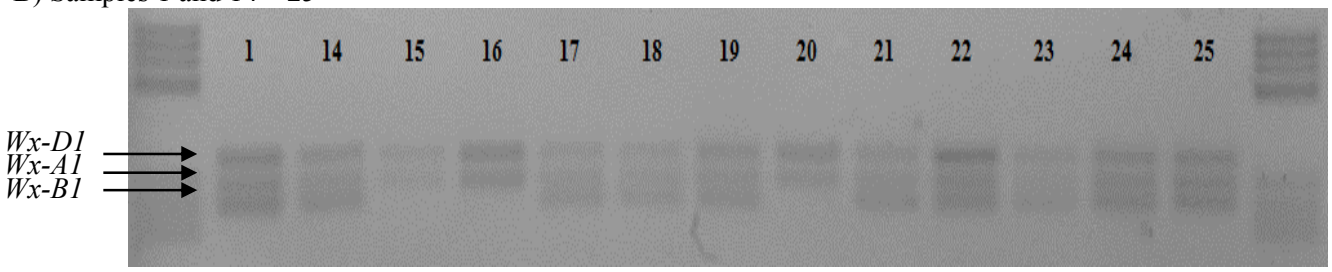
Distribution of the null alleles is the matter of geographical origin. Null allele for A genome is frequent in Turkish genotypes. On the other hand, Korean, Japanese and Australian wheat often carry the null allele *Wx-B1*. Wx-D1 null alleles are very rare [14].

Starch from *Wx-B1* deficient wheat displayed some functional properties in starch pasting [15]. These partial waxy types may have the largest usage in Asian countries for production special noodle types, like udon or ramen [16].

Fig. 1 PCR products of *Wx-A1*, *Wx-B1* and *Wx-D1*
A) Samples 1 – 13



B) Samples 1 and 14 – 25



Mealiness of wheat caryopses

Percentage of mealy caryopses present in samples ranged from 0 to 100%. Figure 2 shows examples of LTm values among 97 caryopses per sample. Two genotypes EF 02-54/9 and TA 4024 with extreme values are present. We can conclude, that 8 genotypes, namely Novosibirskaya 67, ANK-28A, ANK-28B, Konini, Citrus, TA 4024, Barevná 9 and Heroldo, had less than 50% of mealy caryopses in sample and the rest of 17 genotypes had 50% or more caryopses with mealy endosperm, even 8 of them had the percentage of mealy caryopses higher than 90 %. Results are shown in Table 1. Genotypes with mealy endosperm may be interesting material for wheat beer brewing, because increasing mealiness causes increased malting yield [20].

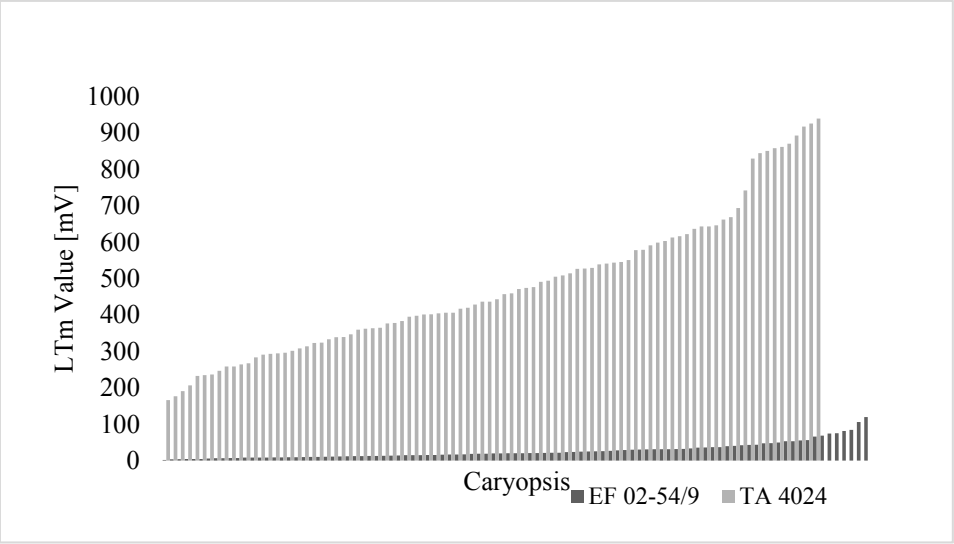
Correlation between presence of *Wx-B1* null allele and endosperm mealiness

Even though many sources claim that *Wx-B1* has the largest effect on amylose content in wheat endosperm [15, 17, 18, 19] and its structure is closely linked to starch amount, no significant correlation was found between mealiness and occurrence *Wx-B1* allele in tested genotypes according to Pearson correlation coefficient.

Possible explanation could be the effect of the other two present *Wx-A1* and *Wx-D1* alleles which could substitute absence of *Wx-B1* product in genotypes with its deficiency.

Another reason could be that *GBSS* genes influence only one constituent of the starch, amylose. The second component, amylopectin, is controlled in other ways.

Fig. 2 Examples of two used genotypes with extreme high and low percentage of mealy endosperm



Legend: EF 02-54/9 has 100 % mealy caryopses, 3 % of TA 4024 caryopses are mealy, thus is considered vitreous.

Conclusion

A set of 25 common wheat (*Triticum aestivum* L.) genotypes with white, purple, yellow and blue caryopses were examined in terms of *GBSS* or *waxy* genes variability. Five of them showed absence in *Wx-B1* locus and may therefore be considered as partially waxy and are potentially valuable in future breeding programs. Collection of wheat with nonstandard colored caryopses showed wide range of endosperm structure.

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Section – Animal Biology

Analysis of microsatellite sequence in major histocompatibility complex region in horses

ELISKA HORECKA [1, 2], CENEK HORECKY[1, 2], PETR HORIN[3, 4], ALES KNOLL[1, 2]
[1]Department of Animal Morfology, Physiology and Genetics
[2]CEITEC MENDELU
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[3]Department of genetics
[4]CEITEC VFU
Veterinary and Pharmaceutical University Brno
Palackeho trida 1/3, 612 48 Brno
CZECH REPUBLIC

eliska.horecka@mendelu.cz

Abstract: Major histocompatibility complex (MHC) is one of the immunologically important mammalian genome regions. Genes in MHC encode proteins, which are involved in innate and adaptive immune response. Genetic changes in this region can affect immune reactions in animals. But complex documentation of genetic variation in MHC is still missing in most domestic animals, including horses. In horses MHC is located on chromosome 20, region 27 403 526 – 33 865 081 bp. The aim of this study was utilisation of described microsatellite markers to analyse the genetic diversity of selected populations of horses. And then to determine number of alleles and compare results with the original authors. Even and odd alleles were identified for microsatellites ABGe17416 a 305-93, thus further sequencing will be performed to reassert in/del in PCR product.

Key-Words: MHC, horse, microsatellite, pathogen, alleles

Introduction

The major histocompatibility complex (MHC) is a fundamental part of the vertebrate immune system, and the high variability in many MHC genes is thought to play an essential role in recognition of parasites [2]. The function of MHC molecules is to bind peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T cells. The consequences are almost always deleterious to the pathogen—virus-infected cells are killed, macrophages are activated to kill bacteria living in their intracellular vesicles, and B cells are activated to produce antibodies that eliminate or neutralize extracellular pathogens. Thus, there is strong selective pressure in favor of any pathogen that has mutated in such a way that it escapes presentation by an MHC molecule [3].

The MHC contains some of the most polymorphic genes of the vertebrate genome, but alleles of some of these genes are ancient and predate speciation of related taxa [4]. Because trans-species polymorphisms confound analysis of MHC history and haplotype structure by single nucleotide polymorphisms (SNPs), many investigators have turned to microsatellite repeats and microsatellite–SNP combinations as a source of more rapidly diversifying gene markers to provide a better understanding of MHC evolution and function [8]. Brinkmeyer-Langford *et al*. [1] states for 69 primer pairs tested, 37 (53%) amplicons were polymorphic and 30 (41%) were monomorphic among the IHRFP horses (International Horse Reference Family Panel). Among the polymorphic microsatellites 31 were newly described markers and six previously described markers [6, 7, 9, 10, 11].

The aim of this study was utilisation of described microsatellite markers to analyse the genetic diversity of selected horses populations.

Material and Methods

Characterization of study population

In this study 325 individuals from two regions with different infection pressure were included.

Populations from pathogen rich regions were: Camarque, Romanian horse, Campolino, Marajo, Galiceno and Mangalarga Marchador. And populations of Gotland, Yakut and Icelandic horse were set as pathogen free region.

Testing of different polymerases

In this study different types of polymerase were tested : AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific Inc., Waltham, USA), PPP Master Mix (Top-Bio, Vestec, Czech Republic), CombiTaq (Top-Bio, Vestec, Czech Republic), HotStarTaqTM MasterMix (Qiagen, Hilden, Germany). Polymerases were used in multiplex with primers taken from Tseng et al. [11].

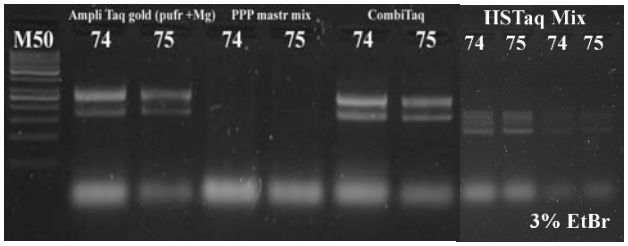
Microsatellite genotyping

PCR amplification was performed in two multiplex reactions, each in the total volume of 6 µl. The first multiplex included primers: 25 pmoles UMN-JH34-2, UM011, COR114 and 40 pmoles COR112, COR113 (primer sequences according to Tseng et al. [11]). The second multiplex included primers 20 pmoles 305-93, ABGe9019, HMS082, ABGe17416 and TKY3324 (primer sequences according to Brinkmeyer-Langford et al. [1]). Multiplex contained 1.6 U CombiTaq polymerase and 1x complete buffer for CombiTaq (Top-Bio, Vestec, Czech Republic), 100 µM dNTP (Thermo Fisher Scientific Inc., Waltham, USA) and PCR water (Top-Bio, Vestec, Czech Republic) adjusted to 6µl. Primers were modified on 5' end by fluorescent labels 6-FAM, PET, NED and VIC so that samples could be separated and analysed in one mix. Temperature profile of PCR reaction 1 was 95/3min; (95/30; 58/30; 72/60) 30x; 72/60min; 7/∞. Temperature profile of PCR reaction 2 was: 95/3min; (95/30; 58/30; 72/30) 30x; 72/60min; 7/∞. Designed panel of the microsatellites was analysed using genetic analyser ABI PRISM® 3500 (Applied Biosystems, Foster City, CA, USA) and sized with LIZ500 dye size standard (Applied Biosystems, Foster City, CA, USA). Results were analysed using the GeneMapper v4.1 software (Applied Biosystems, Foster City, CA, USA).

Results and Discussion

In this study different types of polymerase were tested. Results are displayed in Figure 1. The best results were achieved by using CombiTaq and AmpliTaq Gold DNA polymerases. For further analysis was used only CombiTaq polymerase because the price was only one-third compared to AmpliTaq Gold DNA.

Fig. 1 Using different types of polymerase



Specific microsatellites were selected to cover all three classes of MHC region. From class I located in region 27 403 526 – 30 620 000 bp, two microsatellites were selected: 305-93 (MS_6) and UMN-JH34-2. From class III, which covers region 31 321 086 – 31 572 317 bp, two microsatellites were selected: ABGe9019 (MS_2) and HMS082 (MS_3). From the largest part of MHC class II, which occurs in location 32 621 480 – 33 849 668 bp, six microsatellites were used: ABGe17416 (MS_4), TKY3324 (MS_5), COR112, COR113, UM011 and COR114. Number of alleles were determined for individual microsatellite (Tab. 1).

Table1 Information about microsatellite markers used in this study

Markers	Class MHC	Range of amplikon	Number of alells	Repetition
305-93	I	331-347	16	CA
UMN-JH34-4	I	186-218	17	CA
ABGe9019	III	288-330	13	CA
HMS082	III	189-219	13	TG
ABGe17416	II	276-317	24	CA
TKY3324	II	244-266	12	CA
COR112	II	228-258	13	TG
COR113	II	251-271	10	TG
UM011	II	160-180	11	CA
COR114	II	219-245	12	TG

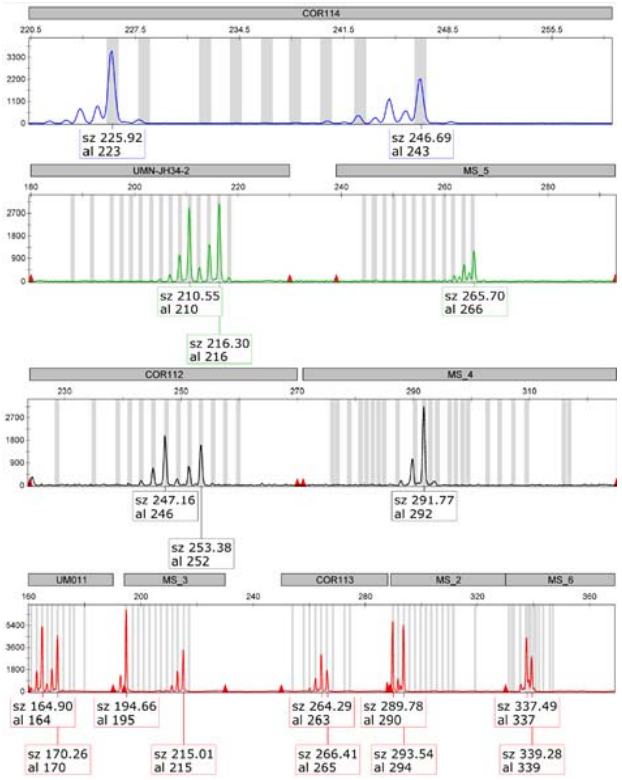
Comparison of our results and alleles by Brinkmeyer-Langford et al. [1], Tseng et al. [11] are listed in Table 2.

Table2 Comparison of the Brinkmeyer-Langford et al. (2012) and our results

Markers	Number of alells by Brinkmeyer-Langford et al./ Tseng *et al*	Number of alells discovered in this study
305-93	7	16
UMN-JH34-4	0/12	17
ABGe9019	11	13
HMS082	10	13
ABGe17416	13	24
TKY3324	13	12
COR112	11/13	13
COR113	10/8	10
UM011	11/13	11
COR114	9/10	12

Tseng et al. [11] describes even and odd alleles in microsatellite UM011, but we confirmed only even alleles. For microsatellite ABGe17416 and 305-93 even and odd alleles occurred, therefore, the numbers of alleles were significantly different than found by Brinkmeyer-Langford et al. (2012). This finding will be specified by sequencing the PCR product to reassert in / del, or odd and even alleles. In the picture 2 is shown an example of genetic analyzer ABI PRISM® 3500 result.

Fig. 2 Examples of microsatellite markers and their size



Conclusion

This study extends the original panel of five microsatellites (Klumplerová et al., 2013) to another five microsatellites for better and uniform coverage of whole MHC region. This panel of ten microsatellites together with SNPs determined in MHC region and with the panel of neutral microsatellites will be used for more accurate analysis of genetic diversity and evolution of MHC in horses.

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NKR genes region and their genetic diversity in horses

CENEK HORECKY[1,2], ELISKA HORECKA[1,2], PETR HORIN[3,4], ALES KNOLL[1,2]
[1]Department of Morphology, Physiology and Animal Genetics
[2]CEITEC - Central European Institute of Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[3]Department of Animal Genetics; Faculty of Veterinary Medicine
[4]CEITEC - Central European Institute of Technology
University of Veterinary and Pharmaceutical Sciences Brno
Palackeho trida 1/3, 612 48 Brno
CZECH REPUBLIC

cool.czenda@gmail.com

Abstract: The genes of the immune response represent a functionally important region of vertebrate genome with demonstrated intense effect of selection. Natural killer cells (NK) are related to antigen recognition process through their highly variable receptors. The genetic variability and different expression of genes for receptors underlies functional variability of individual NK cells. As like in mouse model, the Ly49 receptors on horse NK cells are believed to bind to MHC class I molecules of target cells. The six *Ly49* genes form a gene family located on horse chromosome 6 between 38 200 Kbp – 38 500 Kbp. In this work were identified and genotyped nine microsatellites located in NK cell receptors (NKR) region, which includes genes *Ly49*. Using this methodological work may contribute to the estimate of its genetic diversity in this functionally important region. This methodology will be used for the association analysis of selected diseases in horses.

Key-Words: horse, NK cell, NKR, Ly49, genetic diversity, microsatellite

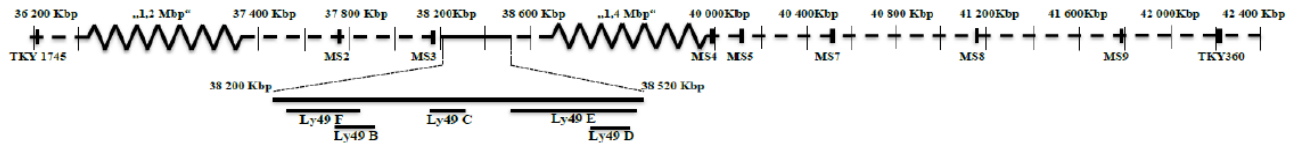
Introduction

Natural killer (NK) cells mediate important functions in innate resistance to pathogens, including direct cytotoxicity of infected cells and secretion of cytokines [1]. NK cells, a subset of lymphocytes, are part of the first line of defence of the innate immune system. They play a crucial role in cell-mediated immune responses in host defence against pathogens and in tumour cell surveillance [2]. The activities of NK cells are regulated by a diverse spectrum of activating and inhibitory receptors, belonging to both immunoglobulin-related (KIR) and lectin-like receptor (Ly49) families and that bind to cognate ligands [1, 3].

NK receptor gene complexes are intimately associated, both genetically and functionally, with major histocompatibility complex (MHC) recognition, and interactions of different combinations of NK receptors and MHC class I molecules may contribute significantly to selection and disease resistance [4]. Radiation hybrid mapping and fluorescence in situ hybridization localized horse *Ly49* genes to chromosomes 6q13 (see Fig. 1) [5].

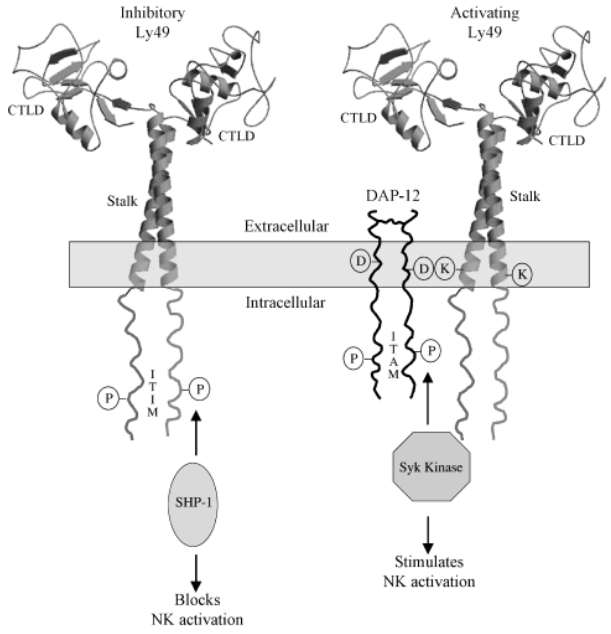
Ly49 receptors are lectin-like type II transmembrane disulfide-bonded homodimers expressed on NK cells and some T-cell subsets and are best known for their role in the regulation of NK cell functions. Cell-mediated cytotoxicity and release of cytokines/chemokines are functions regulated by Ly49 recognition of proteins class I MHC or virus-encoded MHC-like product(s) [1, 6].

Fig. 1 Organization of the studied region on equine chromosome 6



Ly49 expression begins early during NK cell development in the bone marrow (BM) [7]. Activating and inhibitor Ly49 receptors are described as being expressed on both the developing and mature NK cells [2, 6]. The inhibitory Ly49 receptors contain immunoreceptory tyrosine-based inhibitory motifs (ITIMs) on their cytoplasmic tails that become phosphorylated on tyrosine upon receptor engagement (Fig. 1) [8]. The activating Ly49 receptors lack ITIMs, and instead have a positively charged residue in their transmembrane segment that facilitates the association of DAP12 a signalling adapter protein containing immunoreceptor tyrosine-based activating motifs (ITAMs) (Fig. 2) [9, 10].

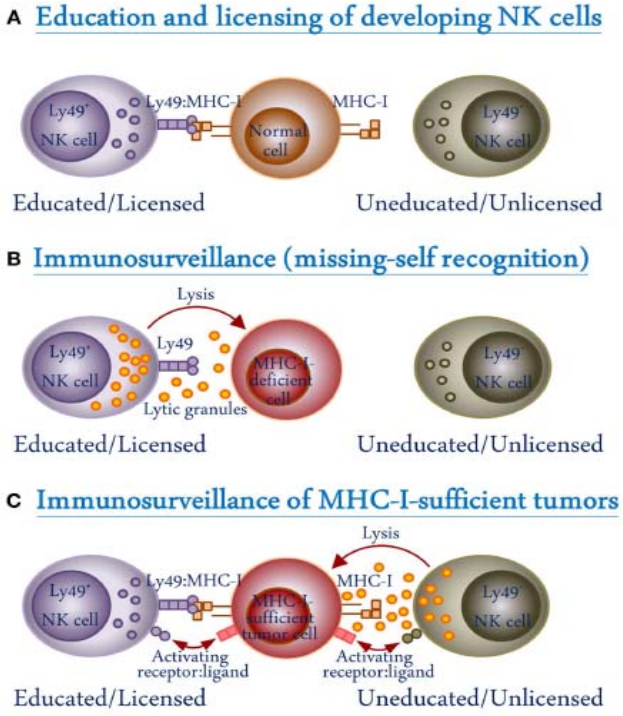
Fig. 2 Inhibitory and activating Ly49 NK cell receptors with their intracellular signalling counterparts [2].



The inhibitory Ly49 receptors are involved in NK cell education, a process in which NK cells acquire function and tolerance toward cells that express "self-MHC-I." On the other hand, the activating Ly49 receptors recognize altered cells expressing activating ligands (Fig. 3) [6]. Inhibitory Ly49 receptors are generally agreed to be important for the prevention of autoimmunity by suppressing NK cell activation [11]. The activating Ly49 receptors recognize ligands that are expressed on abnormal or infected cells and activate cytokine production and cellular cytotoxicity by NK cells [6].

Domestic mammals represent suitable models for evolutionary biology in general. Among them, the family Equidae consisting of a single genus, Equus with different free-living and domesticated species exposed to a variety of pathogens in different habitats is a suitable model for analyzing diversity and evolution of immunity-related genes. It is a rapidly evolving mammalian family, both at the karyotype and molecular level. Therefore, the Equidae might also be interesting models for studying evolution of NKR genes [12].

Fig. 3 Schematic representation of the role of Ly49 receptors in NK cell development and function.



Legend: ***(A)*** *During NK cell development, interactions between the inhibitory Ly49 receptors and their self MHC-I ligands on normal cells result in NK cell functional maturation (education/licensing).* ***(B)*** *Licensed Ly49C but not unlicensed Ly49 NK cells recognize MHC-I- deficient cells and kill them throught here lease of lytic granules (missing-selfrecognition).* ***(C)*** *Tumor cells express ligands which are recognized by activating receptors on NK cells. However, MHC-I- expressing tumor cells can inhibit licensed NK cells through interactions with their inhibitory Ly49 receptors. Unlicensed NK cells will not be inhibited in this way because they lack Ly49 receptors* [3].

The aim of this work is to design genetic markers (microsatellites) to study the genetic diversity of NKR region. Selected microsatellites will also be used to describe the genetic variability NKR region in selected populations and for association analysis of selected diseases in horses.

Material and Methods

Genotyping animals and their DNA

In this study were genotyped 350 individuals from nine populations of different horse breeds (Marajo,

Campolina, Mangalarga Marchador, Galiceno, Camargue, Romanian horse, Gotland, Yakut and Island horse). Samples of isolated DNA were provided from DNA bank of professor Hořín, Department of Animal Genetics, VFU Brno.

Selection of microsatellite markers

The whole genome sequences of six horses [13] in the areas of *Ly49* gene family and adjacent parts (35 – 43 Mbp) were used to select markers *in silico*. Suitable panel of microsatellites for the study of Ly49 region was selected in silico by the number of repeats in available horse whole genome sequences. Only microsatellites with the highest number of alleles were selected.

Design of primers for selected markers

For such markers, primers were designed using the OLIGO software v4.0 (National Biosciences, Inc.; Plymouth, Minnesota).

Fragmentation analysis for selection of microsatellites

Nineteen markers were designed and tested using a fragment analysis with fluorescently labeled nucleotides (fdCTP) on the panel of horse breeds (Hucul, Czech Warmblood, Danish Warmblood, Quarterhorse, American Miniature Horse, Andalusian horse and Camargue).

Nine markers that showed the highest variability in the test panel of animals were selected. This set of markers was subsequently tested using fluorescent fragment analysis on genetic analyzer ABI PRISM 3500 (Life Technologies Corp.; Carlsbad, USA). The obtained data were analysed in GeneMapper software v4.1 (Life Technologies Corp .; Carlsbad, USA) indicators given in Table 1.

Table 1 Microsatellite markers used in present study.

Marker	Repetition	Range of amplicon (bp)	Number of identified alleles
TKY360	$(TG)_n$	288 – 298	6
TKY1745	$(TG)_n$	169 – 193	12
Ly49_2	$(CA)_n$	267 – 273	4
Ly49_3	$(TG)_n$	212 – 222	6
Ly49_4	$(CA)_n$	222 – 244	9
Ly49_5	$(GT)_n$	330 - 364	14
Ly49_7	$(TG)_n$	151 – 165	8
Ly49_8	$(TG)_n$	161 – 167	4
Ly49_9	$(TG)_n$	257 – 271	8

Results and Discussion

Suitable panel of microsatellites for the study of Ly49 region was selected *in silico* by the number of repeats in available horse whole genome sequences. Only microsatellites with the highest number of alleles were selected. In *Ly49* genes, none of the repetitive sequences was polymorphic. Therefore the study area was expanded to 2 Mbp before and 4 Mbp after *Ly49* gene family to reach the known and described microsatellite markers TKY360 and TKY1745[14].

Markers showing a small number of alleles in the test panel of horse breeds were discarded. The numbers of alleles found in microsatellite markers (Table 1) were identified in populations of fifteen horse breeds from different parts of the world, where 400 individuals were included. Genetic diversity of the *Ly49* region will be estimated based on allele frequencies of the selected microsatellites.

Conclusion

This work extends the number of genetic markers for analysis of Ly49 NK cell receptors genetic variability. Previously described alleles of *Ly49* genes [5] could be partly characterized by single nucleotide polymorphisms (SNPs) analysis of this area. We enriched the set of usable markers with 9 microsatellites. The combined genotyping of SNPs and microsatellites may help to define haplotypes of *Ly49* genes. Haplotypes may be more informative for describing the genetic variability in this functionally significant and important part of the immune system.

Acknowledgement

This work was supported by CEITEC - Central European Institute of Technology with research funded infrastructure project CZ.1.05/1.1.00/02.0068 from the European Regional Development Fund.

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Occurrence of cell death in cancer cell line PC-3 after treatment of plumbagin

LENKA MAZALOVA[1], JAN BALVAN[2], HANA POLANSKA[2], ZBYSEK SLADEK[1]
[1] Department of Morphology, Physiology and Animal Genetics, Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2] Department of Pathological Physiology, Faculty of Medicine
Masaryk University
Kamenice 753/5, 625 00 Brno
CZECH REPUBLIC

xmazalo2@node.mendelu.cz

Abstract: The aim of this study, which is focused on occurrence of cell death in cancer cell line PC-3 after treatment of plumbagin is get information about distribution of normal, apoptotic and autophagic cells by exposure to plumbagin. Plumbagin is one of the simplest plant secondary metabolite of three major phylogenic families - Plumbaginaceae, Droseraceae and Ebenceae. Plumbagin assigns highly potent biological activities, for example antioxidant, antiinflammatory, anticancer, antibacterial and antifungal activities. Cancer cell line PC-3 is derived from the 4st degree of adenocarcinoma of prostate. The type of cell death is important for developing of new diagnostical approaches.
In this study types of cell death were studied by using experiment which take 48 hours. During this time cells were exposed to plumbagin, which cause one of type of cell death. Action of this metabolite was finished after certain hour depending on the number of sample and after that samples were analysed by light microscope.
Proportion of type of cell death was following. The highest percentage of apoptotic bodies (46%) was observed 6 hours after start of experiment. Apoptotic cells were in the largest frequency 2 hours after start of experiment (10%). Most of residual cells moved to autophagy 48 hours after start of experiment (20%).
To conclusion, treatment of cancer cell line PC-3 by plumbagin showed that this metabolite can cause cell death of cancer cells. This information is important for further development of treatment of cancer diseases.

Key-Words: apoptosis, necrosis, autophagy, cancer, cell, PC-3, plumbagin

Introduction

Carcinoma of prostate gland is the most expanded oncological disease in developed countries. Incidence of this type of cancer is higher about 300% from 1995. On 100 000 men come on 131 case of incidence prostate cancer according to information from National oncological registr. This intensive increase is cause by ageing of population and preventive medical examination, respectively [1]. More than 65% of all causes prostate cancer were determined in patients, who were older than 65 years [2].

Prostate cancer represent very important social problem. This fact is confirmed by experiments, which are targeted on development of new diagnostical approaches. These can help reveal cancer proliferation exactly and efficiently and punctual treatment can be begin. In this time research in cancer diseases is concentrated on process, which are in association with occurrence of some type of cell death, for example apoptosis, necrosis and autophagy.

Apoptosis, also called programmed cell death, is main mechanism, which helps in physiological elimination of cells from organism. This cell death occurs naturally during development and ageing of organism as homeostatic mechanism and for keeping population of cells in tissues. In consequence of damage by diseases or by noxious influences to apoptosis can occur also during imunological reaction as defence mechanism [3]. Apoptotic process is characterised by specific changes in cells structure – shrinking of cell, condensation of cytoplasm and chromatin in nucleus (karyopyknosis),

bubbling cytoplasmatic membrane (zeiosis), division of apototic cell on apoptotic bodies. Apoptotic bodies are recognised and eliminated by phagocytosis. It is Necrosis is passive form of cell death. It is finally resulting of bioenergetic catastrophe, which follows from depletion of ATP on level incompatible with cell survival. Necrotic process occur in typical sequence – fusion of small membrane bubbles into one big bubble, which is separated from rest of cell substrate [5]. Morphologically necrosis is characterised by production of vacuoles in cytoplasm, falling down of plasmatic membrane and initiating of inflamation around dying cell. Cells which are dying by necrosis exhibit changes in morphology of nucleus too, but not fragmentation and condensation as in apoptosis [4].

Autophagy is catabolic process targeted on cell organels and cytoplasmatic components, which are determined for degradation in lysosome. Autophagy proceeds selectively and is concentrated on liquidation of old and useless structures in cell primarily. It provides recyclation of cell components and participates in maintenance of cell homeostasis and cell integrity [6]. Level of autophagic activity is arranged as response on various intracellular and extracellular stimulus, for example ongoing disease, starvation, lack of oxygen and other form of stress [7].

Plumbagin belongs to group of naphthoquinones. It is organic compound, which is extracted as derivate from root of plant *Plumbago zeylanica*. Coloured pigments are the most frequent forms, which are occured in some kind of bacteria, fungus and higher plants. Naphthoquinones have pharmacological effects, high toxicity, antibacterial, antifungal, antiviral and antiparasitic traits. In tissue and cellular cultures anticancer and antiproliferative effects were observed [8].

The aim of this study, which is focused on occurrence of cell death in cancer cell line PC-3 after treatment of plumbagin is get information about distribution of normal, apoptotic and autophagic cells by exposure to plumbagin.

Materials and Methods

This study was treated on cellular cultures of line PC-3. This cancer line is derived from the 4st degree of adenocarcinoma of prostate, androgen independent (HPA Culture Collections, Salisbury, UK).

Cultivation of cell lines

Cultivation proceeded in cultural, sterile bottles at 37 °C and concentration of CO_2 was 5%. Medium for PC-3 is composed from Ham's F12 medium with 7% FBS and antibiotic.

important for absence of inflammation around dying cell [4].

Passage of cells

1. Extraction of old medium from cultural vessel, where are accumulated cells.
2. Washing by EDTA (2 ml).
3. Extraction of EDTA.
4. Washing by trypsin (2 ml, 1x) – time of reaction 30 seconds.
5. Extraction of trypsin.
6. For 3 minutes put cultural vessel with cells into CO_2 thermobox.
7. Washing away deadherent cells into medium.
8. Cell suspension put into centrifugal tube and rotate for 7 min, 2700 revolution, at 4°C.
9. Extraction of supernatant and resuspending of cells in medium.
10. Sowing of cells into new cultural vessel with fresh medium.

Changing of medium:

1. Extraction of old medium from cultural vessel.
2. Washing by rinsing medium (medium + ATB, 2 ml).
3. Extraction of rinsing medium.
4. Putting of fresh medium.

Application and effect of cytostatic in growth medium:

Cytostaticum plumbagin was applicated into growth medium with prostatic cell culture in concentration, which correspondent with IC50 – 2uM plumbagin. Cultivation was interrupted at expiration of time interval and morphological changes were analysed. Duration of action of cytostatic was following: 1, 2, 3, 5, 6, 7, 9, 9.5, 10, 11, 12.5, 13, 27, 48 hours.

Evaluation of results:

Capture of occurence of characteristic features of cell death was provided by inverse light microscope Olympus IX71. Photographs were taken by camera Nikon D80.

In every time interval representative selective set of cells was analysed. For each measurement one hundred of cell were collected. These cells were divided to five categories by their morphology: normal cells, apoptotic bodies, apoptosis, autophagy and necrosis. From each categories percentage of occurrence was analysed. Study Death by design: apoptosis, necrosis and autophagy by Edinger and Thompson, 2004 was default for sorting of cells into particular categories [4].

Results and Discussion

Percentage of occurrence of cell death by dependency on time of plumbagin treatment is shown in Table 1.

Table 1 Occurrence of cell death [%]

Time of treatment [hours]	Normal cell [%]	Apoptosis [%]	Apoptotic bodies [%]	Autophagy [%]	Necrosis [%]
0	100	0	0	0	0
1	68	8	22	2	0
2	58	10	26	6	0
3	68	4	19	9	0
5	75	3	14	8	0
6	41	5	46	7	1
7	76	3	16	5	0
9	68	2	27	2	1
9.5	70	5	18	6	1
10	66	6	25	3	0
11	67	7	24	2	0
12.5	75	4	17	3	1
13	58	7	33	1	1
27	74	2	20	4	0
48	54	3	23	20	0

As it is apparent from these results, cells with normal morphology achieved the greatest occurrence. Treatment of plumbagin wasn't expressed by changes in morphological structure and evidences of upcoming cell death weren't elicited.

Nevertheless, percentage of occurrence of normal cells declined by 30%. One of the lower worth of unchanged cells was registered 48 hours after start of experiment. This effect is caused mainly by increase of autophagy. Cells succumbed to stress (depletion of growth medium, less of space) and process of delaying cell death-autophagy-was choosen.

Desintegration of apoptotic cell into apoptotic bodies was the most numerous morphological changes. The largest occurrence was observed 6 hours after beginning of experiment. It is in association with fact that apoptotic cells were the most plentiful 2 hours after start of testing. Time intervals needful for causing of morphological changes, from shrinking of cytoplasm in apoptotic cell to disruption into apoptotic bodies, were in correlation absolutely. In remaining measurements apoptosis was about 5% and incidence of apoptotic bodies was about 20-25%. In consequence, increased proportion of apoptotic bodies is caused by identification because one apoptotic cell is broken down into several apoptotic bodies. Apoptotic cell is only one, while apoptotic bodies emerge from it much more. Therefore, number of apoptotic bodies is larger proportionally.

Occurrence of autophagy fluctuate during whole experiment. Whereas in initial measurement proportion of autophagy cells was increased, by end proportion was decreased. After 48 hours stressful factors, which affected cells were emerged and autophagy was achieved maximum – 20%. In remaining time interval 5% occurrence of autophagy wasn't overlaped. It was caused mainly by fact, that cell tried to gain delaying from cell death by autophagy. After certain time, depletion of energy supply occurs and necrosis begins.

Traits of necrosis were observed in the making of experiment rarely. This fact can be explained by proportion of autophagy because it was relatively

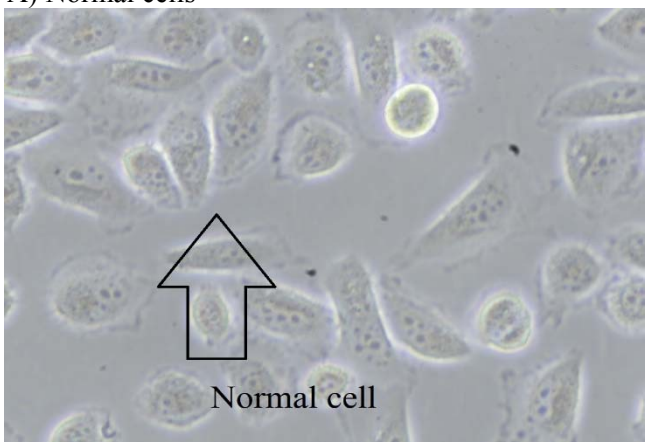
minor and identification of necrotic cell among one hundred other cells is problematic. Moreover, it may be caused by disruption of cell in time between measurements probably and this cell became unrecognizable.

Data of occurrence of cell death in cancer cell line PC-3 after some treatments are not at disposal. This study is the first, which published results of such experiments. But results described above are in correlation with similar experiments with using of different treatment [4].

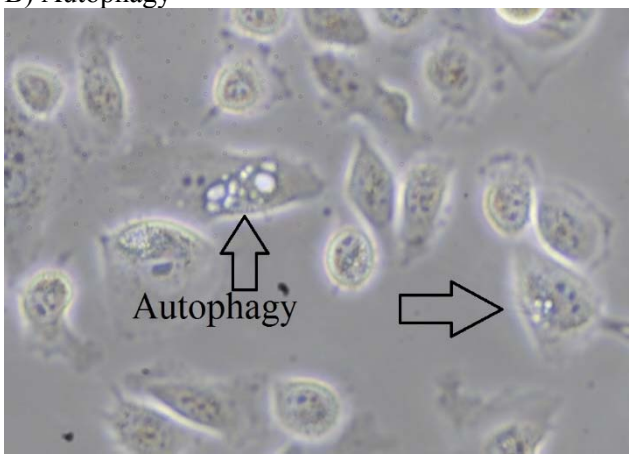
Observed types of cell death are in Figure 1.

Fig. 1 Type of cell morphology associated with cell death

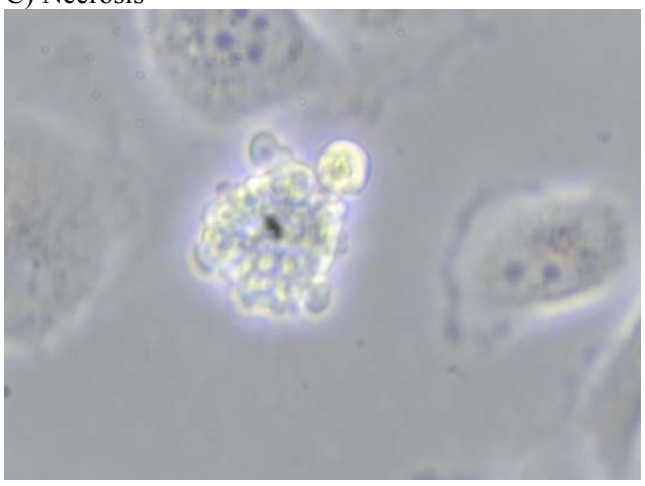
A) Normal cells



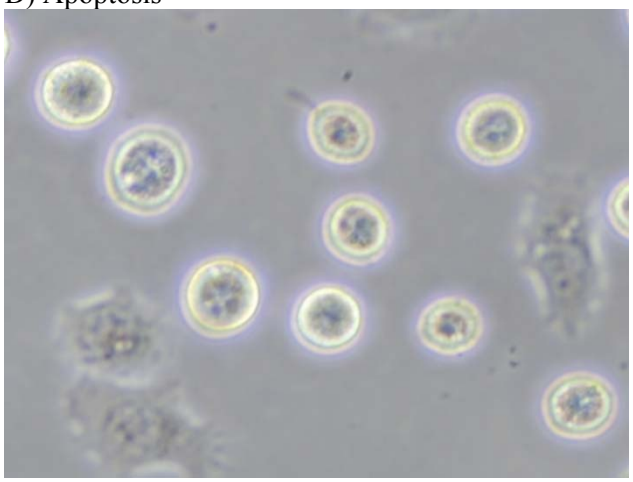
B) Autophagy



C) Necrosis



D) Apoptosis



Conclusion

Treatment of plumbagin on prostate cancer cell line PC-3 indicated that initiating of certain type of cell death was successful. Apoptosis with follow up disruption into apoptotic bodies was mainly observed. This detection confirms importance of using of cytostatics during treatment of cancer diseases.

Current approaches on active service against cancer are concentrated on induction of cell death. By this proliferation and migration of cancer cells would be terminated. It is very important to engage in this question in next study.

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Toxic effect of 1% PHMG on aquatic organisms

EVA POSTULKOVA, RADOVAN KOPP, PETR CHALUPA, LENKA HADASOVA
Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

e.postulkova@seznam.cz

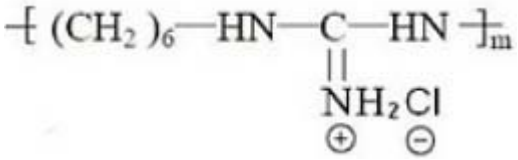
Abstract: Tests of toxicity are part of evaluation of newly developed and into practice introduced algicidal preparations. Using of model organisms, the fish species Zebra fish (*Danio rerio*) and culture of common green alga species *Desmodesmus communis* are recommended for testing the chemical substances in toxicology. Purpose of this study was to examine toxical effects of 1% PHMG (Polyhexamethylene guanidine hydrochloride) preparation on Zebra fish (*Danio rerio*) and green alga (*Desmodesmus communis*). Determination of acute toxicity on Zebra fish was realized according to ČSN EN ISO 7346-1 and test of growth inhibition of freshwater alga was realized according to methodology ČSN ISO 8692. The concentrations of 0.001; 0.005 and 0.1 $ml.l^{-1}$ for 1% PHMG for inhibitory tests with green alga (*Desmodesmus communis*) were chosen. The concentrations of 0.010; 0.025; 0.050; 0.075; 0.100; 0.125 $ml.l^{-1}$ of 1% PHMG for short-term tests of acute toxicity on Zebra fish (*Danio rerio*) were chosen. The values of LC_{50} and IC_{50} were evaluated using graphic probit analyze. For Zebra fish (*Danio rerio*) 48hLC_{50} 1% PHMG was 0.0431 $ml.l^{-1}$ and 48hIC_{50} 1% PHMG was 0.0010 $ml.l^{-1}$ for *Desmodesmus communis*.

Key words: algicide, *Desmodesmus communis,* green algae, IC_{50}, LC_{50}, test of toxicity, Zebra fish (*Danio rerio)*

Introduction

Tests of toxicity are important part of evaluation of newly developed and into practice introduced algicidal preparations [8]. The emergence and development of new algicidal products is caused by the increasingly growing pond construction and use of these products in fish farming especially for the disposal of unwanted algae and cyanobacteria. Cyanobacteria and algae produce a variety of substances that negatively affect the quality of the environment, many are also dangerous to humans [7]. The mass development of cyanobacteria is not a problem just in the Czech Republic but worldwide as well. There are no simple measures that would be effective against the development of cyanobacteria, applicable to different types of water tanks and would prevent damage to the aquatic ecosystem at the same time [4]. One of the most common options in the fight against algae and cyanobacteria includes application of algicidal preparations [6]. Widespread algicide is a blue copperas. The biggest advantage of this algaecide is low cost and rapid effect. However, its disadvantage is a copper contain in it. The effectiveness of copper is strongly influenced by the composition of the water in which is the blue copperas dosed [5].

Fig. 1 The structural formula of polyhexamethylene guanidine hydrochloride (PHMG)



Among the newly evolving algicides belongs also a polyhexamethylene guanidine hydrochloride (PHMG). It is a cationic polyelectrolyte which has unique physico-chemical biocidal effects. This polymer is colourless and odourless and is non-flammable and non-explosive. Furthermore, it is completely soluble in water and soluble in alcohol. It is not perishable at low temperatures and maintains its biocidal effect at temperatures up to 120°C. PHMG is most commonly used in limiting G^- and G^+ bacteria (eg. *Mycobacterium tuberculosis*), against viruses, fungi, including yeasts and moulds and is expected to limit cyanobacteria and algae [10].

For the testing of chemical substances in toxicology is recommended to use a model species of Zebra fish (*Danio rerio*). Recommended use of Zebra fish does not preclude the use of other species. It is

possible to use other species of freshwater, marine or brackish fish, assuming that appropriate adjustments such as dilution water quality and temperature conditions of the test are made [1].

For inhibition tests test organisms of planktonic algae, for example *Desmodesmus communis, Desmodesmus subcapitatus* or *Pseudokirchneriella subcapitata* may be used. These species are planktonic green algae belonging to the order *Chlorococcales* and in culture they are usually unicellular [2]. The algae and cyanobacteria are common testing organisms sensitive to many chemicals, and therefore they are widely used in toxicity tests [14]. The algae are key functional organisms because they are dominant primary producers and therefore they represent basic segment in aquatic food chains [9].

Material and Methods

Median lethal conentration (LC_{50}) of tested algicidal preparatus 1% PHMG for fish (*Danio rerio*) was tested in acute toxicity tests. Tested fish were exposed for 96 hours to various concentrations of the tested substance dissolved in standardly prepared dilution water. For short-term acute toxicity tests we chose six different concentrations of 1% PHMG: 0.010; 0.025; 0.050; 0.075; 0.100 and 0.125 $ml.l^{-1}$. As a control, we used an aquarium with fish filled only with the diluting water without tested preparation. Every 24 hours, mortality of fish was monitored during the test. Also temperature, pH and dissolved oxygen in water were measured using HACH HQ40D device and conductivity using Hanna combo device in 24 hours interval.

4 months old Zebra fish individuals of total body length 15-20 mm were used in the test. Individual fish were randomly selected to test from the stock tank and were not fed during the test. Each test aquarium contained 10 fish in 3000 ml of test solution without aeration. Dilution water was prepared according to ISO 6341 from stock solutions in an amount of 11.76 g of $CaCl_2 \cdot 2H_2O$, 4.93 g of $MgSO_4 \cdot 7H_2O$, 2.59 g of $NaHCO_3$ and 0.23 g of KCl [12]. Such prepared dilution water was saturated with air oxygen for 24 hours (aeration).

Subsequently, we determined the inhibitory effect (IC_{50}) of 1% PHMG on growth of green alga (*Desmodesmus communis*). Before starting the test, the growth medium according to standard ČSN EN ISO 8692 was prepared [2]. Tests were carried out for 168 hours under laboratory conditions in Erlenmeyer flasks with green alga (*Desmodesmus communis*). Concentrations for inhibition tests of 1% PHMG were chosen as follows: 0.001; 0.005 and 0.01 $ml.l^{-1}$. As the control, there were green algae incubated without any additional substances. Each variant contained 50 ml of the tested solution. Test vessels were sealed by cellucotton to prevent airy contamination and reduce evaporation, but in order to maintain gas exchange [2]. To determine the inhibitory or stimulatory effects of a tested substance, a quantitative method of counting cells in Bürker chamber using a fluorescence microscope was used. This counting was carried out for 72 hours in 24 hours intervals. The principle according to Bürker counting is based on the counting in chamber under a cover glass, where a thin layer of water with a height of 0.1 microns is applied [12]. The number of cells counted in the Bürker chamber was further recalculated using the formula for the amount of cells present in 1 ml.

Fig. 2 The formula for recalculating the cells in 1 ml

$$\frac{\text{amount of cell (individuals)}}{\text{area in mm}^3} * 1000$$

Subsequently, chlorophyll-a was determined within 168 hours based on the principle of chlorophyll extraction with hot ethanol and measuring by spectrophotometer at wavelengths ranged between 665 and 750 nm. Hydrochloric acid (HCl) and pure ethanol (C_2H_5OH) is used to determine the chlorophyll-a [3].

Results and Discussion

For acute toxicity test on fish and inhibition test on algae algicidal substance 1% PHMG was chosen. The aim of the test was to determine the median lethal concentration (LC_{50}) and the inhibitory concentration (IC_{50}) by using probit analysis. During the test, the average water temperature in all variants and control was 23.5°C. Temperature fluctuations were not observed. Oxygen saturation ranged from 53.5 to 89.5% (from 4.43 to 7.49 mg^{-1}). pH value in all aquaria showed a slightly alkaline environment which is suitable for fish farming. Conductivity of the water ranged from 39.2 to 43.0 mS. Mortality at concentrations of 0.050; 0.075; 0.100 and 0.125 $ml.l^{-1}$ after 48 hours was 100%. Concentrations up to 0.025 $ml.l^{-1}$ didn´t cause any mortality. The median lethal concentration

Table 1 Average number of cells and ±SD of *Desmodesmus communis* in 1 ml and the amount of chlorophyll-a

Hours	Control	±SD	0.001 ml.l^{-1} 1% PHMG	±SD	0.005 ml.l^{-1} 1% PHMG	±SD	0.01 ml.l^{-1} 1% PHMG	±SD
0	11 500	4 000	11 500-	4 000	11 500	4 000	11 500	4 000
24	14 468	2 165	7 523	5 367	1 763	1 418	0	0
48	8 935	10 449	17 940	9 113	0	0	0	0
72	27 199	6 993	16 204	5 729	0	0	0	0
96	23 727	4 331	24 306	6 496	0	0	0	0
	Chlorophyll a v µg.l^{-1}							
168	70.25	11.33	57.06	3.58	1.97	1.48	0.00	0.00

(48hLC$_{50}$) for 1% PHMG was calculated using probit analysis on 0.0431 ml.l^{-1}. In Table 1, the average cell counts of green alga *Desmodesmus communis* and the amount of chlorophyll-a after 168 hours is presented. For concentration of 0.01 ml.l^{-1} inhibition occurred within 24 hours, and the inhibition was 100%. At concentration of 0.005 ml.l^{-1} occurred 100% inhibition within 48 hours. At concentration of 0.001 ml.l^{-1} inhibitory effect was observed within 24 hours. After 24 hours, the inhibitory effects receded and the substance began to manifest as stimulant, when at the end of the test was found more than double amount of cells compared to the initial amount. After 168 hours from the start of the test chlorophyll-a was determined in all variants [3]. The average concentration of chlorophyll-a in control achieved the value 70.25 µg.l^{-1} which represents 100%. Tests of determination the chlorophyll-a were performed in Erlenmeyer flasks of 100 ml volume with an initial amount of 11 500 cells in 1 ml (± 4000) under the artificial light in the interval of 13 hours light - 11 hours dark. All variants were in three repetitions. In the variant with 0.001 ml.l^{-1} 1% PHMG the value reached 57.06 µg.l^{-1} (81.2%), in the variant with 0.005 ml.l^{-1} was 1.97 µg.l^{-1} (2.8%) and the latest version with 0.01 ml.l^{-1} there was no chlorophyll-a. For 1% PHMG 48hIC$_{50}$ at 0.001 ml.l^{-1} was set.

The results can be compared with Vaněk [13], who tested the same substance (1% PHMG) and achieved similar results. In our tests, 100% inhibition at 0.01 ml.l^{-1} after 24 hours was found. Vaněk [13] presents, that at the same concentration was 100% inhibition after 48 hours. In acute toxicity tests on fish Vaněk [13] used initial concentration of 0.5 ml.l^{-1}, which caused 100% mortality within 24 hours. The same results occurred in our tests, as it is shown in the Table 2.

Svobodová et al. [11] presents, that the widely used algicidal agent for limiting the mass development of cyanobacteria and algae was Kuprikol 50, containing at least 47.5% metallic copper in the form of copper oxychloride. In determination of the acute toxicity of Kuprikol 50 on aquatic organisms they determine the amount of 48hLC$_{50}$, which was for fish *Poecilia reticulata* 129 mg.l^{-1}. In comparison with Kuprikol 50, our tested preparation 1% PHMG has lesser lethal concentration.

Conclusion

Tests of acute toxicity on fishes (*Danio rerio*) and inhibition tests with selected culture of green alga *Desmodesmus communis* was carried out with 1% PHMG. The effective inhibitory concentrations for algae extermination and the median lethal concentration (LC$_{50}$) for fish were tested. We checked the effectiveness of preparation using density measurements in Bürker chamber under the microscope with fluorescence and 48hIC$_{50}$ 1% PHMG on *Desmodesmus communis* were counted. When comparing 48hLC$_{50}$ 1% PHMG, which is 0.043 ml.l^{-1} for fish, with inhibitory concentration required to limit algae 48hIC$_{50}$, which is 0.001 ml.l^{-1}, we could say that this is a sufficiently safe substance that should not have any negative effects on tested fish *Danio rerio* and vice versa should be sufficiently effective for the reduction of green algae. It is very important for algicidal agents not to cause massive mortality of biomass and prevent the releasing cellular contents into the surrounding area.

The aim is to reduce the photosynthetic assimilation, so the colonies will sink to the bottom out of the reach of photosynthetically active solar radiation and gradually began to decompose there. Therefore at the end of the test the content of chlorophyll-a of green algae *Desmodesmus communis* was determined because then it was possible to see, in which variants of the test with different concentrations of 1% PHMG were algae still

photosynthetically active and for which photosynthetic processes ended.

Table 2 Physico-chemical parameters and mortality of acute toxicity test on fish (*Danio rerio*)

Concentration 1% PHMG	Temperature [°C]					O_2 [%]				
[ml.l^{-1}]	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
Control	23.4	23.8	23.5	23.4	23.7	85.6	57.9	70.8	71.2	71.3
0.010	23.4	23.6	23.5	23.5	23.6	82.6	53.5	59.8	66.8	64.1
0.025	23.3	23.6	23.4	23.4	23.6	87.9	57.9	56.6	56.0	56.6
0.050	23.4	23.7	-	-	-	88.5	76.9	-	-	-
0.075	23.3	23.6	-	-	-	88.9	81.5	-	-	-
0.100	23.2	23.6	-	-	-	88.7	83.5	-	-	-
0.125	23.2	23.6	-	-	-	89.5	86.7	-	-	-
Concentration 1% PHMG	pH					Mortality [pcs]				
[ml.l^{-1}]	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
Control	7.43	7.30	7.41	7.50	7.46	0	0	0	0	0
0.010	7.70	7.47	7.63	7.67	7.64	0	0	0	0	0
0.025	7.73	7.44	7.53	7.56	7.57	0	0	0	0	0
0.050	7.73	7.59	-	-	-	0	10	-	-	-
0.075	7.74	7.65	-	-	-	0	10	-	-	-
0.100	7.76	7.63	-	-	-	0	10	-	-	-
0.125	7.76	7.68	-	-	-	0	10	-	-	-

Acknowledgement

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Detection of Aminoglycoside, Sulfonamide and Tetracycline resistance genes in Escherichia coli isolated from bovine milk samples

VLADIMIR PYATOV, IRENA VRTKOVA, ALES KNOLL
Department of Animal Morphology, Physiology and Genetics
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

vladimir.pyatov@mendelu.cz

Abstract: Many studies report that the amount of antibiotic resistant bacteria has increased in recent years. The aim of this study was to evaluate the frequency of aminoglycoside (*strA*, *strB*) sulfonamide (*sulI*, *sulII*) and tetracycline (*tetA*, *tetB*) resistance genes in *E.coli* isolated from milk samples of dairy cows in the Vysočina Region. A total of 72 samples were obtained from dairy farms. Isolates were tested for the presence of afore mentioned genes by using PCR. *StrA* and *strB* were detected most frequently. In 28 samples there were no resistance genes detected, while in 7 isolates all six were identified. The remaining samples showed a varying number of resistance genes and combinations.

Key-words: PCR, *E.coli*, antibiotic resistance, aminoglycoside, sulfonamide, tetracycline.

Introduction

Escherichia coli is one of the most common pathogens causing contagious and environmental mastitis on dairy farms [1]. Antimicrobial therapy is frequently used for treatment and prevention of this disease [2]. Wide usage of antibiotics in veterinary medicine plays a significant role in the increase of resistance [3]. As a consequence, the treatment of diseases caused by bacterial pathogens has become very difficult.

Detection of multiple-antibiotic-resistant *E.coli* strains has been reported in several studies [4, 5]. Resistances to aminoglycosides, sulfonamides and tetracyclines among *E.coli* isolates are the most prevalent [6]. The genes most often responsible for mentioned resistances are *strA* and *strB*, *sulI* and *sulII*, *tetA* and *tetB* respectively [2, 3, 6].

Many genes encoding antimicrobial resistance are located on plasmids and/or transposons [5]. This enables their transfer between and within bacterial species. Therefore, the assessment and surveillance of resistances at the genetic level is very important.

The aim of this study was to assess aminoglycoside, sulfonamide and tetracycline resistance in *E.coli* isolated from bovine milk in the Vysočina region at the genetic level.

Material and Methods

A total of 72 *E.coli* isolates obtained from raw cow milk were used in this study. Samples were collected from dairy farms in the Vysočina Region in the Czech Republic. Total DNA extraction from milk samples and bacterial species identification were provided by using "Thermo Scientific PathoProof ™ Mastitis Complete-12 assay". For detection of amingoglycoside (*strA* and *strB*), sulfonamide (*sulI* and *sulII*) and tetracycline (*tetA* and *tetB*) resistance genes the PCR method was used. The primers (Table 1) were designed using Primer3 and verified by Oligo4 and Primer Express® Software v2.0. The reaction mixture (total volume 10 µl) consisted of 5 µl of PPP Master Mix (Top-Bio, Praque), 0.5 µl of each primer (stock concentration 10 µM), 3.5 µl of PCR water and 0.5 µl of the template DNA (20ng/µl).

PCR was performed under the following conditions: initial denaturation at 95°C for 2 min, 30 cycles of denaturation at 95°C for 30 s, primer annealing at 60°C for 30 s and elongation at 72 °C and final elongation at 72°C for 7 min. Reaction products were detected by electrophoresis using a 2% agarose gel and visualized with ethidium bromide.

Table 1 PCR primers used for detection of antibiotic resistance genes

Gene	Primer name	Primer sequence	Fragment size (bp)	Annealing temp. (C°)	Reference GenBank
strA	StrA-F StrA-R	TACCGGACGAGGACAAGAGT GACCCGTGCATTGAAGAGTT	165	60	NC_001740
strB	StrB-F StrB-R	GGCGATTATAGCCGATCAAA TCAGCCGGATCGTAGAACAT	174	60	NC_001740
sulI	SulI-F SulI-R	GACGAGATTGTGCGGTTCTT CCGACTTCAGCTTTTGAAGG	160	60	X12869
sulII	SulII-F SUlII-R	TGGTGTGGCCTATCTCAATG CGCAATGTGATCCATGATGT	160	60	M36657
tetA	TetA-F TetA-R	TGTCCGACAAGTTGCATGAT CCTTGAACGGCCTCAATTT	178	60	X00006
tetB	TetB-F TetB-R	GCCAGTCTTGCCAACGTTAT CGATGCGCCTATTAATGACA	178	60	J01830

Results and Discussion

Aminoglycoside resistance genes were detected most often. In 35 isolates (48.6%) the *strA* resistance gene was detected, while 42 (58.3%) contained the *strB* gene. Similar results were obtained by Schweiger et al. [3] where *strA* was found in 52.6% porcine and 61.2% human origin samples. The occurrence of *strB* was 54.7% in porcine and 63.8% in human origin samples. This contrasts with Lanz et al. [7], who found both genes in only 4.7% of animal isolates and Srinivason et al. [2], who detected both genes in 8.5% of dairy samples.

Genes responsible for sulfonamide resistance were detected less often: *sulI* in 27 (37.5%) and *sulII* in 22 (30.6%) samples. Some research groups report a significant prevalence of *sulII* over *sulI* in E.coli samples, e.g. Sunde and Norström [8] found sulII in 75% of the meat samples and sulI only in 16%. Karczmarczik et al. [9] detected above mentioned genes in 90% and 26% of the environmental samples collected at a cattle farm, respectively. In contrast, Maynard et al. [5] found sulI in 79% and in 36% of E. coli isolates obtained from pigs. On the other hand Dolejska et al. [10] and Drugdova and Kmet [11] report about low prevalence of both genes in avian E.coli isolates.

Genes responsible for tetracycline resistance were detected least frequently. *TetA* was detected in 14 (19.4%) and *tetB* – in 21 (28.2%) of the isolates. Bryan et al. [12] also observed a prevalence of *tetB*, but at a higher percentage (35% and 63% respectively) in diverse human and animal sources. Mayhard et al. [5] identified a significant prevalence of *TetB* over *TetA* (80% and 25%). This contrasts with Karczmarczyk et al. [9] who reported *tetA* being present twice as often in *E.coli* isolates than *tetB*. Lanz et al. [7], Sunde and Norström [8] and Koo and Woo (2011) also detected *tetA* more frequently. Srinivasan et al. [2] found *TetA* only in 10.9% of their samples and have not detected *TetB*.

In total, 8 *E.coli* isolates carried five antibiotic resistance genes, 10 carried four genes, 4 carried three genes 12 carried two genes and 3 carried one gene, in different combinations. In 7 samples all six antibiotic resistance genes were detected. There were no sought genes identified in 28 *E.coli* isolates.

Conclusion

This study reported about prevalence of aminoglycoside (*strA*, *strB*) sulfonamide (*SulI*, *SulII*) and tetracycline (*TetA*, *TetB*) resistance genes in E.coli isolated from milk samples of dairy cows in the Vysočina Region.

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Endoparasites of wild animals of vineyard and agricultural landscape in the South Moravia

JANA SEDLAKOVA, MARIE BORKOVCOVA
Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of Agriculture
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jani.sedl@seznam.cz

Abstract: A parasite is an organism that is metabolic and physiological dependent on another organism. The aim of the study was to determine the interrelationships between spectrum of endoparasites and species biodiversity in reporting landscape with focus on selected omnivore, carnivore and herbivore representatives. Together 139 excrement samples of animals were collected in selected localities and were investigated by the flotation and subsequently microscopic examination. In Conclusion parasitic infestation of the hare, deer, pheasant and marten of vineyards and fields was compared. Monitoring was carried out on a total of 6 locations, 3 locations were found in vineyards (site A) and 3 locations in intensive agriculturally use (site B). The total number of species of endoparasites on site A was observed in animals 30, at locations B was 24 species. Prevalence of endoparasites were determined at most in deer - in both locations identically, then in pheasant in area B, the lowest prevalence was captured by hares in area A. Using ecological indices were calculated index for biodiversity area A H = 1.1 and for the site B H = 0.8. Index evenness for site A was E = 0.35, for the site BE = 0.21. Index of species dominance evaluate site A as less anthropogenically influenced, but the differences were not very large in comparison with the location B.

Key-Words: endoparasite, excrement sample, flotation, microscopic examination, vineyards

Introduction

There are many works devoted to anthropogenic impacts on species diversity in the scientific literature. There were monitored primarily charismatic species of mammals, birds, butterflies daily ferrous and large beetles. Diversity of parasites in relation to landscape fragmentation and intense agricultural activity is contrary devoted little attention. It is known, however, that just by parasite species spectra can reveal the negative impacts of anthropogenic activities before they react to these influences by main predators [1].

Studies of biodiversity traditionally focus on charismatic mega fauna. Little is known about parasite biodiversity. Relationships of host specific parasites with their hosts should be common and that parasites may even go extinct before their hosts. Examined the coexistence between parasite diversity and habitat quality have focused on parasites that require intermediate hosts and pathogens that require vectors to complete their life-cycles. It shows that parasite amount correlates with size of environment, even among animals that are locally common. The absence of some ectoparasites genera in small habitats suggests that parasites can go locally extinct even if their hosts persist. It is therefore necessary to preserve fragments habitat for the conservation of biological diversity of parasites, but also its size to maintain not only the host population, but also populations of parasites [2].

Parasites tend to be host-specific [3]. They can be found on only a few or even a single host species [4]. The co-extinction of parasites with their hosts may be common because of this high degree of specificity [5]. Host populations are ‘‘islands’’ and host population size should determine parasite diversity, just as classical island biogeography theory predicts [6].

Parasites interact in complex ways with other stressors. The interaction may lead to a disproportionately negative effect on the host population or the stressor may ameliorate the effects of parasitism. Pollutants may increase parasitism by increasing host and decrease parasitism if infected hosts suffer differentially high mortality. Abundance of parasites attendant is either increased or reduced habitat alterations [7]. The rich communities and high abundance may foster parasitism [8]. The results of the study in the United States suggest that by influencing the community composition of vertebrate

hosts for disease-bearing vectors, habitat fragmentation can influence human health. Forest destruction and fragmentation have been shown to reduce mammalian species diversity and to elevate population densities of white-footed mice. It is consequence of reduced species diversity and high mouse density in small fragments is an increase in human exposure to Lyme disease [9].

The aim of this study was to contribute to knowledge in this area.

Material and Methods

Characterization of material

The faeces samples were collected at 6 locations in the South Moravian Region district Brno-Country, Znojmo and Břeclav. At 3 sites designated as localities A is the main agricultural activity viticulture and horticulture, landscape typical of the hilly terrain. On the sunny slopes are cultivated vines, apricots and peaches. We find it significant forest-steppe and steppe communities. These are locations Nosislav, Moravian Krumlov and Kobylí.

Nosislav is located at an average altitude of 192m, the average annual temperature is 9°C, and average rainfall amount is 551mm per year. The site is situated at the river Svratka in Dyjsko-Svratka valley and passes through the territory of the D2 motorway convenient location slopes and warm climate with mild winters want traditional crops in the area - the vine, apricots and peaches. This is a wine-growing village in Velkopavlovické wine region [10].

Moravian Krumlov locates in an altitude of 225m, the average annual rainfall is 500-550mm, the average annual temperature is 9°C. The site lies in a basin that is on three sides wrap river Rokytná. Moravian Krumlov is appealing a varied landscape and impact around the meandering nature of the river Rokytná with rare flora and fauna, as evidenced by a national preserve "Krumlovsko rokytenské conglomerates "in 2005 the number of combinations of abiotic conditions (orientation, slope, humidity, local soil chemistry) in this area has developed a number of communities of different nature, due to which the whole territory acquired by the extraordinary species richness [10]. There is verified 550 species of higher plants. National nature reserve has a warm, dry climate with mild winters and shorter sun shines [11].

Location Kobylí located at an altitude of 205m, the average annual rainfall is 515mm and the average annual temperature is 9°C [11]. Once seabed, the lake basin, together with favorable climatic conditions allow the existence of protected steppe communities. Forest vegetation are insular, discrete, in some parts of the tree vegetation is present only in the form of acacia. Predominant extensive agricultural crops (fields, orchards, vineyards), in recent decades, it was also a lot of terracing slopes. The natural vegetation is preserved spare practically only on steep slopes [10].

At 3 sites designated as B disgust are the main agricultural crop production activities, especially the cultivation of maize, sunflower, cereals and vegetables [9]. Agriculture greatly affects the appearance of the landscape; harvest due to the warm climate takes place twice a year. This is a site Olbramovice, Moutnice and Zaječí [11].

Olbramovice lies at an altitude of 220m. The area is situated between the two areas and a very hot area of T4 and T2 warm area. The average annual temperature is between 8-9°C. The surrounding forests occupy only a tenth of the village, you will find also orchards and vineyards [10].

Moutnice is situated at an altitude of 197m, where the average annual temperature is 9°C, the warmest month is July, where the average temperature is 19.9° C and the coldest month is January, where the temperature is -1.9°C. Average annual precipitation reaches 506mm, the area is among the hottest locations in the country. Due to the proverbial fertility of fields Moutnice the land has always belonged to the basic livelihood of agriculture [10].

The third site is designated as B Zaječí with an average altitude of 187m. The climatic classification of the area is situated in the warm climate of the unit W4 [10], the average annual temperature is between 9-10° C and average annual rainfall is 500-550mm. The site southwest touches the lower reservoir dame system [11].

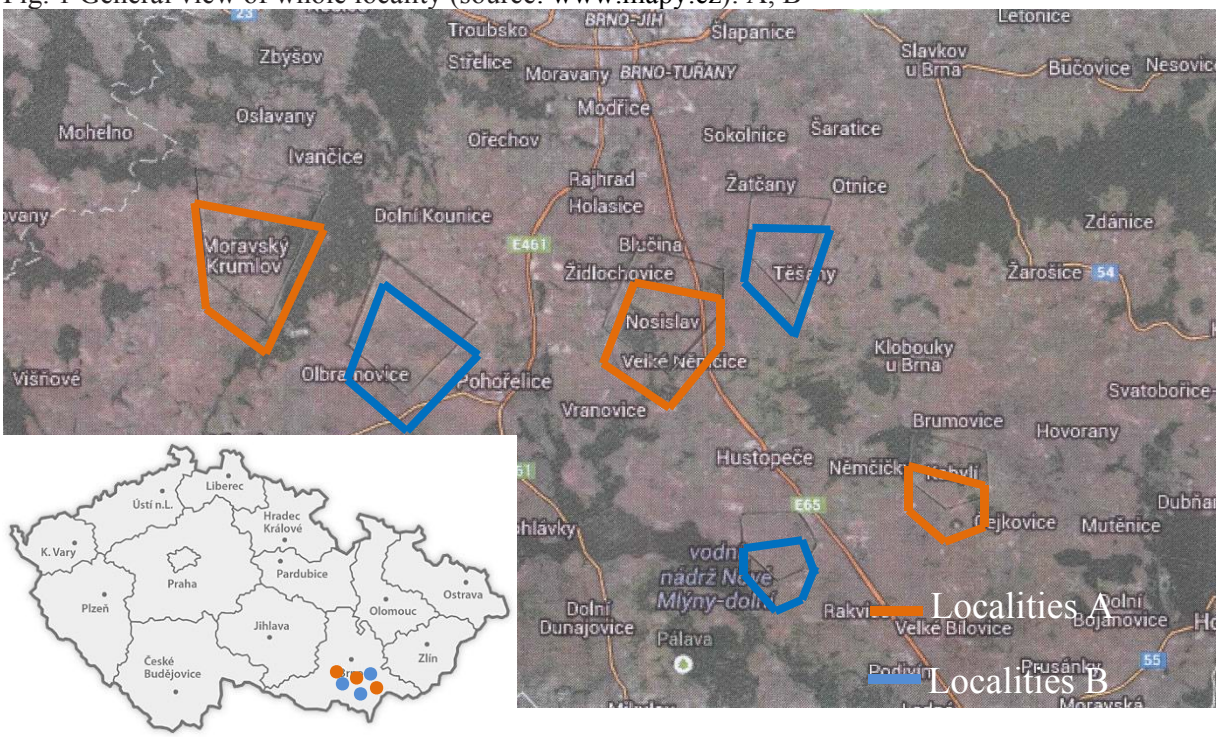
Characterization of method

The faecal samples were collected in the six localities of the South Moravia. Individual sites were all explored and droppings were collected from all parts of the territory.

The faecal specimen was fresh and the collection was done with precautions to reduce the likelihood of transmitting zoonotic infections. The faecal material was stored in the polythene bags. The faecal specimen was inspected visually for consistency, blood, color, mucus, worm segments and undigested food particles first [12].

The faecal samples were investigated by the faecal flotation. This laboratory technique concentrates the eggs present in the faeces into a drop of solution for easy identification. The important factor in the faecal samples is the specific gravity of the flotation solution. Most parasitic stages float at specific gravity of 1.2 to 1.3 [13].

Fig. 1 General view of whole locality (source: www.mapy.cz): A, B



The process of flotation is: Mix 3 – 5g volume of faeces in water in a beaker and stir until faeces are in suspension. Pour the mixture through a 0.5 – 0.8mm sieve into another beaker. Pour the contents into 15 ml centrifuge tube. Centrifuge the tubes at 1500 rpm for 2 minutes. Decant the tubes and fill the tube with flotation solution. Centrifuge the tubes at 1500 rpm for 2 minutes again. Remove the drop from the surface of the centrifuge tube and transfer to microscope slide. Add a cover slip and look for the eggs under the microscope [13].

Results and Discussion

In localities located in southern Moravia were collected faeces samples. Three locations marked as A Nosislav, Moravian Krumlov and Kobylí. Three locations designated as B are Olbramovice, Moutnice and Zaječí. A are locations for a typical hilly landscape of vineyards, fruit and vegetables, vineyards and orchards. In areas are located a significant forest and forest-steppe communities. In areas B is primarily a field where crop production is important that the site and its landscape significantly.

At 3 A locations, a total of 70 fecal samples gathered from the kinds of carnivores, birds and herbivores. A total of 12 faecal samples hare fecal flotation method of detection of 10 parasite species with parasite infestation +, which corresponds to finding 0-4 eggs on microscopic examination of faeces. For the species of deer were collected 18 samples of faeces. There was found 12 species of parasites with prevalence +++, which corresponds to 10 to 14 eggs during microscopic examination. For pheasant were found two species of parasites from the total number of examined samples of faeces 9. Marten was found six species of parasites collected from 31 faecal samples. The location A was gathered a total of 70 fecal samples, which were found 30 species of parasites.

Fig. 2 Prevalence of parasite in localities A and localities B

A localities	Number of samples	Number of parasite species	Prevalence
Hare	12	10	+
Roe	18	12	+++
Pheasant	9	2	++
Marten	31	6	++
Total	70	30	

B localities	Number of samples	Number of parasite species	Prevalence
Hare	10	8	++
Roe	23	11	+++
Pheasant	11	2	+++
Marten	25	3	++
Total	69	24	

In areas designated as B, it was gathered a total of 69 faecal samples. 10 samples hare, 23 samples deer, 11

samples pheasant and 25 samples marten. With hare was found eight species of parasites with prevalence ++, with deer was found 11 species of parasites with prevalence +++, pheasant were found in two species of parasites with prevalence +++ and martens were detected 3 species of parasites with prevalence ++. The total number of species of parasites found in dung in areas designated as B found 24 parasite species.

These results are consistent with the findings of other authors, therefore, that increased anthropogenic activities may negatively affect the species diversity of all parts of the habitat, including endoparasites.

How are they connected parasites with their hosts and the host environment, a study which compare parasite richness on birds from fragmented forests in southern China. It is found that the number of parasites associated with the size of the wood. The absence of certain genera of ectoparasites may be locally extinct, though their hosts are still present. It is necessary to preserve habitat and its fragments of sufficient size for the population of parasites [2].

Also, other authors agree that, the space plays an important role in regulating the population. Heterogeneity has generally positive effects on density and hence the population it is beneficial that improve the best quality habitat at the expense of worst quality habitat [14]. In the presence of spatially structured habitat heterogeneity, increasing local spatial autocorrelation in habitat generally has a beneficial effect on populations, increasing equilibrium population density [15].

More fragmented landscape was detrimental to the parasitic disease invasion and transmission, which implies that the potential of using artificial disturbances as a disease-control agency in biological conservation. Two components of the spatial heterogeneity (the amount and spatial autocorrelation of the lost habitat) formed a trade-off in determining the host–parasite dynamics. It is the possibility of using the spatial arrangement of habitat patches as a conservation tool for guarding focal species against parasitic infection and transmission [16].

It was investigated the influence of condition-specific competition on the specificity of two species of feather lice, that share a host the mourning dove. Humidity restricts the range of one species to the more humid eastern United States. The second species is restricted to drier regions of the western United States. The abiotic factors can determine species distributions. The balance between these factors is subject to change as environmental conditions change, even if the host distribution remains unaffected [17]. Species heterogeneity, species richness, and abundance of final host birds were positively correlated with species heterogeneity, species richness and abundance of trematodes in host snails [8].

I was developed a parasite diversity model based on known host associations with North American carnivores to investigate the spatial heterogeneity of parasite richness, how host composition, its relationship to carnivore richness, and specificity influenced these patterns [18]. Another studies compiled that report data on the relationship between animal population density and island area for individual species and faunas [19].

Conclusion

139 faecal samples were gathered in localities in southern Moravia and examined a total of laboratory equipment. The areas were divided into three locations marked as A and B. These two kinds of sites differ farming, which affects the amount and composition of rainbow endoparasites, which were followed by the species of pheasant, deer, marten and hare. It was found that A locations where the vineyards are more prevalent species represented less than the prevalence of sites designated as B where landscape intensively cultivated. In areas B is a smaller number of species in it with greater prevalence.

It was used True diversity, Richness and Shannon-Wiener index. Prevalence of endoparasites were determined at most in deer - in both locations identically, then in pheasant in B, the lowest prevalence was captured by hares in A. Using ecological indices were calculated index for biodiversity area AH = 1.1 and for the site BH = 0.8. Index evenness for site A was E = 0.35, for the site BE = 0.21. Index of species dominance evaluate site A as less anthropogenically influenced, but the differences were not very large in comparison with the location B.

Although our results are consistent with the findings of other authors, it would still need further monitoring to be able to draw the appropriate conclusions.

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Construction of multiplex quantitative PCR for detection of streptococcal mastitis

JAKUB SURYNEK, ALES KNOLL, IRENA VRTKOVA
Department of Animal Morphology, Physiology and Genetics
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xsurynek@mendelu.cz

Abstract: The objective of this study was to develop a multiplex quantitative PCR-based method for simultaneous detection of *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* in biological samples. The *cfb* gene for CAMP factor was used for detection of *S. agalactiae*. The *cpn60* gene was used for detection of *S. dysgalactiae* and the plasminogen activator gene for *S. uberis* detection. PCR for *S. dysgalactiae* performed optimally, but reactions of *S. agalactiae* and *S. uberis* showed poor and no amplification respectively.

Key words: mastitis, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, qPCR

Introduction

Intramammary infection, also known as mastitis, is the most frequently occurring and economically the most important infectious disease in dairy cattle. Besides health disorders of mammary gland, mastitis causes significant losses in milk yield, degradation of its nutritive and technological properties, fertility disorders and even systemic disease [1]. Numerous microorganisms have been described as causative agents of bovine mastitis. According to their epidemiology, mastitis pathogens can be divided into two groups, contagious and environmental. The primary reservoir of contagious pathogens is an infected udder whereas a contaminated environment is the primary reservoir of pathogens causing environmental mastitis. *Streptococcus agalactiae* is considered as typical contagious pathogen of the mammary gland, where it can survive for a long period of time. *Streptococcus uberis* is a typical environmental pathogen. *Streptococcus dysgalactiae* has been most commonly described as a contagious pathogen but it can also behave as an environmental pathogen [2, 3].

Traditionally, culture methods are considered gold standard in mastitis pathogens identification. However, in last twenty years introduction of molecular biology methods brought new possibilities – identification based on DNA using polymerase chain reaction. Historically, most PCR assays developed for identification of *Streptococcus* sp. targeted the 16S rRNA gene [4, 5, 6]. However, false positive results may occur due to high homology of ribosomal operons (91–93%) thus reducing specificity of this approach to 0.87–0.96 [7].

Polymorphism of *cpn60* gene was commonly used in phylogenetic studies of *Streptococcus* spp. and also differentiation of its species and strains. Product of this gene, the cpn60 protein, also known as GroEL or HSP60, is a 60 kDa heat-shock protein that assists in the correct folding of most bacterial proteins under both normal and stress conditions [8]. The cpn60 proteins showed extensive sequence similarity in bacterial species, typically around 70 %. Dmitriev et al. [7] successfully used polymorphism of *cpn60* for differentiation of *S. agalactiae, S. dysgalactiae,* and *S. uberis*.

For detection of *S. agalactiae* Gillespie and Oliver [9] *cfb* gene encoding the CAMP factor of *S. agalactiae* was used. In case of *S. uberis*, Sazonova et al. [10] described its plasminogen activator gene (*pauA*). Gillespie and Oliver [9] first used *pauA* gene for detection of *S. uberis*. Therefore aim of this study was to assess the performance of our own reaction for *S. dysgalactiae* together with reactions previously used by Gillespie and Oliver [9]. Shome et al. [11] carried out study of potencial molecular targets for detection mastitis pathogens and for detection of S. uberis they used also the *cpn60* gene.

Material and Methods

Bacterial strains

Gemonic DNA of three strains of streptococci was used for preparation of standards: *S. agalactiae* CAPM 5153, *S. dysgalactiae* CAPM 5548, and *S. uberis* CAPM 5675.

Table 1 Primers and probes for multiplex qPCR

Designation	Sequence (5′→3′)	Length, bp	Source
SagCAMP_F	AGCTCTATTAGAAGTACATGCT	22	
SagCAMP_R	CATTTGCTGGGCTTGATTATT	21	Gillespie and Oliver, 2005
SagCAMP_P	FAM-ATCAAGTGACAACTCCACAAGTGGTAA-BHQ1	27	
Sdycpn60_F	GCGATTGCTCAGCCTGTTTCT	21	
Sdycpn60_R	GGCTTCTGAAATGTATTCTCCAA	23	
Sdycpn60_P	Cy5-TTGCTGCTGTGTCATCTCGTTCTG-BHQ2	24	original design
SubpauA_F	AGAGGAATTCATCATGTTTTAACA	24	
SubpauA_R	AATTGTAGAAGAACCATTTGATGT	24	Gillespie and Oliver, 2005
SubpauA_P	HEX-AGCGTCTAACAACTCGGCCTTTG-BHQ1	23	

Primers and probes

Primers and probe for *S. dysgalactieae* were designed using OLIGO 4.0. Length of amplicon was designed to 95 bp. Previously published primers and probes were used in reactions of *S. agalactiae* and *S. uberis* (Tab. 1). Oligonucleotides were purchased from Generi Biotech (Hradec Kralove, Czech Republic)

Triplex qPCR for *S. agalactiae*, *S. dysgalactiae,* and *S. uberis*

Dilution series of *S. agalactiae, S. dysgalactie,* and *S. uberis* genomic DNA ranging from 10^7 to 10^1 genome copies were made. Amplification was carried out in 8-tube strips (Life Technologies, Foster City, CA, USA) using the ABI 7500 real-time PCR system and the PCR conditions were as follows: UDG pre-treatment at 50°C for 2 min, initial denaturation/activation at 95°C for 10 min, 45 cycles of denaturation at 95°C for 15 s and extension at 57°C for 1 min. Each 20-µl reaction contained 10 µl of TaqMan Gene Expression Master Mix (Life Technologies, Foster City, CA, USA), 600-nM primers, 250-nM probes and 2 µl of template. Reactions were run in triplicates (Fig. 2).

Results and Discussion

Optimal annealing temperature for multiplex qPCR

Compared with SagCAMP_F and SagCAMP_R, our original primers Sdycpn60_F and Sdycpn60_R gave strong PCR product of expected size 95 bp at all six annealing temperatures tested. Primers SagCAMP_F and SagCAMP_R gave much weaker PCR product whose intensity is comparable at temperatures 55, 56 and 57°C, and then it gradually decreases until no amplification at 60°C. For unknown reason, primers SubpauA_F and SubpauA_R gave no PCR product at any temperature. Annealing temperature of 57°C was selected for multiplex qPCR.

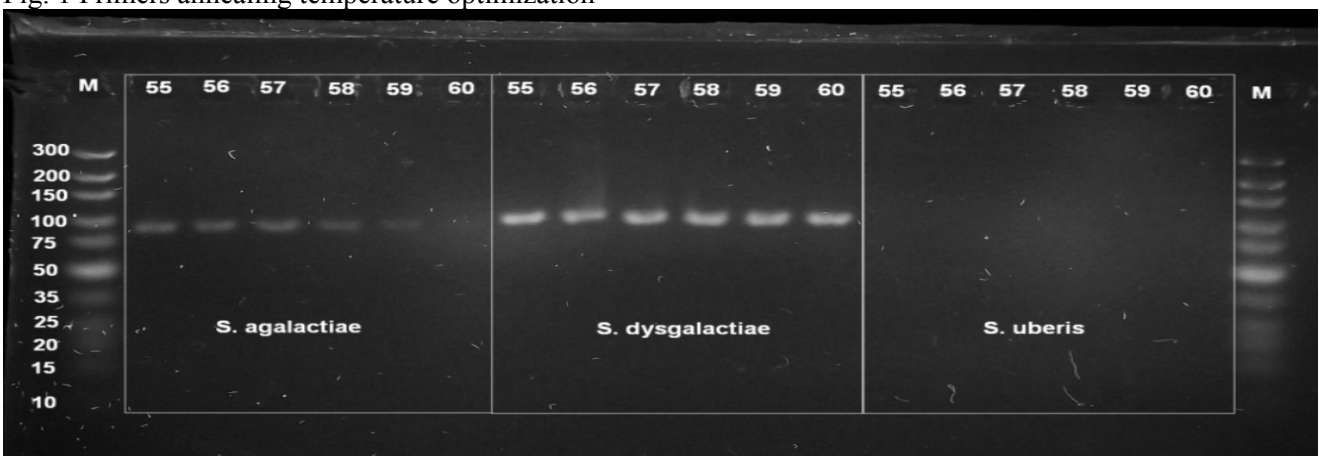
Performance of multiplex qPCR

Reaction of *S. dysgalactiae* performs well in multiplex. Amplification curves have correct shape; there is expected ΔCt of dilutions and good fit of replicates. However reaction of *S. agalactiae* does not meet these criteria and no reaction of *S. uberis* occured. These results are in congruence with results obtained in annealing temperature optimization by gel electrophoresis.

PCR detection of streptococcal mastitis

Muliplex PCR and multiplex qPCR assays that can simultaneously detect different mastitis-causing organisms in milk and other samples have been reported on. Phuektes et al. [4, 5] designed multiplex PCR assay for detection of *S. aureus*. *S agalactiae*, *S. dysgalactiae*, and *S. uberis*. Their assay was based on 16S rRNA genes. These assays might be not specific enough due to known problems of 16S rRNA-based bacterial identification. Different approach was chosen by Gillespie and Oliver [9] who in their multiplex qPCR used *cfb* gene coding CAMP factor of *S. agalactiae* and plasminogen-activator gene for *S. uberis* detection. However these reactions according to our results are not suitable for quantitative detection. Dmitriev et al. [7] were able to distinguish *S. agalactiae*, *S. dysgalactiae* and *S. uberis* in single tube based on sequences of their *cpn60* genes. Their results hold promise for design of multiplex qPCR for streptococcal mastitis detection.

Fig. 1 Primers annealing temperature optimization

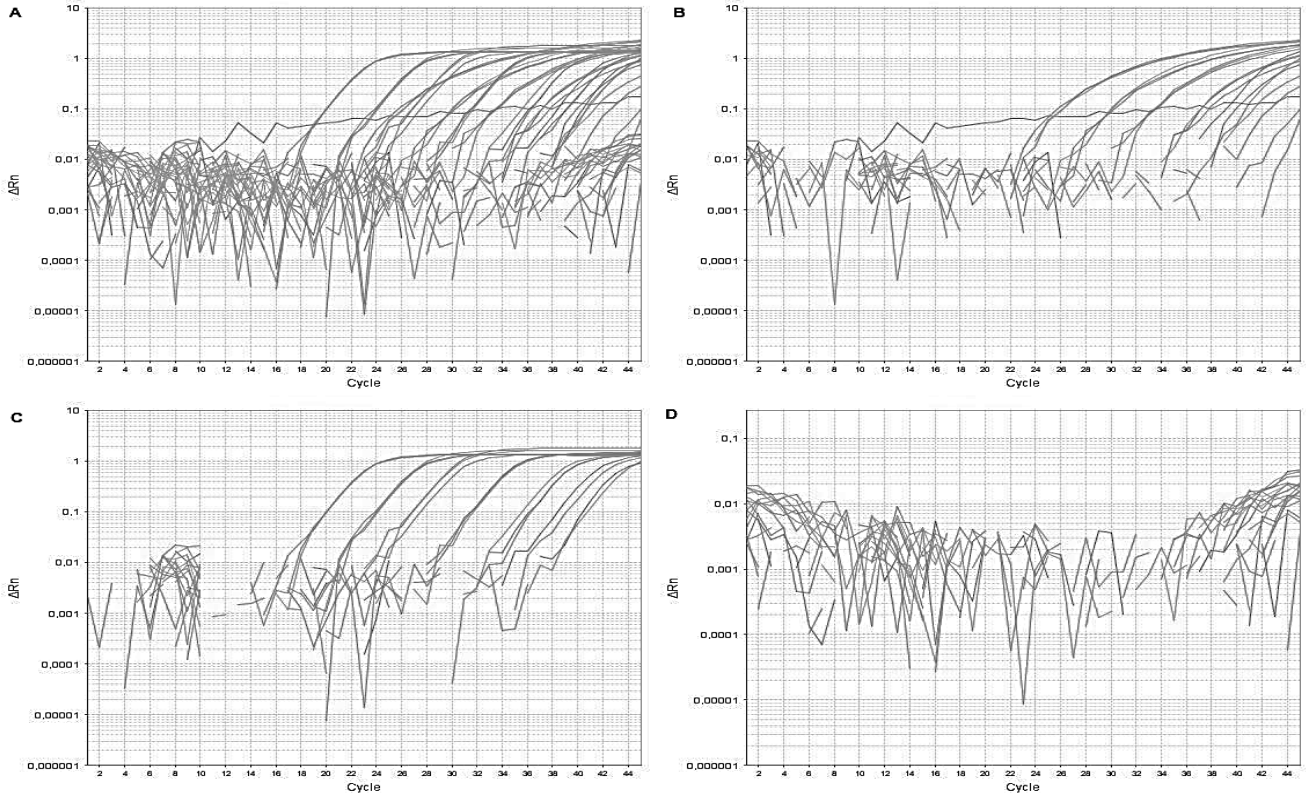


Legend: M – size marker (bp); figures indicate annealing temperature tested.

Conclusion

qPCR for *S. dysgalactie* targeting cpn60 gene seem to be performing well in multiplex conditions. Results of reactions of *S. agalactiae* and *S. uberis* are unsatisfactory. Primers and probes most likely need to be redesigned and tested for specificity. Another option could be to base the multiplex qPCR on *cpn60* gene and design primers and probes in sites with enough heterogeneity to distinguish species of interest from other *Streptococcus* species.

Fig. 2 Amplification plots of *S. agalactiae*, *S. dysgalactiae* and *S. uberis* reactions



Legend: A – combined plot of all three reactions, B – reaction of S. agalactiae, C – reaction of S. dysgalactiae, D – reaction of S. uberis.

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Isolation techniques of neutrophils and peripheral blood mononuclear cells for the comparative experiments in humans and pigs model organisms in flow cytometry

SUSTROVA TEREZA[1], ONDRACKOVA PETRA[2], LEVA LENKA[2], SLADEK ZBYSEK[1]
[1]Department of Morphology, Physiology and Animal Genetics
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Department of Immunology
Veterinary Research Institute in Brno
Hudcova 70, 621 00 Brno
CZECH REPUBLIC

tereza.sustrova@mendelu.cz

Abstract: The pig is due to the morfology of their bodies often used as a model organism from the experimental organisms for comparison with man. The aim of this study ist o determine which isolation technique can be used for comparative imunological experiments focused on human and porcine neutrophils and monocytes . In comparative studies, it is necessary that both cells compared species were isolated by the same technique. Selected isolation techniques are the first step for subsequent detection of selected parameters in flow cytometry and for cultivation of peripheral blood mononuclear cells to profit monocyte-derivated macrophages. For isolation of neutrophils were chosen these techniques: isolation of double density gradient centrifugation Histopaque 11191 a 1077, isolation of double density gradient centrifugation Histopaque 11191 a 1077 after dextran and only dextran sedimentation. Monocytes were isolated by density gradient centrifugation Histopaque 1077, density gradient centrifugation Histopaque 1077 with immunomagnetic separation of $CD14^+$ cells, double density gradient centrifugation Histopaque 11191 a 1077 and double density gradient centrifugation Histopaque 11191 a 1077 after dextran sedimentation. For the isolation of neutrophils is the best tested technique dextran sedimentation. For the isolation of monocytes from peripheral blood show the best results density gradient centrifugation Histopaque 1077. Techniques were evaluated by purity and yield isolated cells during comparison both organisms.

Key-Words: dextran sedimentation, density gradient isolation techniques, clearance, yield, $CD14^+$

Introduction

Flow cytometry is a highly sophisticated method capable of sorting cells according to their size and granularity and subsequently selected by marking of selected cells surface receptors. Flow cytometry are often analyzed blood samples. Therefore, the work addresses the isolation of neutrophils and monocytes from peripheral blood. Due to the fact that the pig is often used as a model organism for comparative studies of human medicine is this work aimed at two organisms - human and pig.

The aim was to choose the technique that will be most suitable for isolating cells of the immune system needs further study in the analysis of flow cytometry. As a cells of interest were chosen human and porcine neutrophils and monocytes. This thesis is focused on the comparison of isolation techniques in terms of purity and yield of the isolated cells. Whereas in comparative studies must follow the same procedure for isolating cells from both species were the most successful methods chosen which showed high purity and yield in both species.

Material and Methods

For isolation of neutrophils and monocytes in pigs has been used 10 healthy pigs stabled in the experimental barn in Veterinary Research Institute in Brno. Pigs were fed a standard diet. Peripheral blood was collected them in the morning, from *vena cava cranialis*. Human neutrophils and monocytes were isolated from peripheral blood of 10 healthy individuals. Cells were isolated from heparin-anticoagulated blood.

For isolation of neutrophils were chosen these techniques: isolation of double density gradient centrifugation Histopaque 11191 a 1077 (sterile-

filtered, denstity 1.119 g/mL and 1.007 g/mL, Sigma-Aldrich, USA), isolation of double density gradient centrifugation Histopaque 11191 a 1077 after dextran (non-pyrogenic, MP biomedicals, France) and only dextran sedimentation.

Isolation technique of monocytes is unknown. Therefore this thesis is focused on isolation techniques of peripheral blood mononuclear cells (PBMC). Monocytes will be selected in the next step. These techniques were chosen: density gradient centrifugation Histopaque 1077, density gradient centrifugation Histopaque 1077 with immunomagnetic separation of $CD14^+$ cells, double density gradient centrifugation Histopaque 11191 a 1077 and double density gradient centrifugation Histopaque 11191 a 1077 after dextran sedimentation. It is assumed that obtained monocytes isolates will be cultured to macrophages. During cultivation, the exchange of media leads to wash lymphocytes. Monocytes differentiate into macrophages and due to adherence are not washed during exchange of media. All techniques were performed in standard contidions according to data sheets. Cells isolations were performed in sterile box (S@FEFLOW 1.8, EuroClone, 2012, Italy). Results were statistically evaluated by pair t-test.

Table 1 Isolation of neutrophils (significant differences are marked by asterisks *$P < 0.05$, **$P < 0.01$)

	* 10^6 cells in 10 ml periferal blood	* 10^6 neutrophils in 10 ml periferal blood	% of neutrophils from isolate	* 10^6 cells in 10 ml periferal blood	* 10^6 neutrophils in 10 ml periferal blood	% of neutrophils from isolate
Isolation technique	human	human	human	pig	pig	pig
Histopaque 11191 and 1077	7.48**	0.58**	7.8**	10.38	0.16	1.6
Dextran sed. + Histopaque 11191 and 1077	0.2	0.0002	0.1	9.1	0.03	0.4
Dextran sedimentation	17.41**	0.48**	2.8**	89.46**	1.07*	1.2

Table 2 Isolation of monocytes (significant differences are marked by asterisks *$P < 0.05$, **$P < 0.01$)

	* 10^6 cells in 10 ml periferal blood	* 10^6 monocytes in 10 ml periferal blood	% of monocytes from isolate	* 10^6 cells in 10 ml periferal blood	* 10^6 monocytes in 10 ml periferal blood	% of monocytes from isolate
Isolation technique	human	human	human	pig	pig	pig
Histopaque 1077	20.48*	0.38	1.9	55.29**	0.11	0.2
Histopaque 11191 and 1077	11.6	0.09	0.8	62.4**	0.18	0.3
Dextran sedimentation + Histopaque 11191 and 1077	4.2	0.07	1.7	8.85	0.03	0.4

Results and Discussion

Techniques of isolation of neutrophils

Research laboratories worldwide most frequently employ linear or discontinuous gradients of serum albumin, Percoll, Ficoll, dextran, Ficoll-Hypaque, Mono-Poly Resolving Medium, dextran/Ficoll, or gelatin, to isolate human peripheral blood neutrophils [1, 3, 5, 7, 8]. Most commonly is used dextran sedimentation, Ficoll-Hypaque centrifugation and hypotonic lysis of residual erythrocytes [1, 3, 7, 8]. Porcine neutrophils are isolated by dextran sedimentation followed by further erythrocyte purification by resuspending the pellet in ice cold and use Histopaque 1083[5]. This study evaluates the selected techniques in detail and compared. In this study, we have demonstrated that the double density gradient centrifugation Histopaque 11191 a 1077 achieves the best scores in humans. Of all the techniques give the highest yield and the highest purity of isolated (Tab. 1, Fig. 1). Almost the same yied achieved in the dextran sedimentation (Tab. 1, Fig. 5). Inappropriate technique for the isolation of human neutrophils is isolation of double density gradient centrifugation Histopaque 11191 a 1077 after dextran sedimentation (Tab. 1, Fig. 3). The cells show a very low yield and purity. For isolation of porcine neutrophils is the best technique dextran sedimentation. This technique has got good purity and yield too (Tab. 1, Fig. 6). Although isolation of neutrophils by double density gradient centrifugation Histopaque 11191 a 1077 has the best purity but yield is very low (Tab. 1, Fig. 2). Inappropriate technique for the isolation of porcine neutrophils is isolation of double density gradient centrifugation Histopaque 11191 a 1077 after dextran sedimentation (Tab. 1, Fig. 4).

Fig. 1 Histopaque 11191 and 1077

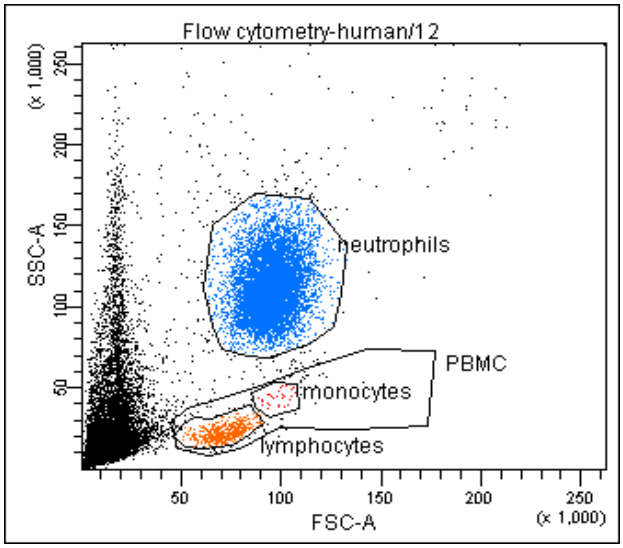


Fig. 2 Histopaque 11191 and 1077

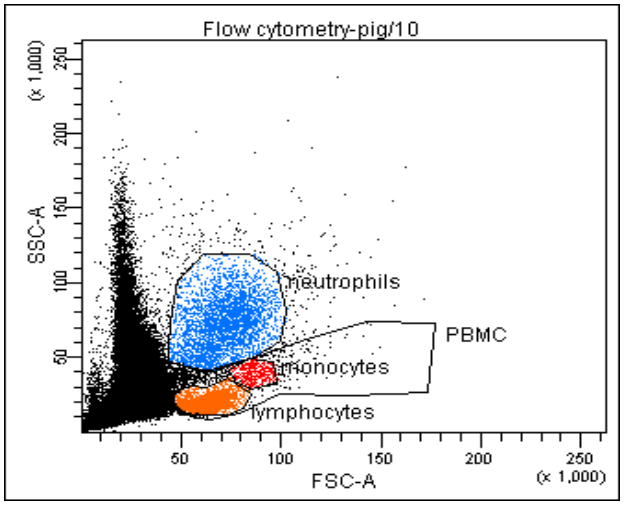


Fig. 3 Histopaque 11191 and 1077 after dextran sedimentation

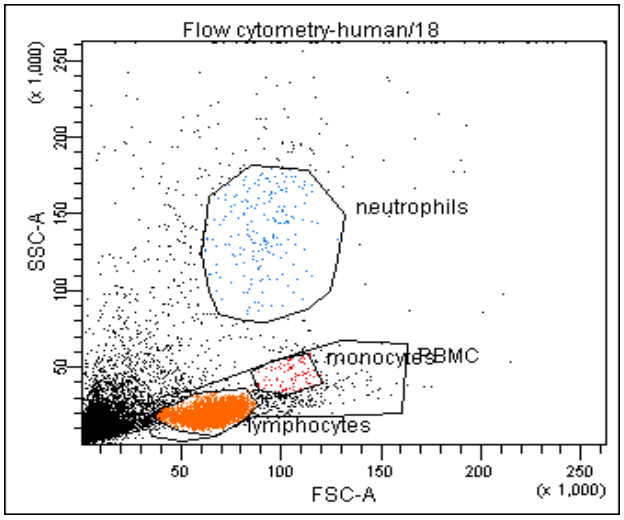


Fig. 4 Histopaque 11191 and 1077 after dextran sedimentation

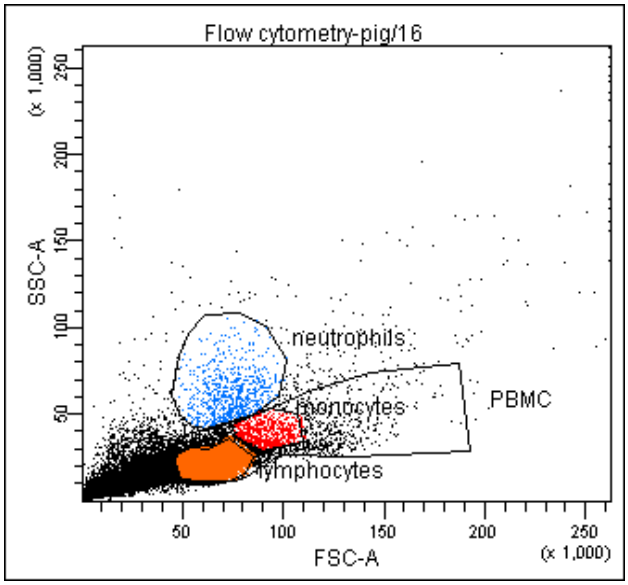


Fig. 5 Dextran sedimentation

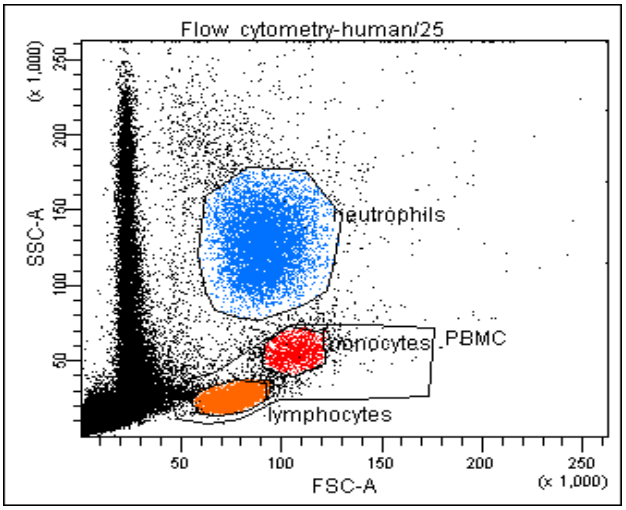
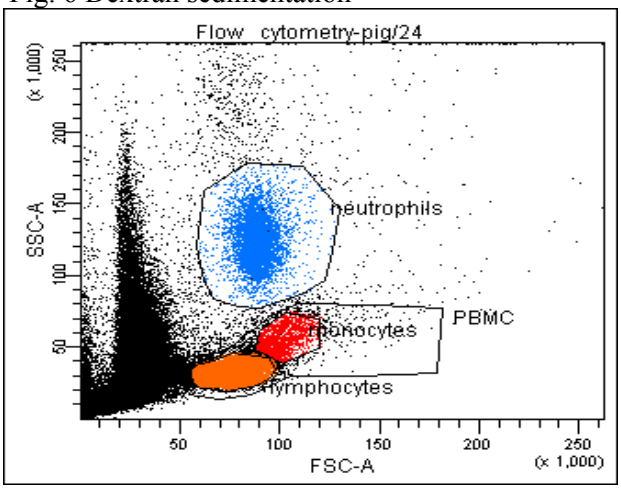


Fig. 6 Dextran sedimentation



Techniques for isolation of monocytes

Many different techniques can be used for monocyte isolation. For example double gradient centrifugation [4], dextran sedimentation and Ficoll-Hipaque density gradient centrifugation [8] or density gradient centrifugation with and without further purification by plastic adherence or immunomagnetic separation of $CD14^+$ cells [2]. This study compares these methods and evaluates their use in comparative studies of human and porcine monocytes. In the isolation of monocytes from human peripheral blood achieves the best scores density gradient centrifugation Histopaque 1077 (Tab. 2, Fig. 7). It shows also the highest yield and purity of the isolated cells best of all the tested methods. Good purity is showed by double density gradient centrifugation Histopaque 11191 a 1077 after dextran sedimentation too, but the method has got low yield of monocytes (Tab. 2, Fig. 11). Inappropriate technique for the isolation of human monocytes is double density gradient centrifugation Histopaque 11191 a 1077 (Tab. 2, Fig. 9). But this method is the best for isolation of porcine monocytes (Tab. 2, Fig. 10). Good purity but low yield of porcine monocytes is achieved by double density gradient centrifugation Histopaque 11191 a 1077 after dextran sedimentation (Tab. 2, Fig. 12). Good yield but lowest purity is achieved by density gradient centrifugation Histopaque 1077 (Tab. 2, Fig. 8). Last test methods for the isolation of peripheral blood monocytes is a technique of density gradient centrifugation Histopaque 1077 which is followed by immunomagnetic separation of $CD14^+$ cells which is typical for monocytes. As the Fig. 13 shows in human and Fig. 14 show in porcine monocytes, this isolation techniques shows hight impurity of lymphocytes in comparison with other techniques and it doesn´t bring better results. Difficulty of techniques and cost per sample are higher than in other techniques and therefore no advantages over other techniques of isolation.

Fig. 7 Histopaque 1077

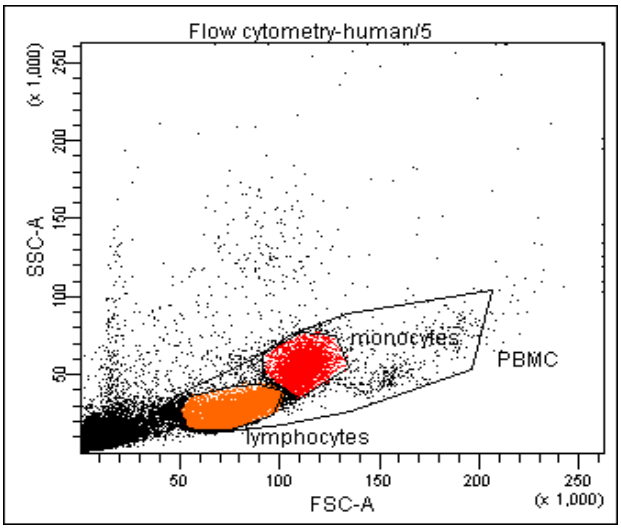


Fig. 8 Histopaque 1077

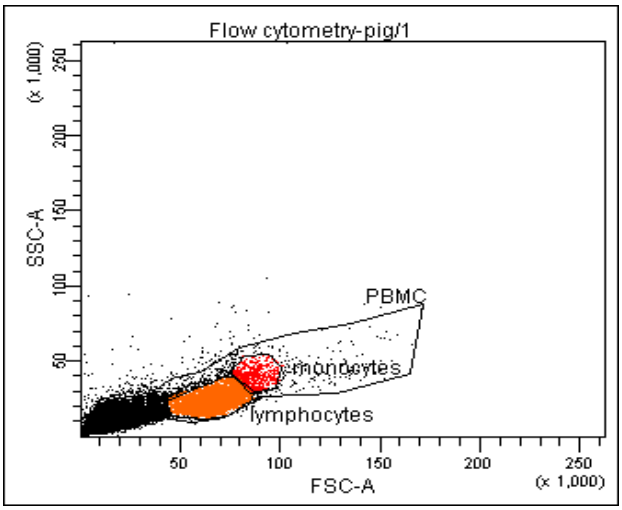


Fig. 9 Histopaque 11191 and 1077

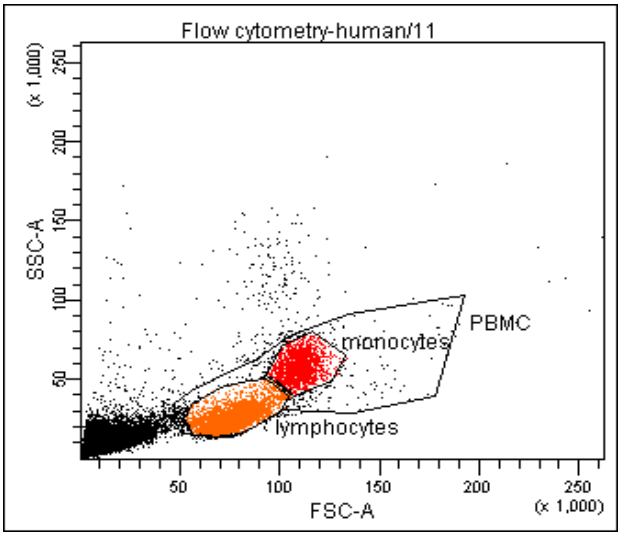


Fig. 10 Histopaque 11191 and 1077

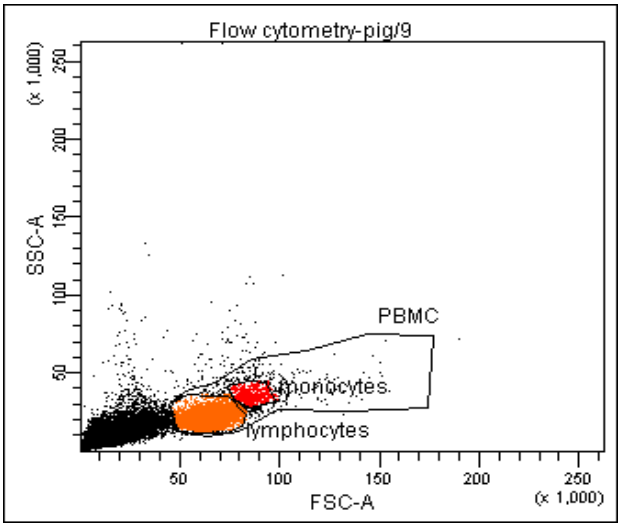


Fig.11 Histopaque 11191 and 1077 after dextran sedimentation

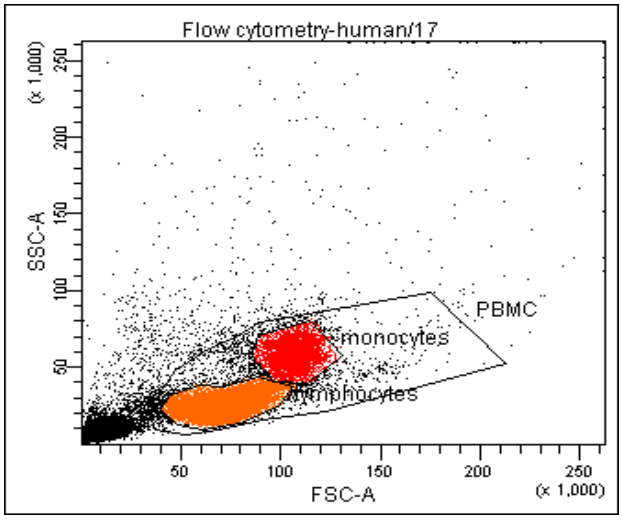


Fig. 12 Histopaque 11191 and 1077 after dextran sedimentation

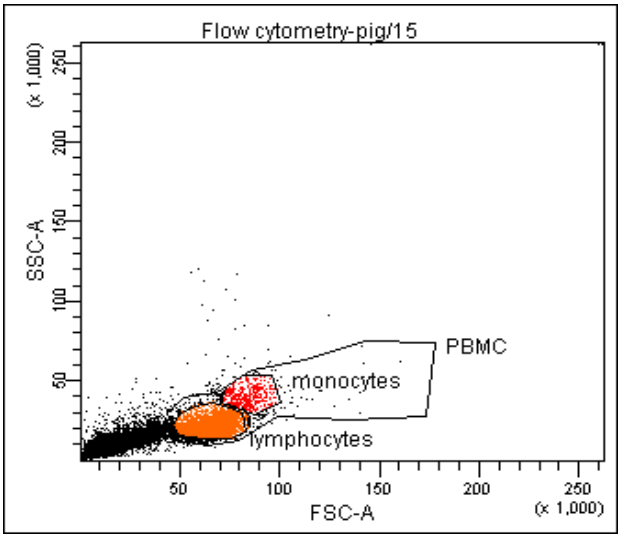


Fig. 13 immunomagnetic separation od CD14+

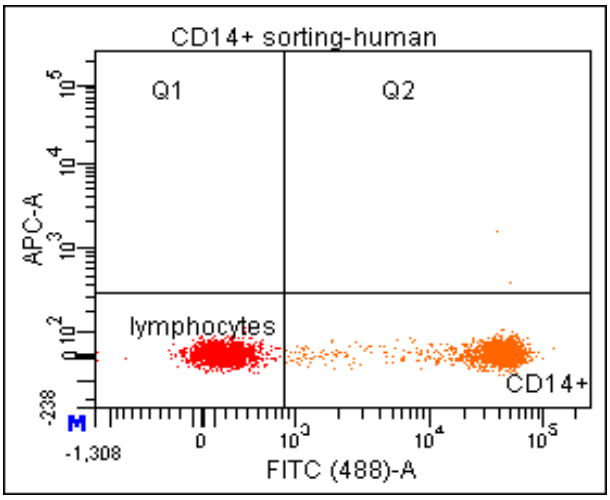
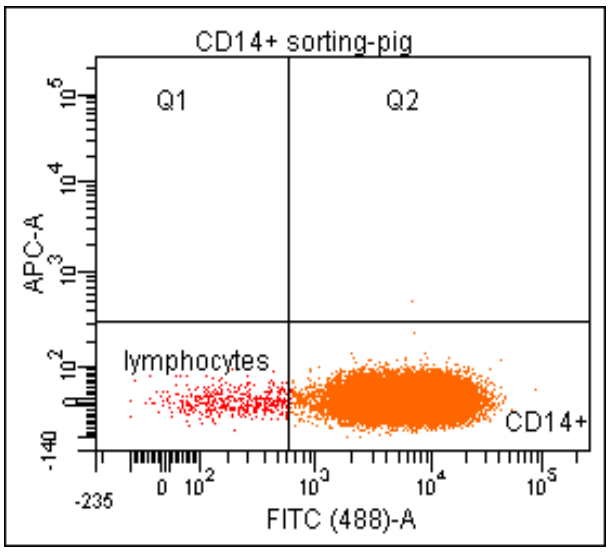


Fig. 14 Immunomagnetic separation of CD14+



Conclusion

The best way to obtain neutrophils from porcine blood is the dextran sedimentation technique. In human this technique shows almost identical neutrophil yield as the double density gradient centrifugation.

Comparing the yield and purity of the compared isolation techniques is the most appropriate technique density gradient centrifugation Histopaque 1077. This is technically simple test method isolating peripheral blood monocytes and gives satisfactory results in humans and pigs.

Acknowledgement

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Heavy metals in the common carp (*Cyprinus carpion L.*) from two reservoirs in the Czech Republic

PETRA VICAROVA, ANDREA KLECKEROVA, HANA DOCEKALOVA, PAVLINA PELCOVA
Department of Chemistry and Biochemistry
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

petra.vicarova@mendelu.cz

Abstract: Concentrations of heavy metals (cadmium, lead and mercury) in selected tissues of common carp (*Cyprinus carpio L.*) fished from two reservoirs in the Czech-Moravian Highland (Pilska and Domanin reservoirs) were measured during the period April - August 2013. A difference in the contents of heavy metals in fish samples from the two reservoirs (Pilska and Domanin reservoirs) was demonstrated. The content of all analyzed heavy metals in carp tissues was higher in the Domanin reservoir than in Pilska reservoir. The difference in mercury concentrations was also found; it was three times higher in tail muscles than in the liver. Cadmium concentration in the liver was higher that in the muscles. No difference was found in lead contents between muscles and the liver. The obtained data was in agreement with the standards except for cadmium contents in the liver. Therefore human consumption of the common carp from Czech-Moravian Highland does not pose any health risk concerning concentrations mercury and lead.

Key-Words: fish, mercury, cadmium, lead, atomic absorption spectrometry

Introduction

Fish is considered as an integral component of healthy diet, the source of high quality protein, vitamins, minerals, omega-3 fatty acids and a wide range of other important nutrients. On the other hand, fish generally accumulates contaminants from the aquatic environment and can transfer toxic metals to humans via the food chain. Fish are widely consumed and for this reason the contents of heavy metals in fish tissues is highly important for human health safety. Water pollution resulted in fish affection with toxic metals. This pollution has different causes and different sources e.g. accidental spillage of chemical wastes, periodic precipitation contaminated with air-borne pollutants, discharge of industrial or sewerage effluents, agricultural drainage, domestic wastewater and gasoline from fishery boots [1,2].

Cadmium, mercury and lead are of particular interest for fisheries and consequently may pose health risk to humans [3]. Heavy metals enter the bodies of fish in three ways: through the body surface, the gills or the digestive tract [4]. In fish, the toxic effects of heavy metals may influence the physiological functions, individual growth rates, reproduction and mortality [5].

The *Cyprinus carpio L.* fish species, inhabiting the Pilska and Domanin reservoirs and being extensively consumed by the local population, were selected for the analysis. The goal of the study is to evaluate health safety of fish for human consumption.

Material and Methods

Sampling and storage

Fish (*Cyprinus carpio L.*) were caught from April to August 2013 in two locations of Czech-Moravian highland, Czech Republic. First site – Pilska reservoir (Fig. 1) – is located 3.8 km north of Žďár nad Sázavou. The second place – Domanin reservoir (Fig. 1) – is located 6.8 km west of Bystřice nad Pernštejnem. Possible contamination of water system - major roads, inflows to the reservoirs and the near uranium mines.

Fish were caught on bait containing bread and potatoes. Caught fish were killed, and samples tail muscle and liver were collected. Samples were kept in a plastic bag at -20°C in the freezer. For each fish was recorded: weight and length of the fish, sex and sampling location.

Determination of mercury

For the determination of total mercury in the samples was used atomic absorption spectrometer AMA 254 (Altec, Czech Republic). The samples were directly weighted (100 mg ± 0.1 mg) into pre-cleaned combustion boats, and inserted into the AMA 254 analyser.

Fig. 1 Designation locations on the map

The samples were died at 120°C for 60 s and thermally decomposed at 550°C for 150s. The limit of detection for the determination of mercury is 0.01 ng Hg.

Determination of cadmium and lead

Before analysis the fish samples were lyophilized (Power Dry LL 3000, Thermo Scientific, USA) for 7 days and subsequently mineralized in microwave oven for 20 min. For mineralization was used 0.4 mg and 8 ml nitric acid (1:1). Cadmium and lead were determined by electrothermal atomic absorption spectrometry (CONTRAA 700, Analytik Jena, Germany). Cadmium was measured at the wavelength 228.8 nm and lead was measured at the wavelength 283.3 nm.

Table 1 Limits of detection

	Tissue	$\mu g.kg^{-1}$ FW
LOD cadmium	Muscle	0.51
	Liver	0.62
LOD lead	Muscle	4.93
	Liver	5.96

**FW – fresh weight*

Statistical analyses

Statistical analyses of metal content in tissues were made using one-way analysis of variance (ANOVA) in program STATISTICA. Statistical significance was declared when *p* value was equal to or less than 0.05.

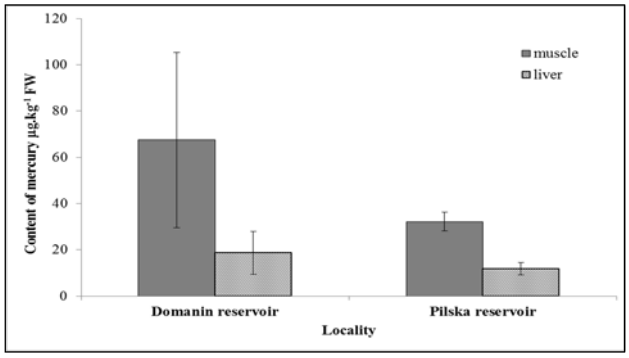
Results and Discussion

Carp *(Cyprinus carpio L.)* was selected for monitoring bioaccumulation of mercury, cadmium and lead in selected water ecosystems. Carp is omnivorous fish with the widest food web (aquatic plants, aquatic worms, protozoa,…) and is one of the most consumed fish in the Czech Republic.

In Pilska reservoir were analyzed 12 samples (3 female and 3 male, in length range 46 – 52 cm, in the range of weight 1.0 – 3.1 kg), in Domanin reservoir were analyzed total 18 samples (4 female and 5 male, in length range 42 – 52cm, in the range of weight 1.7 – 3.1 kg).

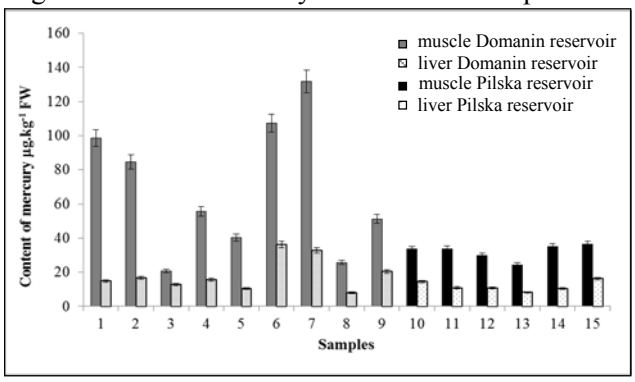
The average mercury content was in Domanin reservoir statistically higher than in Pilska reservoir in both tested tissues *($p<0.05$)* (Fig. 2).

Fig. 2 Content of mercury in tail muscle and liver fish of both locations



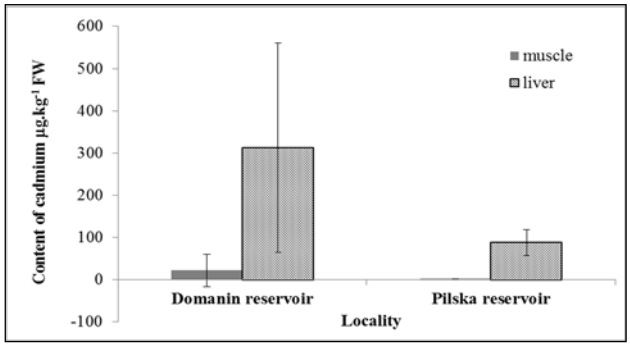
Content of mercury was statistically significant higher in tail muscle than in the liver *($p<0.05$)* (Fig. 3). Svobodová et al. [3] presents that higher mercury content in muscle than in the liver shows low or no contamination of the locations. On the basis of this argument, is possible to consider both reservoirs (Domanin, Pilska) as uncontaminated.

Fig. 3 Content of mercury in individual samples



Content of cadmium in Domanin reservoir was significantly higher *($p<0.05$)* than in Pilska reservoir (Fig. 4).

Fig. 4 Content of cadmium in tail muscle and liver fish of both locations

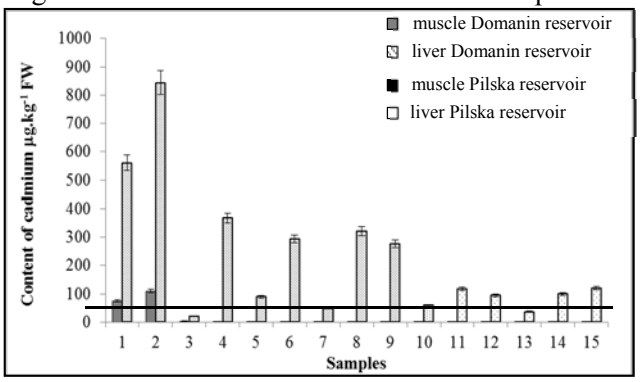


Cadmium content was significantly higher in the liver than in the muscle samples (Fig. 5). Visnjic-Jeftic et al. [6] presented very similar conclusions. They studied heavy metal and trace element accumulation in muscle, liver and gills of the Pontic shad (*Alosa immaculata* Bennet 1835) from the Danube River (Serbia). They found the highest concentration of cadmium in liver (0.714 ± 0.323 µg/g dry weight) and approximately half the amount in muscle (0.433a ± 0.181 µg/g dry weight). Cadmium is metabolized in the liver, what caused the higher content in this tissue [7].

Two fish samples contained statistically significantly higher cadmium contents in both tested tissues *($p<0.05$)*. This fish were caught in the spring, when the *Cyprinus carpio L.* are still living at the bottom of reservoir and in the sediment.

Cadmium concentration in fifteen liver samples exceeded the Czech standards (50 $\mu g.kg^{-1}$), see the bold line in the Fig. 6. The livers are not normally consumed by humans, so there is no recommendation for limit concentration.

Fig. 5 Content of cadmium in individual samples



The lead content in carp tissues in Domanin reservoir was statistically higher than in Pilska reservoir *($p<0.05$)* and no difference in lead concentration was found between muscles and liver (Fig. 6, 7).

Fig. 6 Content of lead in tail muscle and liver fish of both locations

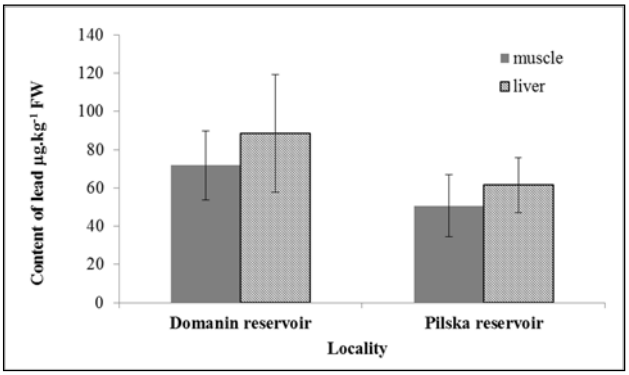
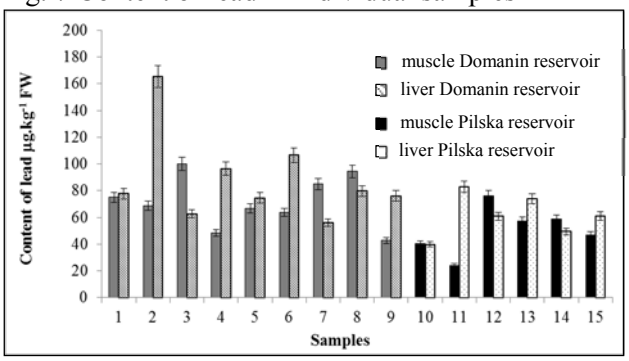


Fig. 7 Content of lead in individual samples



Conclusion

The results obtained in this work showed the differences in the contents of heavy metals between the two locations (Pilska and Domanin reservoirs). The contents of all the metals under analysis were higher in samples from the Domanin reservoir than from the Pilska reservoir. The major cause may be a recent clearing of the Skalsky reservoir, which is the main tributary of the Domanin reservoir. Another cause may be the vicinity of major routes. The difference in mercury concentrations in the analyzed tissues was also found; the concentrations in tail muscles were three times higher than in the liver. Cadmium content in the liver was higher than in the muscles. Lead concentrations did not differ in the studied samples.

The total mercury concentration in muscle tissues ranged from 20 to 132 $\mu g.kg^{-1}$ and in the liver from 8 to 36 $\mu g.kg^{-1}$ in both reservoirs. Cadmium concentrations in tail muscles ranged from 1 to 110 $\mu g.kg^{-1}$, in the liver from 19 to 844 $\mu g.kg^{-1}$ from both reservoirs. Lead concentrations in muscle tissues ranged between 24 and 101 $\mu g.kg^{-1}$ and in the liver between 39 and 166 $\mu g.kg^{-1}$. The contents of individual elements in carp tissues from the two reservoirs were compared. The obtained data were in agreement with the standards except for cadmium contents in the liver. Therefore human consumption of the common carp does not pose any health risk concerning mercury and lead concentrations. In most liver samples, cadmium concentrations exceeded the health safety limit. With regard to the fact, that the liver is not massively consumed this does not represent a health risk. In two carps caught in the spring, increased content of cadmium was also found in the muscles, which could be caused by fish over-wintering in the reservoir's sediments.

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Section – Techniques and Technology

Quantitative Assessment of Climate Change Adaptive Capacity For East Nile Locality – Khartoum State-Sudan

AHMED ALHUSEEN
Faculty of Natural Sciences
Department of Landscape Ecology
Comenius University in Bratislava
Bratislava 842, 154

ahmedalhuseen@yahoo.com

Abstract: Adaptive capacities of urban societies and economies should be increased so as to be able to cope with expected climate change impacts which are heading. This study aimed to produce generic research findings that will be widely applicable in assessing adaptive capacity for whole Khartoum State - capital of Sudan. However, the specific objectives were to test the applicability of the toolkit of quantitative assessment of climate change adaptive capacity proposed by United Nation Habitat to the case of Khartoum state-Sudan and to quantitatively assess the climate change adaptive capacity for East Nile locality (District) as one of seven localities that compose Khartoum state. This study has used a toolkit developed by UN-Habitat for quantitative assessment of city's adaptive capacity to climate change as main methodology to gain the results. The toolkit composes of group of indicators or dimensions necessary to measure city's adaptive capacity. A quantitative analysis has been done through desk assessment using secondary data driven from localities/city profiles, national and state reports and research results. The result shows that from the highest possible value of 1, East Nile locality's adaptive capacity is found to be below the mid-point as it only registers with a 0.36 rating. It shows also that the toolkit is found to be flexible and practical. The study concluded that; in spite of national and sub-national efforts to adapt with climate change in Sudan, however, these effort so far deemed to be inefficient and inadequate, resulting in lack of capacity and capability to avoid current climate variability at both local and national levels and in supporting vulnerable communities.

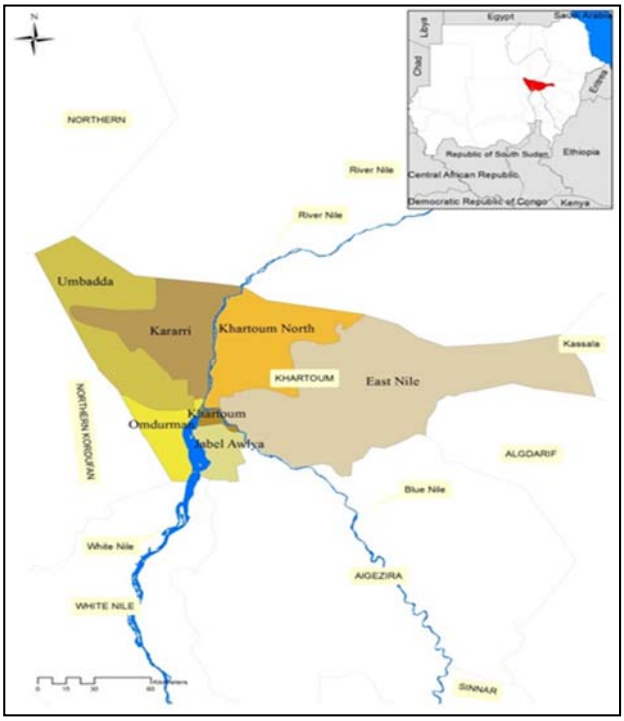
Key-Words: Quantitative analysis, East Nile locality, Adaptive capacity, Khartoum state

Introduction

The human race is fast becoming an urban species: cities occupy less than 3% of the Earth's land surface but now house just over 50% of the world's population, a figure that was only 14% in 1900 and is estimated to increase to 60% by 2030. The rate of growth in developing countries is faster than in industrialized nations [1]. Cities face significant impacts from climate change, both now and into the future. These impacts have potentially serious consequences for human health, livelihoods, and assets, especially for the urban poor, informal settlements, and other vulnerable groups. Climate change impacts range from an increase in extreme weather events and flooding to hotter temperatures and public health concerns [2]. Urgent action is required to reduce greenhouse gas emissions if the worst consequences of climate change are to be avoided. Similarly, early interventions are required to ensure that urban societies and economies are able to cope with inevitable changes in climate that will occur even if emissions are sharply curbed now [3].

Sudan has federal governing system, according to it; the country has been divided into 17 states each states divided into localities (Districts), localities divided into administrative units (Counties) and popular committees (Neighborhoods) represent the smallest governance units in this hierarchy. Khartoum State — Sudan; represents the capital of the country and it has been considered as the most populated area of Sudan, its population is estimated as 5,274,321 million inhabitants according to official statistics [4]. Khartoum state extends approximately over an area of 22,000 Km² and it has been divided into 7 localities and each locality divided into a number of administrative units which in total are equal to 36 administrative units (Figure 1) East Nile locality falls at the northeast part of Khartoum state; it has an area of 8188 km2 which represent 30% of the total area of Khartoum state. It has a population of 868147 inhabitants resemble to 16% of the total population of the state and they are distributed over 8 administrative units that compose the locality. Urban dwellers represent 63% and rural is 37% of the total population of the locality [5]. Khartoum city

Fig. 1 Khartoum state and its seven localities



experiences four climatic seasons [6]. The winter season extends from mid-November to March, with clear skies, cool and dry air from the north-east, a minimum temperature ranging between 8°C and 10°C which falls to 5°C during night, and maximum temperatures varying from 23°C to 25°C, and a relative humidity which may sometime
be as low as 20 per cent a monsoonal dust storm is common to occur during this season of the year specially in the period from January-March locally called Haboob. The hot, dry summer season starts at the end of March. The maximum temperatures may exceed 45°C by the end of May. Weather instability is indicated by the reoccurrence of dust storms. The rainy season covers the period from July to September, with August being the rainiest month, Generally, annual rainfall ranges between 110 and 200 mm, but a minimum of only 4 mm was recorded in 1984 and an exceptional maximum of 420 mm in 1988. A short, hot (about 40°C) transitional season occurs between mid-September and the beginning of winter. This changeover season from south-westerly to north-easterly winds is accompanied by dust storms [7].

Khartoum Sate suffers from increased emissions of Green House Gases (GHGs); these emissions have been considered as major contributors to the heating up of microclimate of the city as well as being responsible in deteriorating the air quality of the city. Climate scenario analyses for whole Sudan conducted in 2003 by Sudan's First National Communication to the UNFCCC indicated that; there will be rise in average temperature, between 1.5°C - 3.1°C during August i.e. hot season, and between 1.1°C 1°C during January i.e. cold season by 2060 [8]. In addition to that; there will be reduced average rainfall by about 6mm per month during the rainy season. Hazard and vulnerability assessment conducted during carrying out the National Adaptation Program of Action in 2007 has predicted Khartoum state's region will experience extreme weather events and variability as a result of climate change [9]. Flash and river floods, heatwaves and dust storms are most frequently expected climate change impacts to occur in the state. In autumn of 2013 and 2014 successively, Khartoum state has witnessed excessive rainfall, flash and river floods which caused a catastrophic effects on the life and properties of the city inhabitants. East Nile locality was the most badly affected locality among the others with the highest incidences of death toll and houses destruction.

Aim and objectives

The general aim of this study is to produce generic research findings that will be widely applicable in assessing adaptive capacity for whole Khartoum State the capital of Sudan. However, the specific objectives of this study are confined to:

- Test the applicability of the toolkit of quantitative assessment of climate change adaptive capacity proposed by United Nation Habitat to the case of Khartoum state-Sudan.

- Quantitatively assess the climate change adaptive capacity for East Nile locality (District) as one of seven localities that compose Khartoum state.

Material and Methods

This study has used a toolkit developed by UN-Habitat for quantitative assessment of city's adaptive capacity to climate change; it composes of group of indicators or dimensions necessary to measure city's adaptive capacity (Table 1). This toolkit introduces both qualitative and quantitative methodologies that could be used in the climate change assessment [10]. Quantitative analysis has been done through desk assessment using secondary data driven from localities/city profiles, national and state reports and research results. The selected dimensions and indicators to be used in the assessment may vary from one city to another depending on data availability. In this study, socio-economic, technology and infrastructure have been used as major indicators and each indicator have been assigned a weight; the total weight for the three major indictors is equal to 1.

Then each one of the three indicators has been fragmented into sub-indicator and each has assigned score driven from localities/city profiles, national and state reports and research results.

Weighted score has been calculated by multiplying weight per indicator by score driven from reports. Total score has been acquired by multiplying total weight by sum of weighted score per indicator then the summation of the total score gives the score for adaptive capacity of study area.

Table 1 Adaptive capacity assessment Toolkit

	A	B	C	D	E	F
	Dimension of Adaptive Capacity And select Indicators	Weight per indicator	Total Weight	Score	Weighted Score Per indicator	Total Score
	Wealth		**.50**			(Sum in G x C)
	(Possible Indicators) -GDP -% HH earning above Poverty Threshold			(rate/score based on city data)	(=score in D x B)	
G					(=Total Score)	
	Technology		**.25**			(Sum in H x C)
	(Possible Indicators) -% HH with access to communication -% HH with access to electricity			(rate/score based on city data)	(=score in D x B)	
H					(=Total Score)	
	Infrastructure		**.25**			(Sum in I x C)
	(Possible Indicators) - Road Density - % paved road - % HH with safe housing unit			(rate/score based on city data	(=score in D x B)	
I					(=Total Score)	
	TOTAL		**1.00**			(= Sum of Above)

Results and Discussion

Table 2 illustrates the quantitative assessment of adaptive capacity by using reference template shown in table 1. The gained results represent the quantitative assessment of the East Nile locality's adaptive capacity. The assessment used selected socio-economic indicators and proxy indicators for technology and infrastructure as measures of adaptive capacity. The result shows that from the highest possible value of 1, the locality's adaptive capacity is found to be below the mid-point as it only registers with a 0.36 rating. This explains the catastrophic consequences which the locality has been subject to during autumn 2013 and 2014 consecutively when the death toll reached thousand and hundreds of families lost their homes.

This result proves that; in spite of national and sub-national efforts to adapt with climate change in Sudan, however, these efforts so far deemed to be inefficient and inadequate, resulting in lack of capacity and capability to avoid current climate variability at both local and national levels and in supporting vulnerable communities. Urban centers in Sudan have been considered as a major affected sector by climate change very recently. For instance, Khartoum state is considered as a major affected sector by climate change in 2013 and adaptive capacity building is carried in three different sectors which are; agriculture and food security, water and health. In November 2013, Khartoum state has designed its climate change adaptation plan as partial part of the National Adaptation Plan (NAP) and it has been submitted to the cabinet of ministers for endorsement as a climate change adaptation policy. The assessment toolkit found to be flexible and practical and it gives realistic image of how far is city

prepared to address the adverse impacts of climate change.

Conclusion

study concluded that; in spite of national and sub-national efforts to adapt with climate change in Sudan, however, these effort so far deemed to be inefficient and inadequate, resulting in lack of capacity and capability to avoid current climate variability at both local and national levels and in supporting vulnerable communities.

Table 2 Results of adaptive capacity assessment

Dimensions, indicators and rating used			Locality / District Adaptive Capacity		
Socio-economic		**.50**			**0.13**
- %Poverty Incidence.	0.30		0.037	0.0111	
- %Literacy rate.	0.20		0.046	0.0092	
- %Slum squatter.	0.20		0.32	0.064	
- %Development expenditure from locality's allocations.	0.20		0.81	0.162	
- %Informal sector.	0.10		0.12	0.012	
				0.25	
Technology		**.25**			**0.16**
- %Access to telecommunications.	0.30		0.45	0.135	
- %Access to electricity.	0.30		0.40	0.12	
- %Shortage in disaster risk reduction units coverage.	0.40		0.95	0.38	
				.63	
Infrastructure		**.25**			**0.08**
-%HH with safe water access.	0.20		0.45	0.09	
-%paved roads.	0.20		0.055	0.011	
-%constructed surface rain water drainage system.	0.20		0.15	0.03	
- %Greenery and forestation.	0.10		0.30	0.03	
-% Houses with improper sewage system	0.10		0.78	0.078	
-%Houses with poor condition.	0.20		0.32	0.064	
				.30	
		1.00			**0.36**

The adaptive capacity of the study area is low the thing that necessitate a comprehensive rigorous adaptation plan for the whole city. Inclusion of the city adaptation plan to climate change in development process it may be one possible solution and it may cost less. Provision of high quality city infrastructure and raise awareness of the locality inhabitants represents prerequisites to increase their adaptive capacity to climate change. The toolkit for assessing the adaptive capacity found to be applicable, effective and flexible.

Acknowledgement

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Using of the nanotextiles for removing of microbial pollution

LENKA DETVANOVA[1], JITKA PRICHYSTALOVA[1], MICHAL DOSEK[2], LIBOR KALHOTKA[1]
[1]Depatrment of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemědělská 1, 613 00 Brno
CZECH REPUBLIC
[2] ASIO spol. s r.o.
Kšírova 552/45, 619 00 Brno
CZECH REPUBLIC

lenka.detvanova@mendelu.cz

Abstract: This paper describes using of nanotextile materials in the filtration device to remove microbial pollution. Nanotextile is non-woven textile formed by fibres with average length 50-300 nm. The size of pores is 0.30 μm ± 0.12 μm, it means membrane should be able to catch every microorganisms. For microbial simulation was selected *Escherichia coli,* occurs in digestive tract of warm-blooded animals and it is indicator of fecal contamination of water. Contaminated water was filtered under pressure 0.5 bar (50 kPa) and subsequently was performed microbial analysis of filtrate. As control sample was used unfiltered contaminated water. A high efficiency of selected filtration materials are shown in the conclusions of our paper.

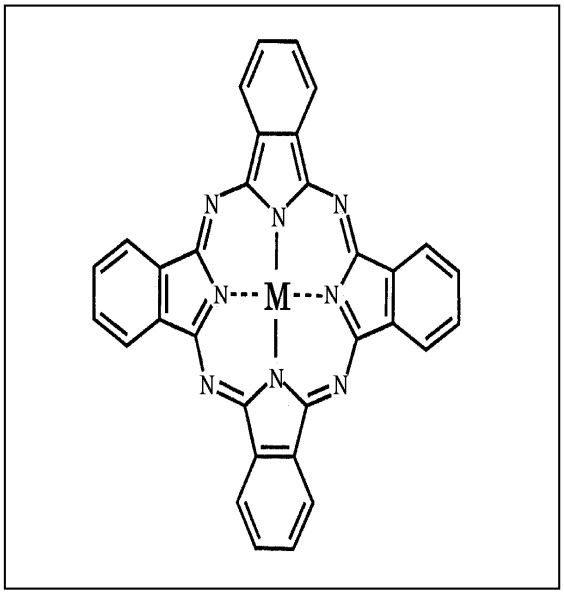
Key-Words: Escherichia coli, nanotextiles, filtration

Introduction

In recent years, nanomaterials (NM) are in centre of scientific interest and began to be used in various fields of industry, agriculture, pharmacy or medicine [1]. The basic building elements of NM are nanoparticles [2]. Nanoparticles (NP) are characterized by size about 1-100 nm at least in one dimension and they have different shapes: nanotubes, nanofibres, crystalline structures or fullerenes. NP occur in nature as secondary products of industrial processes. They are part of atmospheric particles and natural events as eruptions, erosions and fires. The count of products made from NM is estimated to be hundred to thousands tons per year and these numbers will increase [3]. NM can be made from any substance. In nanoform is available 44 elements of The Periodic Table of Elements and their compounds. Nowadays, are most commonly used NM from metals (nanosilver, nanoiron), silicon, carbon (dendrimers, carbon nanotubes) and from metal oxides (titanium, zinc, cerium) [4]. NM apply as support for catalysts, sunscreen components, components into silicon chips or to strengthen tennis rackets [5]. To NM are added phtalocyanines (FTC) due to their antimicrobial properties. FTC are 2D synthetic analogues of porphyrine, consisting from four isoindol subunits connect with nitrogen atoms. FTC and their metal derivates are used in preparation of pigments and dyes, they are also applicable in the manufacture of electronic and optoelectronic devices [6].

Fig. 1 Phtalocyanine molecule (Internet)

This paper deals with the usage of nanofibrous materials modified with FTC. The paper follows



previous experiments with nanofibrous structures, designed for the filtration of water. With FTC modification we wish to increase the removal of microorganisms.

Material and Methods

FTC derivatives was synthesized in The Centre of organic Chemistry (COC, Rybitví, Czech republic). The water-soluble FTC derivates are prepared by substitution of the FTC skeleton in order to prepare a cationic FTC with antimicrobial effect. Microfiltering material SPURTEX was made in SPUR a.s. Zlín.

In the experiment, selected materials were used (see tab. 1), which were drenched in distilled water before the experiment. For the simulation of microbial pollution, the bacterium *Escherichia coli* was selected. *E. coli* was incubated in TSB (Biokar Diagnostics, France) for 18 h at 37 ˚C. Subsequently, *E. coli* was centrifuged (3000 RPM) and washed with solution saline. This procedure was repeated twice. From the resulting suspension was made solution for experiment with density 10^6 cells/ml.

Saline solution with addition of *E. coli* CCM 7929 (Czech collection of microorganisms) was poured into a filtration device in the upper part of the apparatus. Pressure was applied through the filling valve and during the experiment was rectified by manometer. Filtration material was anchored to the filtration head. The sample of water was transported through the filter and after 100 ml of water was added, the filling valve closed. During the experiment, the time of flow of the filtrate was measured. In the sample, coliform bacteria were established by plate cultivation method on VRBL agar (Biokar Diagnostics, France) for 24 h at 37 ºC.

Results

In figure 1 is the comparison of selected membranes and their antibacterial abilities.

Fig. 2 Selected membranes and their antibacterial abilities

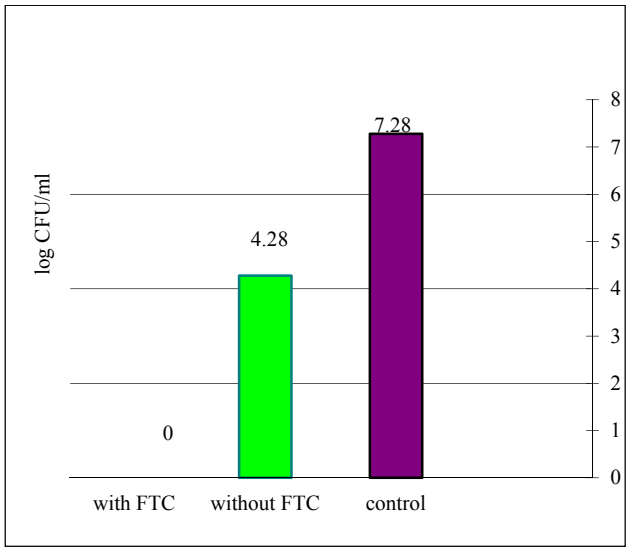


Table 1 Selected materials and their parametres

sample	material	volume [ml]	time [s]	efficiency [%]
without FTC	PVDF	60	8.10	99.88
with FTC	FTC 177F1z1 O2BC	60	7.31	100

Discussion

During the experiment was confirmed our assumption high efficiency of NM with addition of FTC to remove microbial pollution of water. Material without addition of FTC reduced *E. coli* counts for three degrees comparing with controls, material with addition FTC had 100% success. High efficiency of membrane with addition FTC can be caused by positive charge of FTC membrane, which makes better connections with negative ions in cell wall of *E. coli*.

A similar conclusion had been reached Merchat et al. [7] in their work with porphyrins, which are similar photosensitive agents such as FTC. Minnock et al. [8] found out gram-pozitive bacteria are more sensitive to photosensitive agents then gram-negative, because of the cell wall structure. This conclusions confirm Banfi´s et al. [9] paper, who when examine synthetic FTC found, that gram-positive *Staphylococcus aureus* was more sensitive than gram-negative *E.coli*. Mikula et al. [10] discovered that FTC with concentration FTC 2 $g.l^{-1}$ causes serious damaging cell wall after 3 hours exposition. Using metalophtalocyanines was achieved reducing for five degrees comparing with control. This suspension was lit by white light. This same type of FTC reduced after illuminating by red light counts of microorganisms for 5.5 degrees [11]. Došek et al. [12] reached filtration efficiency 98.84-99.31% at the same filter condition.

Conclusion

In this paper filtration materials with and without addition of FTC were compared. We analysed their ability to capture, or more precisely, to leak *E.coli*, which is natural colonizer of the digestive system of warm-blooding animals and it is an indicator of faecal contamination in water. The results of microbial analysis show high filtration efficiency of selected materials. The next objectives of our papers will be testing FTC durability and resistance to washout, the development and testing of membranes with functionalized surfaces and nanobiocides that could be a more effective alternative for cleaning and water treatment.

Acknowledgement
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Use of urine for recycling of phosphorus in the form of soil conditioner

MICHAL DOSEK[1, 2], MICHAL CERNY[1], PETR DOSTAL [1]
[1]Department of Engineering and Automobile Transport Mendel University in Brno
Zemedelska 1, 613 00 Brno
[2]ASIO, spol. s r.o.
Ksirova 552/45, 617 00 Brno - Komarov
CZECH REPUBLIC

xdosek1@node.mendelu.cz

Abstract: Household wastes and landfill is recently very discussed topic not only in this country but in all European countries. Taking the statistics for waste management in Europe, we find that on average 38% of production goes to landfill. Not so in Sweden, where Eurostat statistics indicate only a percentage. Swedes have perfectly disciplined and sophisticated system of waste separation, recycling and composting could be a good example not only for the Czech Republic.
Currently, it seems that things are starting to move in our country. Examples include projects such as research organizations and companies that are interested in dealing with the issue of waste and recycling and sees potential in them rather than the problem. One example is the project "From waste to materials", which is funded by the support of the National Agency for Agricultural Research. The project aims to recycle materials that are currently considered as waste materials applicable especially in agriculture
Recycling helps save both renewable and non-renewable resources and often can reduce the burden on the environment. EU Directive no. 98/2008 (EC) defines recycling as any recovery operation by which waste materials are reprocessed into products, materials or substances whether for the original or other purposes. It includes the reprocessing of organic material but does not include energy recovery.
This holds even solving the project consortium, which is divided into several work packages. The first is focused on the development of recycling technologies phosphorus soon scarce and essential nutrients. Phosphorus is extracted from treated waste water from other streams rich in phosphorus. There are already a number of technologies that are capable of phosphorus from wastewater recycle predominantly to form of struvite, exceptionally, to form hydroxyapatite. From the activated sludge, phosphorus is most often in the form of fly ash is recycled as fertilizer, sometimes also after acidic hydrolysis to form other fertilizers.
Furthermore, the project aims to optimize the management of yellow waters for the purpose of direct fertilization using them or getting phosphorus soil conditioner. This work, however, is focused on the acquisition of human urine concentrate, which will serve as a source waning and non-renewable phosphorus. For quickly and relatively cheaply preconcentration of urine is used in this case solar energy.

Key-Words: urine, solar energy, struvite, phosphorus, precipitation, nutrients

Introduction

The development of society, whether demographic, economic and technological developments affect not only water consumption, but also the production and composition of waste water, hence the liquid waste.
Successive extraction of phosphate ore as the main source of phosphorus for the production of phosphate fertilizers may lead to a global collapse because darkest scenarios predict that this will happen in the next 20 to 30 years [1] Is mainly to blame completely upset the cycle of phosphorus, which is incorrect management of this element came into surface waters by 75% more phosphorus than they were before the Industrial revolution. [2] A significant proportion of nutrients is carrying wastewater from faeces, urine and biodegradable solids. If these resources are managed (urine, feces, gray water, biodegradable substance) separated, can be recycled 92% nitrogen, 82% phosphorous and 62% potassium of the total flow of chemicals. [3]
Our hypothesis is based on the use of yellow water as an excellent source of nutrients N, P, K. It can be used to produce soil conditioner and applied directly to the soil or to the crystallization of magnesium phosphate-ammon, otherwise known as struvite. This is a highly prized fertilizer. For successful crystallization, as used, for example, a Canadian company Ostara, you must achieve at least a phosphorus concentration of 50 mg / l, (according to Chris Howorth from the company Ostara), yellow water which meets the

listing. The resulting fertilizer Crystal Green® - based struvite then contains 5% nitrogen and 28% phosphorus. Using the technology can be recycled 80% to 90% of dissolved phosphorus from the waste water and 20% ammonia.
There are several extensive review, for example. Morse et al. [2] or de Bashan [3], which are compared in detail how each technology in terms of removal and recycling of view. Currently phosphorus from wastewater frequently removed by precipitation using salts of aluminum or iron and the larger sewage through increased biological phosphorus removal.
Although yellow water comprises less than 1% of the total waste water from households, are the source of almost 80% nitrogen and at least 50% of the total phosphorus amounts of these nutrients occurring in domestic wastewater. [4] Since the nutrients are concentrated in such a small volume for separation is inviting.

Reasons for separation of urine
The main reasons for the separation and purification of yellow plants in European countries:

- Reduction of nutrient with respect to subsequent problems with eutrophization with regard to the costs associated with the removal of their consequences
- Minimize the amount of run-off of nutrients in WW with regard to the economy of clean water, especially reducing the concentration of nitrogen cheaper cleaning process
- Strain the inflow parameter input to the WWTP, which makes it possible to consider other, less economically advanced technologies such as wastewater treatment plants. Domestic wastewater at
- Recycling nutrients contained in urine as fertilizers - phosphorus reserves are falling and the price of phosphate and nitrate fertilizers repeatedly increasing [4]

Urine as a source of raw materials for agriculture
The average person annually produces 50 L of faeces, 500L urine and 100,000L of gray water (depending on local conditions) [5] For specific about the composition of the urine shows the contents of the most widely used in agriculture nutrients contained in urine, relative to the need for these substances necessary for the production of grain weighing 250 kg.

Table 1 Nutrients contained in human feces and their contents in a standard fertilizer needed to produce 250 kg of grain per year. [7]

Most important nutrients	Urine (500 L)	Faeces (50Ll)	Urine + Faeces	Fertilizer for 250kg corn production
N (kg)	5,6	0,09	5,7	5,6
P (kg)	0,4	0,19	0,6	0,7
K (kg)	1,0	0,17	1,2	1,2
N+P+K (kg)	7,6 (94%)	0,45 (6%)	7,5 (100%)	7,5

Material and Methods
In 2013, a project from waste materials taken the first experiments with separation yellow water (urine) and their hygienisation. The next step in this experiment is verify their fertilizing effects in a field experiment and crystallization of phosphorus fertilizers in bioavailable form of struvite and the use of solar energy for decrease the amount of the water and prepare soil conditioner, which is rich to nutrients N,P,K.
Because fresh urine can be a source of various pathogens, depending on the health of producers, or it may be contaminated with feces, is needed before further handling urine hygienization.. The simplest method is the storage for several months. WHO Handbook for disposal of excreta determined period of stabilization for 6 months at 20 ° C. [9]
It was founded by experiment, which was clean urine of male origin, collected in music festival in Brno, Majáles. The volume of urine 10 m3 was stored in tanks and regularly monitored. The key parameters was microbial indicators prescribed by Czech legislation and contents of N, P, K. The results show the following two tables.

Table 2 Microbial indicators monitored in urine during hygienisation

Microbial indicators	**1. month (CFU/100 mL)**	**6. months (CFU/100 mL)**
Enterococus	85	0
Escherischia Coli	0	0
Salmonela	negative	negative
Thermotolerant coliforms	0	0

Table 3 nutrient content in urine after 6 months storage

Parametr	Unit (mg/L)
Total P	180
Kjeldahl Nitrogen	2200
Nitrate nitrogen	< 0,2
Potassium	301
COD_{Cr}	2280

Note: Accuracy 10%

During this simple experiment it was shown that after 6 months storage of urine is sufficiently hygienized and retained and key nutrients and is therefore very suitable for further treatment, eg. Soil conditioner and struvite crystallization. Folloving part will describe, how we conducted in the experiments whit preparing soil conditioner using solar energy. Struvit precipitation will be verified experimentally at the end of the year 2014.

Soil conditioner, urine precontration

The second part of the experiment was designed to concentrate the separated urine and verify the use of solar photovoltaic panels for the production of concentrate and evaporation of the water content of the urine. This is very useful, because in future, when we will have some struvite or soil conditioner factory, will be important also the price per urine transport, so for this reason is necessary to remove water and prepare concentrate rich to N,P,K nutrients.

The experiment was performed with 50 l hygienized urine, which was placed in the storage tank. Urine was heated by a heating cable (75 W), connected to two pieces of photovoltaic solar panels KC120-1 with accessories (battery and elektrical convertor).

During the experiment was continuously recorded temperature in the tank and the outdoor temperature within 1h, using dataloger. The tank was covered with urine rain due to the solar panels and was protected against the entry of dirt with a mesh with diameter of 1 mm. It was also recorded the amount of electrical current supplied by solar panels to the heating cables using electricity meter.

Loss of water compound of the urine in the reservoir was recorded daily. Periodically (once a week) was analyzed concentrate in the tank with respect to the contents of nutrients (N, P, K)

Picture 1 Model devices for urine preconcentration

Conclusion

The choice of solution draining separated urine has a significant influence on the trailing parameters sewage treatment plants, water, energy and the possibility of recycling nutrients. Today there are a large range of separation equipment to enable the separation of powers and minimize dilution. There are also technologies and products to enable the collection, storage and processing of urine. From the economic point of view it seems also important to minimize the amount of nutrients in the outflowing WW and especially input to the WWTP through separation of urine. This reduces the overall energy consumption required for sewage treatment. Due to

the decentralized approach, we can also reduce the transport distance of wastewater sewers and minimize transported and pumped volumes of wastewater or sludge weighed and thus save more energy. [8]

Problems with phosphates is closely associated not only with its efficient use of in agriculture, but also his removal from aqueous environment where the increased concentration of undesirable because it causes the formation of algal blooms and deterioration of surface water quality. The threat of depletion of phosphate in the near future, leads to the need for introduction of recycling technologies where the most interesting ways of recycling are the concentrated liquid sources suitable for struvite crystallization which could be subsequently desired commodity among farmers, because it is a slowly dissolving fertilizer.

Struvite is often associated with a blocked duct, which is usually caused by a spontaneous crystallization. Currently is developing a range of technologies for the recycling of waste water right - most of the current sludge water. Crystallization of struvite, control its production and transformation technology from laboratory scale through pilot plant to real plants is an interesting technological challenge.

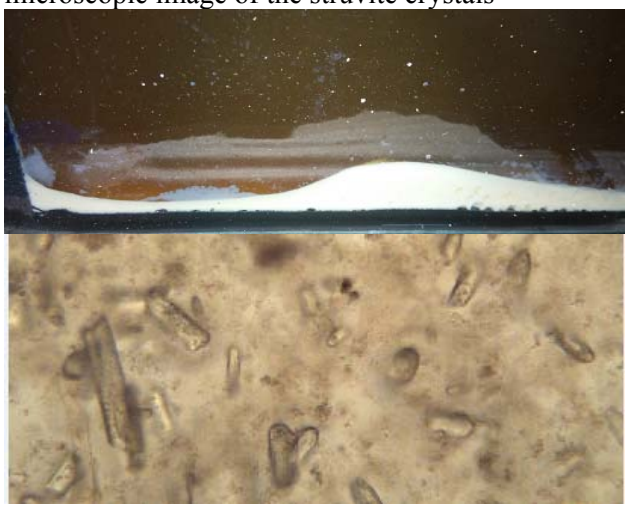
Our experiment shows that the preparation of a concentrated solution of urine realizable. During the experiment were also determined nutrient values in the urine. See the table below.

Table 4 Urine analysis during experiment

Sample	Sampling date	pH	Conductivity mS/m	COD mg/L	P total mg/L	N-NH4 mg/L	N total mg/L
urnie	29.9.2014	9,37	4360	6654	364	7040	7780
urnie	7.10.2014	9,15	5120	6455	389	7800	8500

Already during the first week was recorded decrease in urine volume. Although this was due to less sunny weather, only 10% of the tank volume. However, the value of nutrients is increased, as shown in the table. Since the experiment is not yet finished, it is not possible to present the complete results. However, these existing results seem very promising. The next step is to manage struvite crystallization to the form of commercially salable fertilizer, prepared from urine concentrate.

Picture 2 sediment at the bottom of the urine tank, (second week of the experiment) and below microscopic image of the struvite crystals



The biggest hurdle to this work is public perception of lingering buckets of pee, or the so-called “ick factor.” Urine is actually sterile when it exits the body, save for rare cases of a bladder infection or salmonella poising. So we developed two strategies for eliminating the risk of pathogens, either by solar pasteurization or long-term storage. Both have proven effective.

Second problem are pharmaceuticals that end up in urine. Because it´s difficult to remove hormons, it´s more safty at this stage use only mans separated urine. But even if there is minimal risk to personal or public health, we realizes that only a chosen few will ever be willing to collect their own urine with jugs and funnels. A much more realistic option is urine-diverting toilets.

Acknowledgement

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Cutting performance of cemented carbide tools with nano-crystalline pvd coatings

PAVEL HUDECEK, PETR DOSTAL
Department of Technology and Automotive Transport
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xhudecek@node.mendelu.cz

Abstract: This resource deals with the analysis of properties of hard coatings and wear resistance of chemical vapour deposition (PVD) coated technology. It focuses on the preparation, execution and evaluation of test coatings on the front ball-milling cutters. Examination of these characteristic properties may give into an insight to the reason why some systems show excellent wear characteristic.

Key-Words: PVD, properties of coatings, testing, analysis, frontal spherical milling cutter, durability

Introduction

The coating of cutting tools by hard material layers with the aim to rise their durability has reached a high level. Modern coating methods are have become an important part of industry. Wear resistance, durability, toughness (breakage resistance) and hot hardness (high hardness and chemical stability at high temperature) are the four main properties necessary for high speed use of cemented carbide. These proprieties are for productivity, economy and ecology.

The high-purity, solid coating material (metals such as titanium, chromium and aluminium) is either evaporated by heat or by bombardment with ions (sputtering). At the same time, a reactive gas (e.g. nitrogen or a gas containing carbon) is introduced; it forms a compound with the metal vapour and is deposited on the tools or components as a thin, highly adherent coating. In order to obtain a uniform coating thickness, the parts are rotated at uniform speed about several axes. The properties of the coating (such as hardness, structure, chemical and temperature resistance, adhesion) can be accurately controlled. The physical vapour deposition processes include arc evaporation, sputtering, ion plating, and enhanced sputtering [1].

Physically vapour deposited coatings offer a powerful alternative to improve further the cutting performance of the cutting materials. The flexibility of coating processes of the physical vapour deposition (PVD) method, well supported by the superior and controllable properties of coatings are responsible for the almost exclusive worldwide application of coated tools. [2]

Physical vapour deposition may have a considerable influence on performance in practical use and thus on protective qualities. Coatings allow excellent cutting performance allowing a beneficial of modern CNC machine tools. PVD coatings protect cutting tools against abrasion, adhesion, diffusion, formation of comb cracks and other wear phenomena. The choice of the proper substrate or the correct protective coating in the specific machining operation is very important.

Material and Methods

The goal of testing was to compare cutting performance of cemented carbide frontal spherical milling cutter FW412.16.100.10 with four cutting edge, diameter Ø16, length 100 mm and full cemented carbide (two fabrications):

Wear resistance: cutting force 300 N and observable effects of wear (sparking of tool, cutting edge stability and vibration).

1. Cemented carbide milling cutter (reference)

2. Cemented carbide milling cutter with:
   Nc-gradient coating (tumbling by granulate with grinding paste)
   nc- monolayer coating (tumbling by granulate with grinding paste)
   Nc-multilayer (tumbling by granulate with grinding paste)
   Nc-monolayer coating (no tumbling)

Fig. 1 Cemented carbide front ball-milling cutter

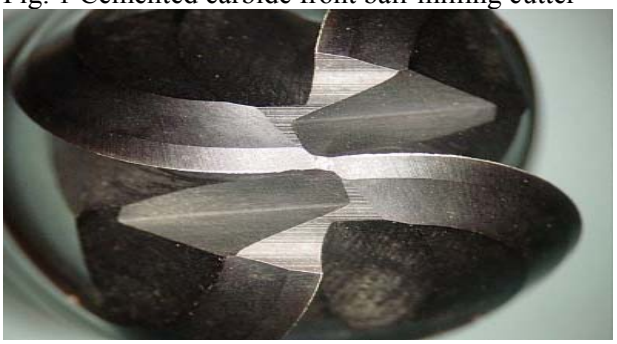


Fig. 2 Micrograph of a surface of the coating deposited tool at 8x

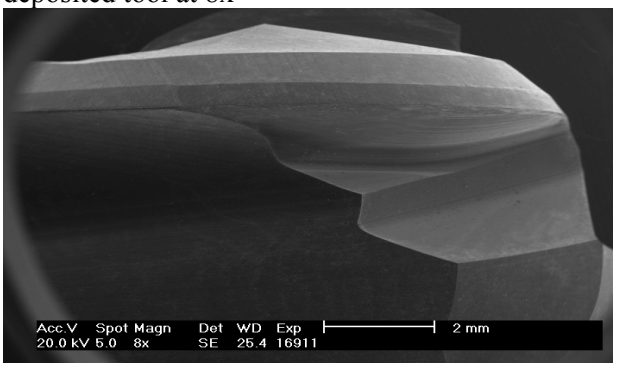


Fig. 3 Micrograph of a surface of the coating deposited tool at 50x

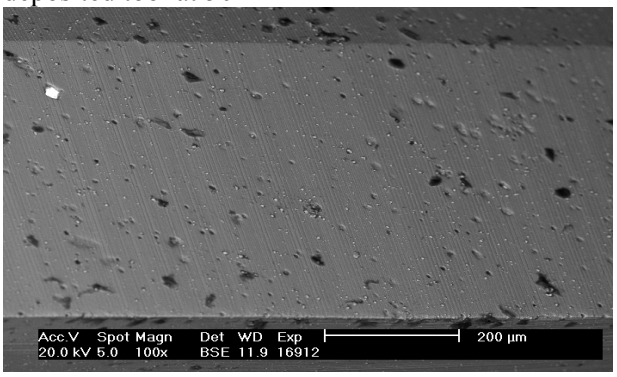


Fig. 4 Micrograph of a surface of the coating deposited tool at 100x

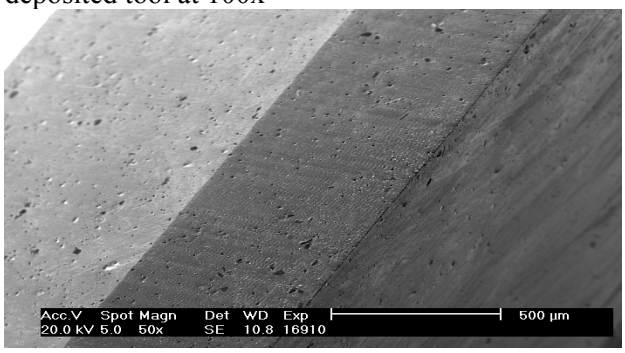
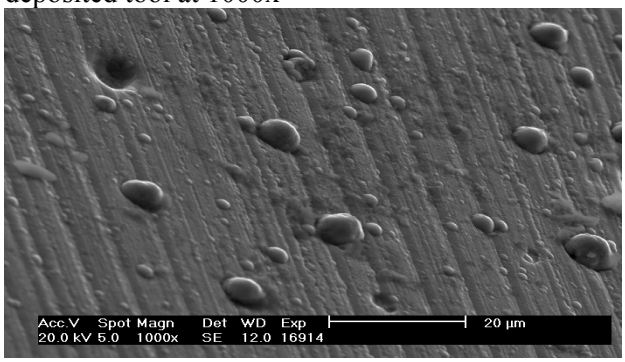


Fig. 5 Micrograph of a surface of the coating deposited tool at 1000x



Workpiece material

The cutting performances are evaluated by machining cast steel GGG70 (ductile iron with spheroid nodular graphite), semi-finished product 90 x 80 – 300 mm. The surface was not clear of casting, inclusion and other surface defects.

Fastening of cutting tools

The cemented carbide frontal spherical milling cutters were fastened with minimal extends in hydraulic tool holder RÖHM 40x16, DIN 69871Fr. This hydraulic tool holder was hydraulically fastened to the spindle head. Tight fastening was main tainted throughout the experiment.

Cutting conditions

Table 1 Cutting conditions during experimental

Cutting conditions	Period	Value
Cutting speed - vc	[m/min]	250 m/min
Rotational speed - n	[rpm]	4980
Feed speed - vf	[mm/min]	400
Feed per tooth - fz	[mm]	0,02
Cutting depth - ap	[mm]	8
Cutting width - ae	[mm]	0,5
Cooling, lubricating	-	-
Type of milling		conventional milling/ climb milling
Wear criteria		Wear effect(spark of tool, cutting edge stability, ineffective cut, colour of cut and vibration)

Milling machine

A console milling machine type FV 25 CNC with control system Heidenhain 315 TNC manufactured by O.S.O. ltd. Olomouc was used.

Reading method of loading tool, methods of measurement and analysis of measure data

In general, tool life is dependent on temperature, pressure compatibility between tool and work materials, and the structural inhomogeneity of the tool material. Axial force, radial force and tangential force were measured using a piezoelectric silicon dynamometer KISTLER 9257B supplied by charge amplifier KISTLER 9011A controlled by PC. Sampling rate was 50 Hz.

This method provided basic source signals of force loading in coordinate system of milling machine. Measurement was focused to axes X, Y. Axis Z

Force (axis of spindle) was about 5 – 10% of those on the significant are axes X and Y. The advantage of measurement of cutting forces during milling was that it allowed of progress of wear as a consequence force change. This allowed observation of the gradual wear of the tool without the need for measurement, disassembly, cooling and reassembly.

Comparison of results

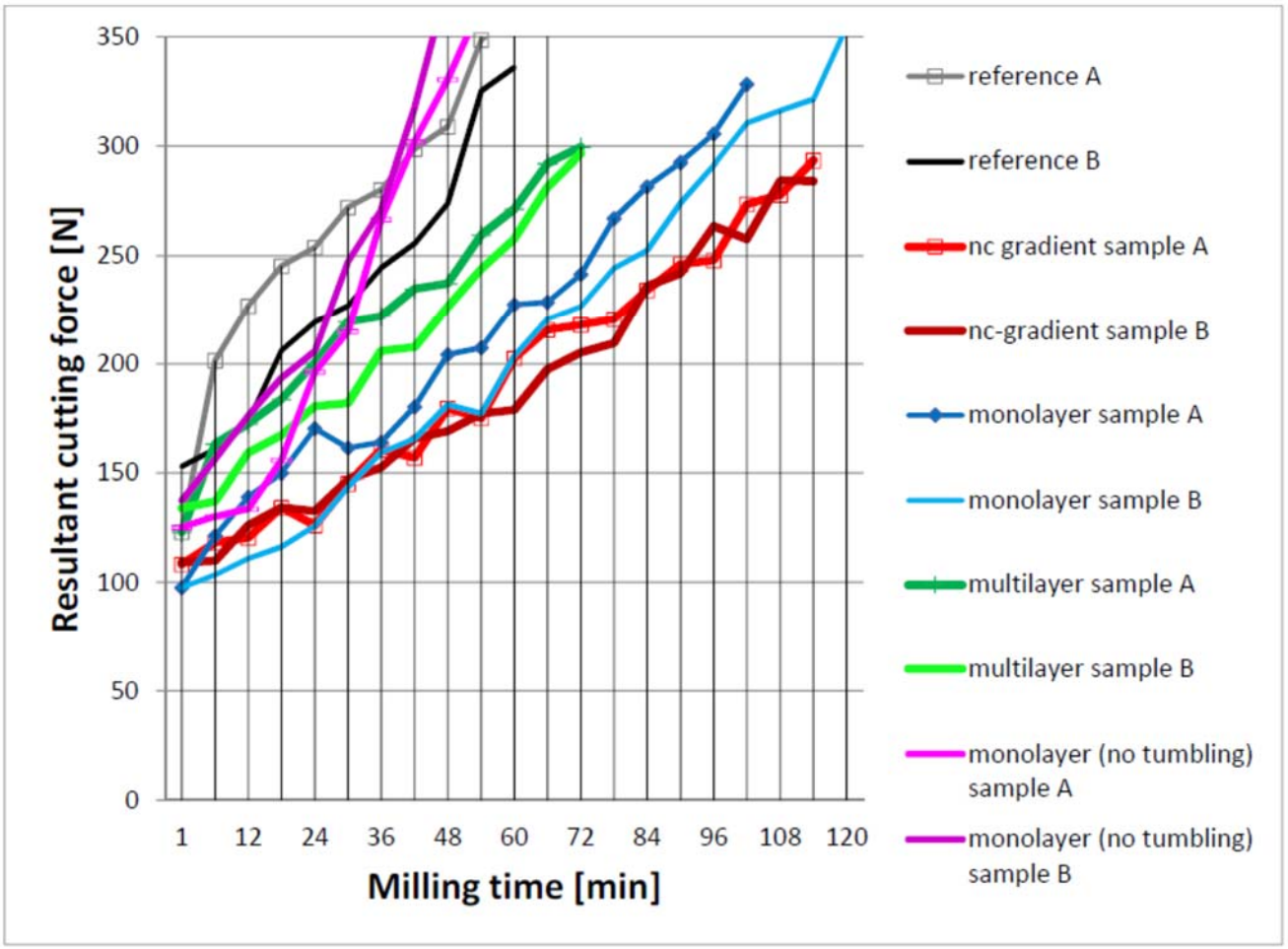
Achieved results and integrated results for individual milling tools are shows in table 2.

See graph 1, which shows progression of average value of resultant cutting forces achieved during milling.

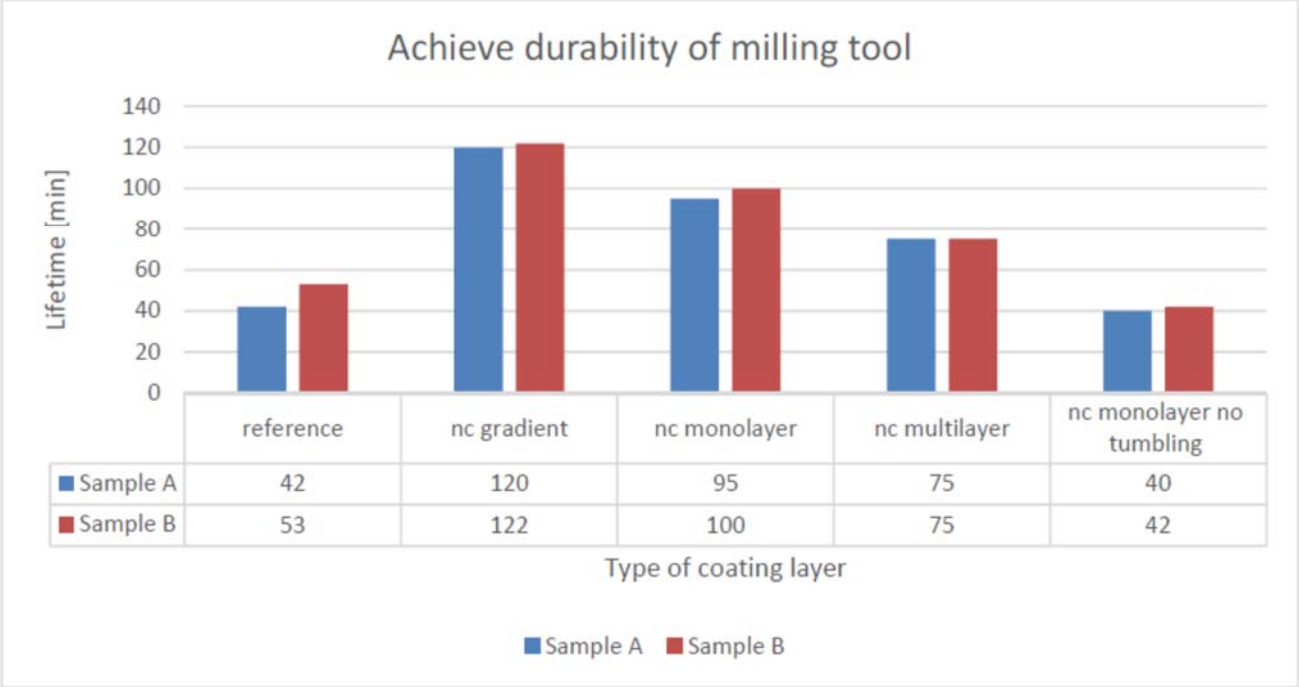
Table 2 Achieved average value of resultant cutting forces

Time [min]	Refer. A	Refer. B	1A	1B	2A	2B	3A	3B	4A	4B
1	123,1	152,9	108,2	109,1	97,5	97,5	123,1	134	125,2	137,6
6	201,6	160,8	118,2	109,8	121,4	103,4	163,3	137,1	130,2	156,3
12	226,9	173,5	120,5	126,2	139,1	110,8	172,5	159,3	133,8	176,3
18	245,3	206,2	134,4	134,4	149,9	116,2	183,8	167,3	155,9	193,6
24	253,8	219,3	126,1	132,7	170,4	125,9	200,6	180,6	196,3	205,9
30	272,3	227	145,2	147,1	161,4	143,7	219,5	181,9	215,1	247,4
36	280,2	244,4	160,7	152,5	164,1	159,2	222,2	206,1	266,6	271
42	298,7	255,5	156,9	165,7	180,2	165,7	234,8	207,8	302,1	317,4
48	308,9	274	179,6	169,2	204,4	181,4	237,3	226,5	330,5	375,1
54	348,6	325,1	175,1	177,2	207,5	177,4	259,5	243,9	364,6	
60	360,2	336	202,7	178,9	227,5	203,7	271,4	257,7		
66	371,6		215,8	197,5	228,7	220,7	292,2	281,4		
72			218,1	205,3	241,5	226,6	299,6	296,6		
78			220,8	209,6	267,1	244,3				
84			234,2	236	281,7	252,4				
90			246,1	242	292,7	274,3				
96			247,9	263,6	305,6	291,4				
102			273,6	257,7	328,4	310,4				
108			277,9	284,4		316,1				
114			293,5	284,2		321,4				
120						354,7				

Graph 1 Progression average value of resultant cutting forces



Graph 2 Achieved durability of milling tool



Results and Discussion

Experiments of cutting power were made by selection of coatings on cemented carbide frontal spherical milling cutters FW412.16.100.10, made of cast steel GGG70 (ČSN 422307).

Procedure which was used precisely describes wear resistance of milling tools during machining process.

The samples of milling cutters showed very little variation between tools, which indicates very good manufacture (sharpening) of tools, PVD coating, methods of calibration and measure of forces.

Conditions during experiments were less sophisticated than could be expected in manufacturing conditions. In practise other benefit and cost reduction can be expected.

As additional control criterion was observation of sparking of milling cutter in climb milling – if achieved there was occurrence of improvement cutting forces.

In terms of side effects, sparking of graphite reference tool was detected after five feeds of tooth in conventional milling cutters, while in the other tools the sparking of graphite was detected much later (**50 feeds of tooth**).

Conclusion

The best result is for a milling cutter with a gradient coating – machining process was very stable with good results of forces. Forces had linear progress without high amplitude during milling all the time.

The best coating can be achieved by both monolayer coating and multilayer coating. The worst coatings were reference tool and monolayer coating without tumbling (granulate with grinding paste).

The durability of the mill cutting tool coated by the gradient method exceeded useful milling-cutter´s durability by 200% (120 minutes) compared with the reference milling-cutter (53 minutes)

Acknowledgement

The research has been supported by the project TP 4/2014: Analysis of degradation processes of modern materials used in agricultural technology; financed by IGA AF MENDELU.

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Modern approaches in car diagnostics

MICHAL JUKL, ADAM POLCAR, DUSAN SLIMARIK
Department of Engineering and Automobile Transport Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

michal.jukl@mendelu.cz

Abstract: Modern vehicles such as agricuture tractor with ECU controlled engine has many electronic parts (sensors, actuators). This parts are usually connected to an ECU. Sensor converts physical quantity to an electrical signal. This signals are used with ECU to manage the operation of the engine with help of actuators. Every signal can be measured with modern diagnostics equipment to verify the functionality of the ECU system. Modern motormanagement systems are supported by serial diagnostics (diagnostics equipment from the manufacturer) but there are still problems that serial diagnostic is not able to solve. Hence, the modern diagnostics techniques should be supported by parallel diagnostics. This article is focused on parallel diagnostics of engine.

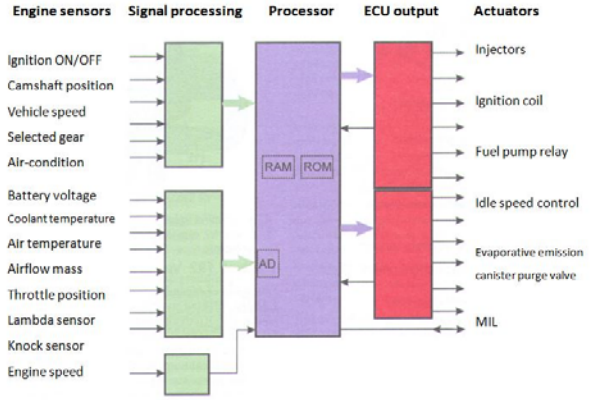
Key-Words: oscilloscope, waveform, sensor, parallel diagnostics

Introduction

The engine management system can be viewed as a closed control loop. In the system there is a control unit that is responsible for managing the operation of the engine. Because the combustion engine is operated in different modes, the controller has to be able to adapt the engine run to these conditions. An example of such a system may be, for example, running the engine shortly after cranking. If it will be a cold start, it is necessary to adjust the fuel injection that enable the smooth start. Said simply, the engine control unit needs to know what the conditions are in the engine, then this state evaluate and set the output parameters of the actuators. Actuators carry out the orders they have received from the control unit. Engine sensors are used to find out input parameters for the control unit. The sensors convert the input parameters such as speed, pressure and temperature into an electrical signal. These signals are then processed in the control unit with input circuits.

As is evident from Fig.1 the control unit has to be connected to a large number of sensors, which differ only its purpose (speed sensing, throttle position sensing, pressure sensing, etc.). They are also different in their output parameters. Many sensors have voltage outputs - eg temperature sensors. The voltage on the sensor then corresponds to a specific temperature (coolant, oil, air, etc.). Each of such sensor has characteristic waveform in the workshop manual or in the datasheet. This waveform can describe the dependence of sensor's resistance on temperature (coolant temperature sensor), or the dependence of generated voltage on engine speed (inductive crankshaft position sensor). These values should be used for the first diagnostics of this component. The basic diagnostic of such sensor can be made using an ohmmeter.

Fig. 1 ECU inputs and outputs [7]



With the increasing complexity of engine management systems also its internal diagnostic is beeing improoved. A milestone in the field of internal diagnostics was the establishment of standards OBD (On Board Diagnostic), respectively OBD-2. The European form of the system is known as the E-OBD [1]. The purpose of introducing these independently operating programs was prevention of excess pollution produced from internal combustion engines. In such a motor management system run tests of individual components which are emission-relevant. Tests of emission-relevant components occur after meeting predetermined conditions, such as coolant temperature, engine speed, vehicle speed, etc. All runs independently of the driver's will. The aim of these tests is to check all components and

create the Readiness code. This code is in binary form and every individual position represents the component of the drive unit, for example catalytic convertor etc. [2, 3] This allows find out what test has not been executed.

For repairs and fault detection is important fault memory. The fault memory keeps written faults and after reading with the diagnostic equipment is supposed to help with repair process. The control unit can show what type of problem has occurred (for example exceeding upper voltage limits, lower voltage limits, or open circuit). With this information is operator able to focus on the origin of defects and speed up the return of the machine to its normal condition. For the OBD-2 standard respectively E-OBD is typical that with any written fault the Freeze Frames are recorded. The Freeze Frames includes specific information about failure issue – e.g. engine speed, coolant temperature, vehicle speed, etc.

Material and Methods

Characteristics of Parallel Diagnostics

Parallel diagnostic consists of control of the basic properties of the component. In other words, it is an independent checking of component whether his behavior is correct. Tests can be performed on all elements, which are connected to the control unit. The control unit (ECU) is an essential element of motor management system. There are a microprocessor and memory in which characteristics (essential to engine running) are stored. According to the data stored in these characteristics ECU controls its outputs – injectors, fuel pump, relays.

A suitable instrument for parallel diagnostics of such components is a digital oscilloscope. The oscilloscope displays the captured signal in time. Then this signal can be analyzed. The big advantage of digital oscilloscope is the ability of recording the signal for later analysis. The kit of the oscilloscope designed for automotive diagnostic is in Fig.2.

Fig. 2 Pico Technology USB oscilloscope

The oscilloscope is able to display only the voltage waveforms. To view the current waveform it has to be connected a current clamp to the oscilloscope. The current clamp converts the magnitude of the current to voltage. In the previous figure (Fig. 2) is also showed other accessories required for oscilloscope in the automotive industry. This equipment includes the already mentioned current clamp, back pinning probes, test leads, attenuators, etc.

Oscilloscope in Automotive Diagnostics

The oscilloscope also finds utilization by checking other devices in the car. An example might be checking the work of the alternator, maximum current of starter, battery informative test, CAN-BUS signal checking or losses in the wiring. You can also control the actuators that have a feedback signal. To check the alternator work is necessary to focus on voltage or current checking. Externally the problem in the alternator may show a gradual reduction of the battery capacity due to insufficient charging or glowing charging indicator. The voltage checking can be done with a voltmeter but the voltage may be within the normal range of alternator regulated voltage even with faulty diode of rectifier. The consequence of faulty diode in the rectifier bridge can be seen just on the oscilloscope in the voltage or current waveform.

Components testing (sensors and actuators) that are connected to the control unit must be adapted according to the type of component. It was mentioned that sensors vary in their output (voltage / current, analog / digital). If we are focused on a petrol engine with fuel injection, the entire engine management system can be checked by using an oscilloscope. The following table shows components suitable for testing with oscilloscope.

Table 1 Tested parameters of sensors / actuators

Sensor / Actuator Type	Parameter
Inductive sensors	voltage
Injector	voltage, current
MAP sensor	voltage
Oxygen sensor	votage
MAF sensor	votage
Fuel pump	current
Ignition	voltage, current

Results and Discussion

Position sensor testing (crankshaft, camshaft)

Test consists of checking: peak to peak voltage, frequency, interference of signal (Fig. 3). Frequency

and amplitude of the signal depends on the speed of the pulse wheel. The pulse wheel has teeth and gaps on its perimeter (Fig. 4), which change the magnetic flux in the coil of the sensor. The output voltage of the sensor can be described with help of the Equation 1, where can be seen that induced voltage is directly proportional to the change of the magnetic flux in time. [6]

Equation 1: Equation of induced voltage

$$U_i = n.\frac{d\phi}{dt} \quad [V],$$

where: U_i – induced voltage,
n – number of winding,
dϕ - magnetic flux change,
dt – time change.

Fig. 3 Crankshaft position sensor (red), camshaft position sensor (yellow)

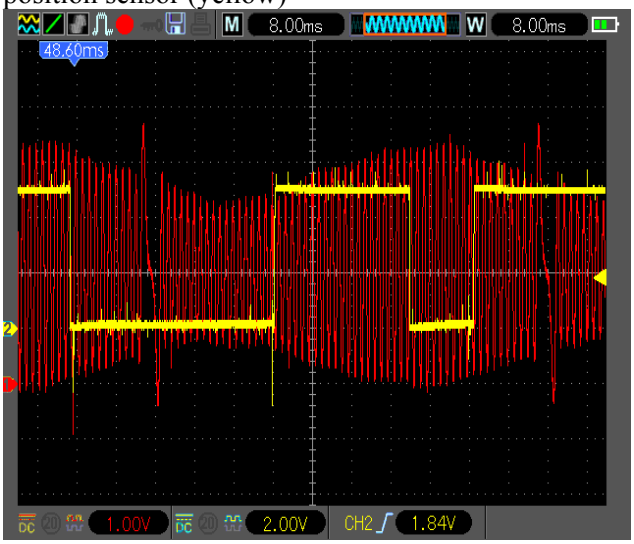
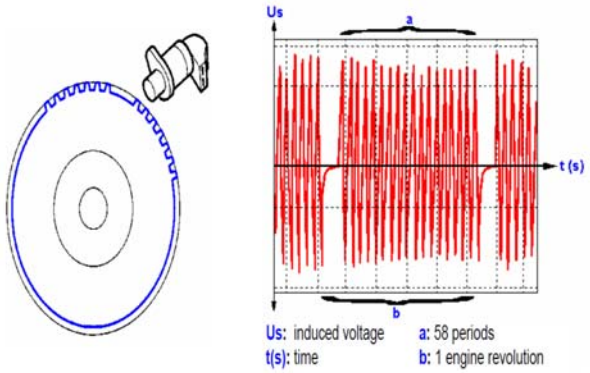


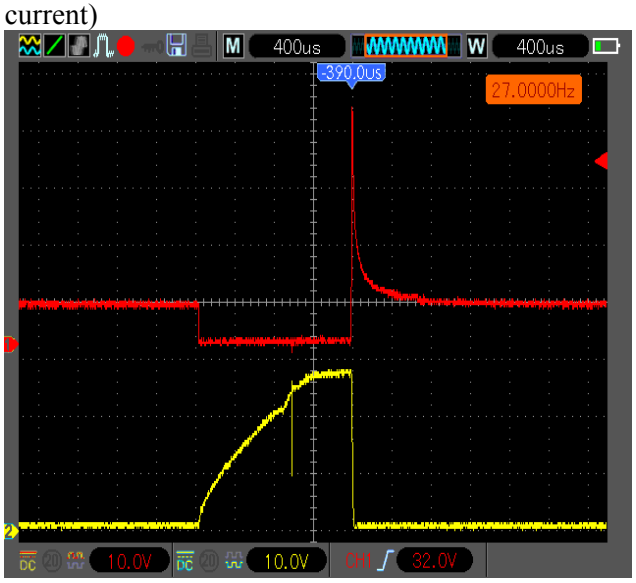
Fig. 4 Pulse wheel and typical waveform of position sensor [5]



Injection testing

On the injectors waveform could be seen the function of injectors or injector needle lift. Injectors can be compared with each other if are measured on another channel (Fig. 5).

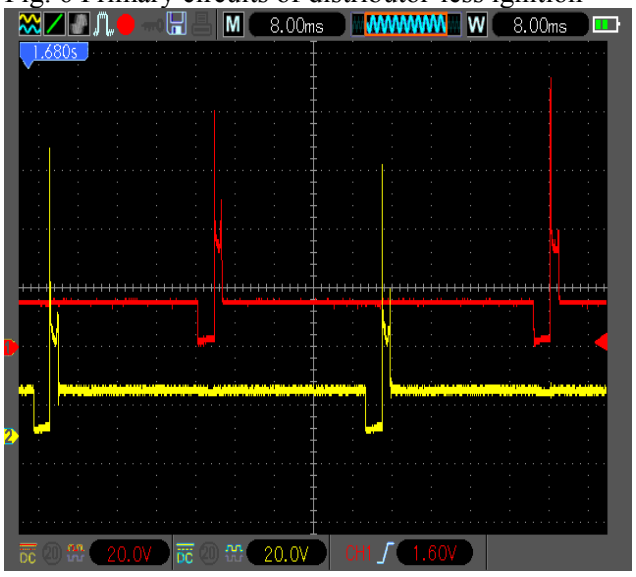
Fig. 5 Injector waveform (red – voltage, yellow-current)



Ignition testing

It exists many ways how to test the ignition. First of all it has to be obvious what type of ignition will be tested (ignition with distributor / distributor-less ignition / COP ignition). In Fig.6 there are showed primary circuits of modern distributor-less ignition.

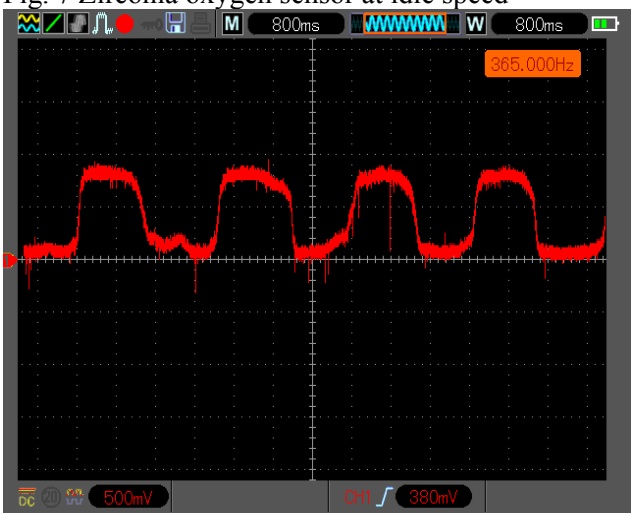
Fig. 6 Primary circuits of distributor-less ignition



Oxygen sensor testing

Oxygen sensor is tested at changing acceleration and deceleration. In the Fig.7 is shown zirconia oxygen sensor at idle speed.

Fig. 7 Zirconia oxygen sensor at idle speed



Fuel pump current testing

Typical fuel pump is an actuator which is controlled by multifunciton relay. In Fig. 8 is shown good fuel pump current waveform. In wrong waveform would be characteristic decreases of current (see Fig. 9).

Fig. 8 Fuel pump current

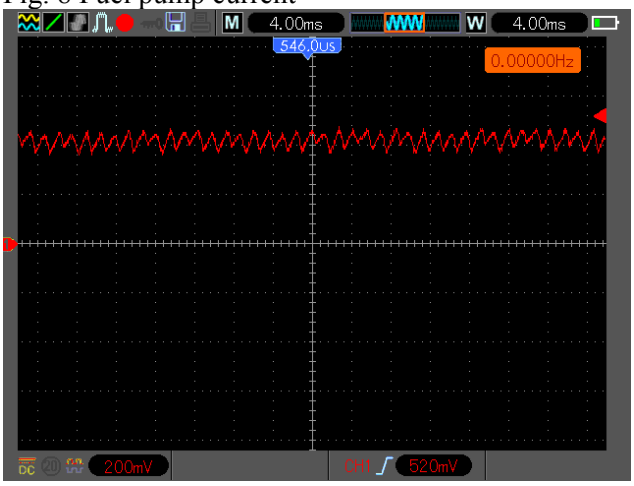
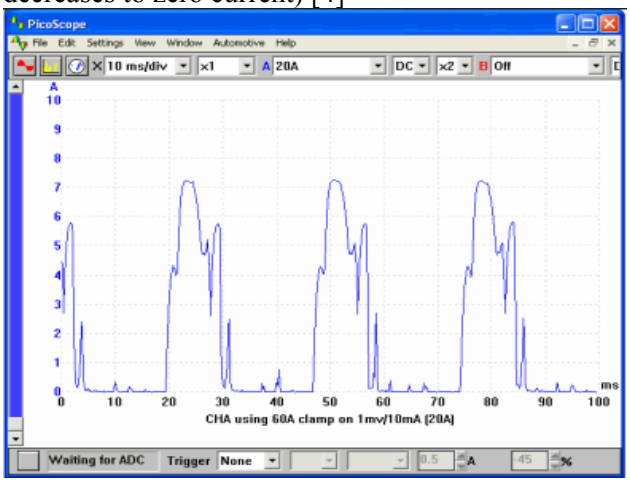


Fig. 9 Wrong waveform of fuel pump current (large decreases to zero current) [4]



Alternator testing

To the objective detection of a problem it is necessary to measure the voltage using an oscilloscope or current using a current clamp. Fig. 10 and Fig. 11 show the voltage and current at the faulty alternator. The alternator current waveform shows current declines to zero. The right waveform is just characterized by sharply defined half-waves of voltage or current.

To remove the DC component of the voltage the first channel was coupled with AC. The CH1 shows voltage at terminal B+ and CH2 shows the alternator current.

Fig. 10 Wrong waveform of alternator current (CH2) and voltage (AC coupled CH1)

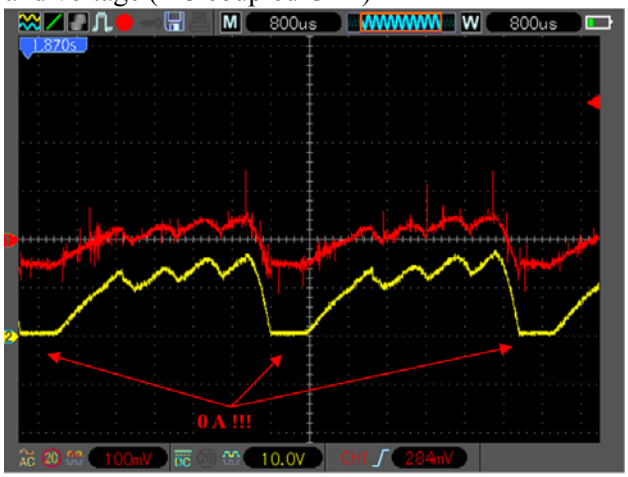
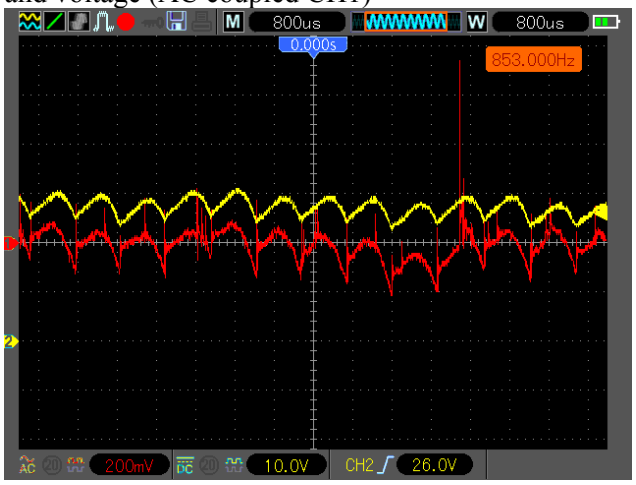


Fig. 11 Correct waveform of alternator current (CH2) and voltage (AC coupled CH1)



Conclusion

This article shows a brief overview of parallel diagnostic of sensors and actuators of motor management system. To verify the functionality of the component is important to assess the shape of curve. In other cases the voltage value or frequency is assessed. Parallel diagnostic with an oscilloscope

requires a certain amount of the operator experience. To the detection of defects on the sensor or actuator is not enough to measure the waveform, but it is important to assess it. Equally important is the knowledge of the engine control system. If the service network is connected to direct support from the manufacturer, it is possible to search for properties of sensors and their outputs in datasheet. The overview shows the measurements with help of 2-CH oscilloscope. Modern oscilloscopes used in the car repair services can be equipped with 4, 8 or more channels. The number of channels is crucial in the cases where random faults appear. If it is possible to observe more channels you can see the connections that occur for example during the engine misfiring and see the fault. Today, the essential properties of a digital oscilloscope are ability of waveform recording. Records are then re-used to locate faults and this feature can help to speed up the diagnostic process.

Acknowledgement

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Optimizing operating parameters for flat-plate milk coolers

VLASTIMIL NEJTEK, JIRI FRYC, JOSEF LOS, RADOVAN KUKLA
Department of Agricultural, Food and Environmental Engineering
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

vlastimil.nejtek@mendelu.cz

Abstrakt: Measuring in laboratory conditions was performed with the aim to collect a sufficient quantity of measured data for the qualified application of flat-plate coolers in measuring under real operating conditions. The cooling water tank was filled with tap water; the second tank was filled with water at a temperature equivalent to freshly milked milk. At the same time, pumps were activated that delivered the liquids into the flat-plate cooler where heat energy was exchanged between the two media. Two containers for receiving the run-out liquid were placed on the outputs from the cooler; here, temperature was measured with electronic thermometer and weight was measured with calibrated scales. Flow rate was regulated both on the side of the cooling fluid and on the side of the cooled liquid by means of a throttle valve. The measurements of regulated flow-rates were repeated five times and the final values were calculated using arithmetic mean and optimum flow ratio calculated by program MATLAB. The measured values show that the volume of exchanged heat per weight unit increases with the decreasing flow-rate. With the increasing flow-rate on the throttled side, the flow-rate increases on the side without the throttle valve. This phenomenon is caused by pressure increase during throttling and by the consequent increase of the diameter of channels in the cooler at the expense of the opposite channels of the non-throttled part of the circuit. If the pressure is reduced, there is a pressure decrease on the external walls of opposite channels and the flow-rate increases again. This feature could be utilised in practice: a pressure regulator on one side could regulate the flow-rate on the other side.

Key-Words: plate cooler, milk cooling

Introduction

Milk is a valuable agricultural product and, after its finalisation, an irreplaceable component of human nutrition. Milk contains a balanced score of proteins, fat, milk sugar, minerals, 14 trace elements, and numerous vitamins [1]. To maintain its quality, milk is quickly cooled after drawing, from approximately 36°C down to 5°C [2, 3]. This process consumes a lot of energy due to the high difference in temperatures and the milk volume [4]. If it were possible to reduce the cost of cooling, e.g. by using preliminary flat-plate flow coolers, the saving would reflect in the overall costs per unit of milk. Another advantage is faster achievement of the required temperature than by standard means, and therefore higher level of hygiene will be reached [5]. Furthermore, the application of the flat-plate coolers offers the desirable extended lifespan of the cooling system thanks to the slower degradation as the average age of cooling systems used in livestock production is 8.9 years [6]. In this way, producers could yield higher profits and enhanced competitiveness [7]. The environmental perspective is important as well. With the ever-growing global population and increasing quality of people's lives a higher need for energy is expected, therefore energy-saving measures become increasingly important [8].

Material and methods

Diagram of the flat-plate cooler connection for the purpose of measuring is presented in Fig. 1. The cooling fluid tank was filled with 13 °C tap water; the second tank, which simulated freshly drawn milk, was filled with water at a temperature of 35 °C. Before the measurement, tanks were filled. The pumps were simultaneously activated, the pumps were activated for 30 seconds. Fluids entered the flat-plate cooler where heat energy was exchanged between the two media. Two containers for receiving the run-out liquid were installed on the output pipes where temperature and weight were measured. Flow-rate was regulated by the throttle valve both on the side of the cooling fluid and on the side of the cooled liquid.

Material used in the measurements:

Cooled and cooling water pumps: Manufacturer: AL-KO, Type: DRAIN 8001, Power input: 550 W, Performance: 10 000 l/h

Flat-plate flow cooler (counter-flows): Manufacturer: SAC Nederland B. V., Finish: Stainless steel, Type: 42, Heat-exchange area: 2.1 m^2
Electronic thermometer: TESTO 922, Resolution: 0.1 °C, Temperature range: -50 to +1000°C,
Stop watch: Manufacturer: JVD, Type: VST31, Accuracy: 1/1000 sec.
Electrometer - HT-353M mechanical, voltage: 3 x 230 / 400 V, Operating temperature: -20 ~ 60°C
Electromagnetic valve - MP116, 230V AC, directly controlled
Hanging calibrated weights OCS-20A/50
The measurements were repeated five times for each regulated flow-rate setup; data shown in tables are calculated as arithmetic means from the measured values. Flow-rate were calculated from the measured values according to equation (1) The 3D graph has three axis, of which two are independent variables (the horizontal axes) and one is a dependent variable (the vertical axis). The measured data were plotted in the space using the points and the grid between them was calculated using the 'griddata' feature.
Feature griddata: ZI = griddata (x,y,z,XI,YI) fits a surface of the form z = f(x,y) to the data in the (usually) nonuniformly spaced vectors (x,y,z).
Griddata interpolates this surface at the points specified by (XI,YI) to produce ZI. The surface always passes through the data points. XI and YI usually form a uniform grid (as produced by meshgrid). XI can be a row vector, in which case it specifies a matrix with constant columns. Similarly, YI can be a column vector, and it specifies a matrix with constant rows.
[XI,YI,ZI] = griddata(x,y,z,xi,yi) returns the interpolated matrix ZI as above, and also returns the matrices XI and YI formed from row vector xi and column vector yi. These latter are the same as the matrices returned by meshgrid.

[...] = griddata(...,method) uses the specified interpolation method:

'linear'	Triangle-based linear interpolation (default)
'cubic'	Triangle-based cubic interpolation
'nearest'	Nearest neighbor interpolation
'v4'	MATLAB 4 griddata method

The method defines the type of surface fit to the data. The 'cubic' and 'v4' methods produce smooth surfaces while 'linear' and 'nearest' have discontinuities in the first and zero'th derivatives, respectively. All the methods except 'v4' are based on a Delaunay triangulation of the data.

Heat amount

$Q = m.c.(t_e - t_i)$ [J] (1)

m – weight of substance [kg]
c – specific heat capacity [$Jkg^{-1}K^{-1}$]
t_e - final temperature [°C]
t_i – initial temperature [°C]

Heat exchange coefficient

$$K = A\frac{\frac{Q}{(t_{Ai}-t_{Be})-(t_{Ae}-t_{Bi})}}{ln\frac{(t_{Ai}-t_{Be})}{(t_{Ae}-t_{Bi})}} \quad [W.m^{-2}.K^{-1}] \quad (2)$$

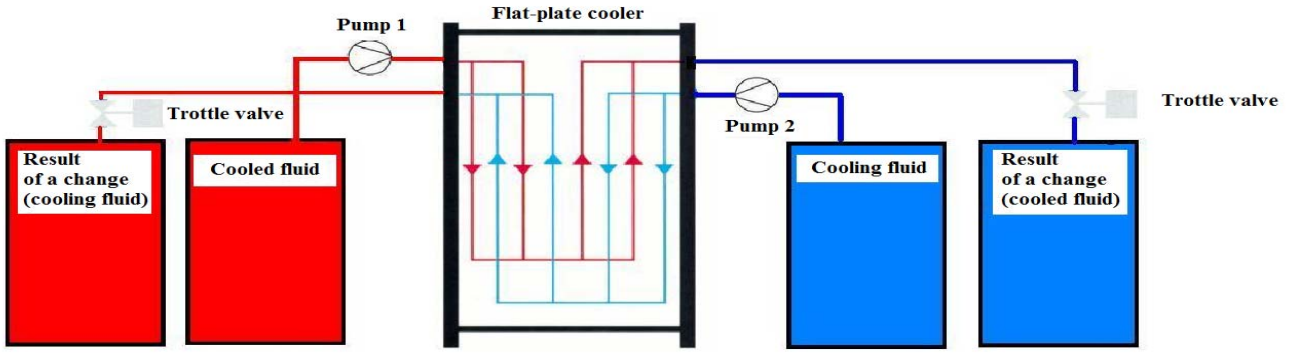
Q – heat amount [J]
A - area [m^2]
t_{Ai} – input temperature of cooled fluid [K]
t_{Ae} – output temperature of cooled fluid [K]
t_{Be} - input temperature of cooling fluid [K]
t_{Bi} - output temperature of cooling fluid [K]

Power plate cooler

$P = \frac{Q}{t}$ [W] (3)

Q – heat amount [J]
t - time [s^{-1}]

Fig. 1 Diagram of laboratory measurements, Brno, CR, 2014



Results and discussion

Table 1 Change of cooling water parameters on passing through the cooler (cooled water throttling regime)

T_{Bi} [°C]	T_{Be} [°C]	m [kg]	Q [J]	P [W]	i [J.kg^{-1}]	K [W.m^{-2}.K^{-1}]
13.0	17.4	22.88	420884	14029	18392	969
13.0	23.6	23.15	1032664	34422	44600	2805
13.0	28.9	23.95	1598906	53296	66754	4265
13.0	30.3	24.15	1746498	58216	72314	4584
13.0	30.2	26.65	1923580	64119	72188	4818

Table 2 Change of cooled water parameters on passing through the cooler (cooled water throttling regime)

T_{Ai} [°C]	T_{Ae} [°C]	m [kg]	Q [J]	P [W]	i [J.kg^{-1}]	K [W.m^{-2}.K^{-1}]
35.0	14.7	4.59	388204	12940	84561	894
35.0	15.5	11.51	937927	31264	81510	2547
35.0	18.8	23.98	1616941	53898	67423	4313
35.0	20.6	31.07	1866131	62204	60066	4898
35.0	21.2	35.03	2010409	67013	57391	5036

Table 3 Change of cooled water parameters on passing through the cooler (cooling water throttling regime)

T_{Ai} [°C]	T_{Ae} [°C]	m [kg]	Q [J]	P [W]	i [J.kg^{-1}]	K [W.m^{-2}.K^{-1}]
35.0	30.0	24.75	513826	17127	20760	1402
35.0	26.7	24.68	856247	28541	34694	2332
35.0	20.1	24.98	1548843	51628	62003	3962
35.0	18.4	24.88	1719440	57314	69109	4411
35.0	17.7	27.28	1968924	65630	72174	4964

Table 4 Change of cooling water parameters on passing through the cooler (cooling water throttling regime)

T_{Bi} [°C]	T_{Be} [°C]	m [kg]	Q [J]	P [W]	i [J.kg^{-1}]	K [W.m^{-2}.K^{-1}]
13.0	33.9	5.86	511941	17064	87362	1397
13.0	33.2	9.85	833067	27768	84575	2269
13.0	29.6	21.52	1499226	49974	69666	3835
13.0	28.0	27.67	1738764	57958	62839	4461
13.0	26.8	35.23	2037116	67903	57823	5136

Fig. 2 Graph showing the dependence of the cooled water heat content on changes cooling water flow, Brno, CR, 2014

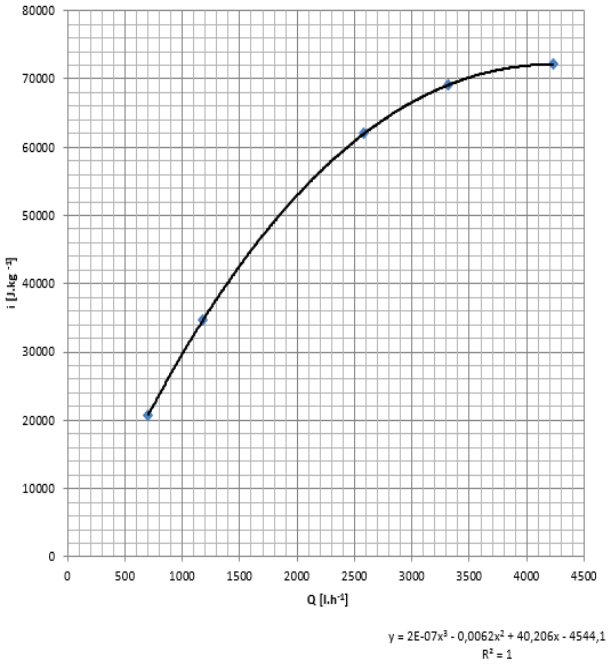


Fig. 3 Graph showing the dependence of cooled water temperature reduction on cooling water flow, Brno, CR, 2014Fig. 2 shows the dependence of the cooled water heat content change on the flow-rate of cooling water – over the flow-rate 3000 $l.h^{-1}$ the rate of increasing is lower. Fig. 3 clearly indicates that the cooled water temperature reduction is minimal at flow rates above 3,000 $l.h^{-1}$. Therefore, the value of 3,000 $l.h^{-1}$ of cooling water was set as optimal.

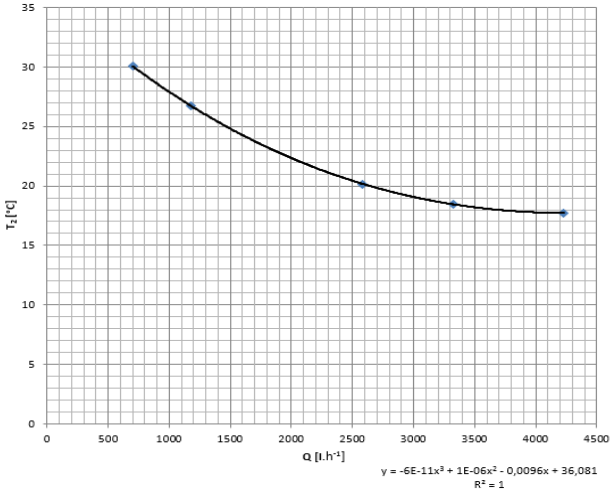
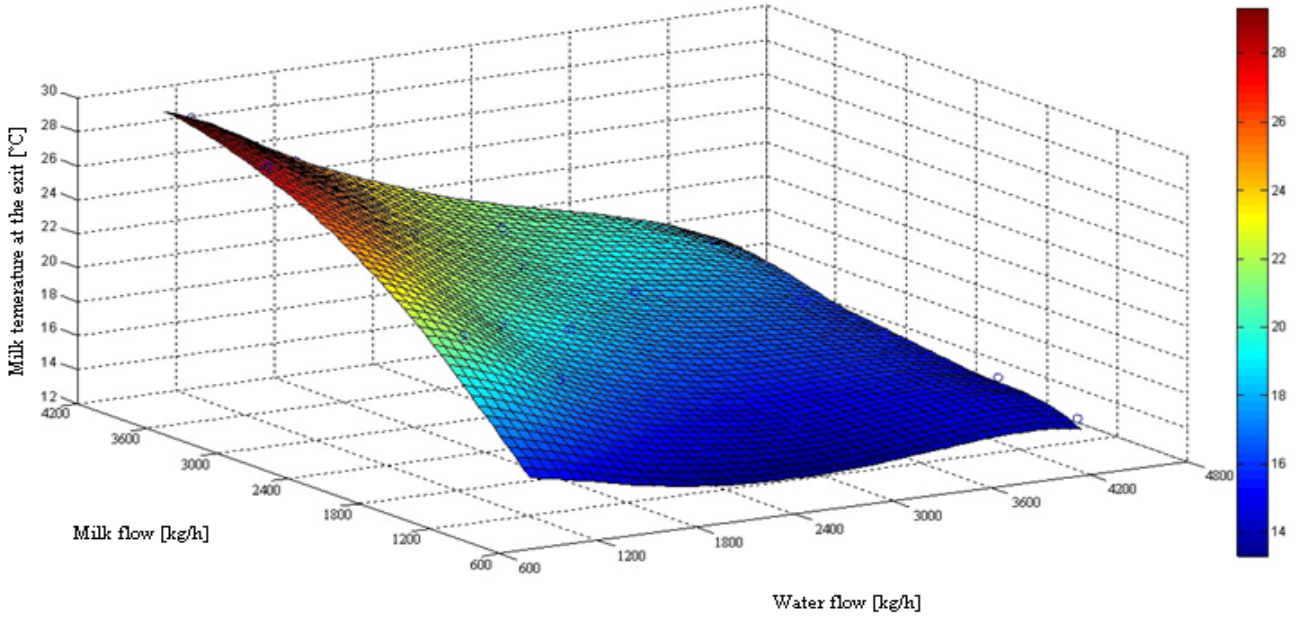


Fig. 4 shows the outlet temperature of milk depending on the flow rate of cooling water (13 °C) and flow rate of the inlet milk (35 °C). Comparing the resulting values with parameters provided by the manufacturer, we can conclude that the values were achieved during the measurements. For the type of the used flat-plate cooler (Type 42) the manufacturer claims milk temperature of milk on the output by 2 – 4°C higher than the temperature of cooling water on the input at a flow-rate of 4,000 litres of milk per hour. In our case, this temperature difference ranged near the upper limit provided by the manufacturer. [9] Fig. 4 makes it apparent that the best level of milk cooling occurs when the flow rate of cooling water is 3,000 litres per hour, while that of milk reaches 1,200 litres per hour. This best ratio can be achieved using a frequency converter that regulates the speed of the milk pump and that of the water pump in order to achieve optimal flow rates.

Fig. 4 Determining optimum flow rates, Brno, CR, 2014



Conclusion

The measured values demonstrate that the volume of exchanged heat per weight unit increases with the decreasing flow-rate. The measured values show that with the increasing flow-rate on the throttled side the flow-rate increases on the side without the throttle valve. This phenomenon is caused by pressure increase during throttling and by consequent increase of the diameter of channels in the cooler that reduce the diameter of adjacent channels with non-throttled liquid. With the decreasing pressure, there is a pressure decrease on the external walls of the opposite channels and the flow-rate increases. This property of the cooler could be utilised in practice when a pressure regulator on one side would regulate flow-rate on the other side and vice versa. We can deduce from the measured values that using flat-plate milk coolers in practice could bring a major cost-reduction of electricity needed for the cooling aggregate. Other presumed benefits include lower cost of the cooling system maintenance and repairs due to lower load, longer service life of the entire system, positive impacts on the quality of milk in terms of rate of its cooling down to the required temperature. It is also possible to consider using heated water for watering the milking cows (this is beneficial especially in winter) as well as for washing, floor hygiene, etc.

Acknowledgements

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Laboratory equipment for the measurement of cereals drying on Mendelu

VLASTIMIL NEJTEK, JOSEF LOS, JIRI FRYC
Department of Agricultural, Food and Environmental Engineering
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

vlastimil.nejtek@mendelu.cz

Abstract: The report focuses on the drying of cereals in connection with the laboratory equipment designed at Mendel University in Brno and used for drying samples of wheat and barley grain. The samples were placed in six cartridges thus forming a high layer of the dried material. Hot air was the drying medium and was blown through the high layer of grain by means of a fan. Samples in each of the cartridges were weighed and the grain temperature was sensed at periodical intervals. Drying of the material in the high layer at a constant flow direction of the drying medium was unevenly, with the highest rate of drying occurring at the bottom layer; subsequently, drying continued in other layers. The measured data were used for producing drying rate curves and the results were compared with the results of measurements achieved with a similar apparatus at a foreign university. The data make it apparent that the drying device is functioning and the measured data match the foreign references. A minor deviation occurred in the resulting values (a large increase in drying rate at the beginning of measurements) and was caused by using grain harvested after the rain. The subsequent heating in a drying facility resulted in a significant evaporation of the surface water between the grains. Subsequently, when measuring was done within the next time interval, the drying rate returned to expected values. The small possibility of regulating air flow is a certain drawback of the current unit. The high layer of the material creates too much resistance for the fan, the device subsequently ensuring maximum air velocity of 0.3 metres per second even at the highest speed. Enhancing the unit with a suction pump to suck the air might be some sort of way out in that this would turn the unit from a high-pressure unit into a vacuum device. Save the weakness described above, the apparatus allows creating suitable settings for scientific measurements and is capable of providing measurement in even more complex areas in the field of drying.

Key-Words: drying, cereals, quality, energy intensiveness

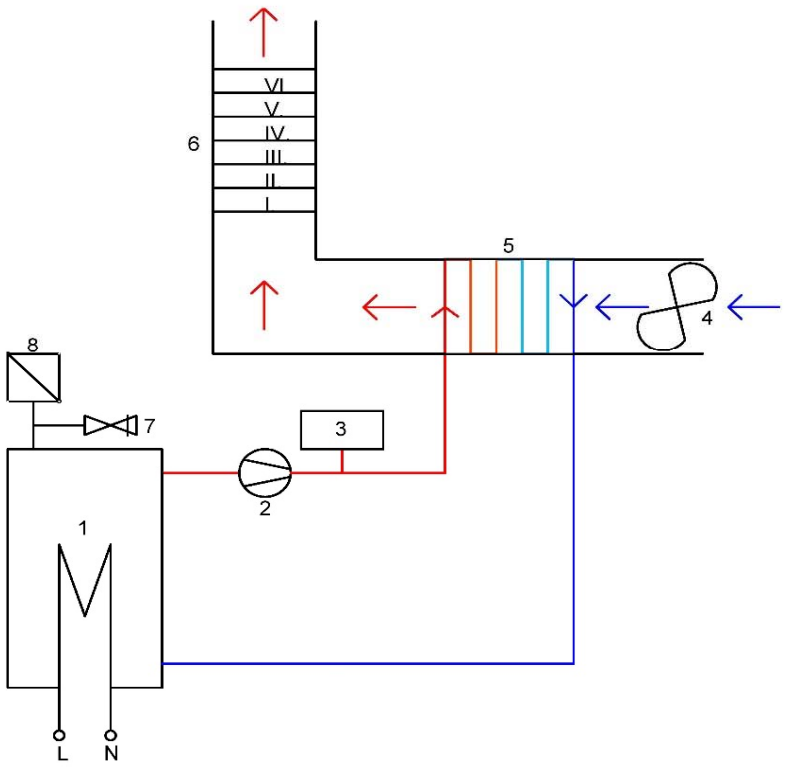
Introduction

Drying is a physical process, in which the water content of the dried product is reduced through the effect of heat, and any change in the chemical composition of the product is not desirable [1]. Since moisture (i.e. water) is removed by evaporation, drying involves a change in the water phase, from liquid to gas (vapour) [2]. This makes drying fundamentally different from other means of reducing moisture of products, which particularly involves mechanical methods such as centrifuging, pressing, etc. [3]. The gradual development of drying technology in each of the industry and farming sectors and related requirements as to drying plant parameters and quality is closely linked to deepening knowledge of the general theory of drying [4]. For this reason, we decided to construct a drying laboratory apparatus at Mendel University. Its function and the potential of using the measured parameters within rather complex experiments in the field were validated by measuring basic parameters in drying cereals, particularly wheat and barley. In future measurements, the apparatus may be possible to use e.g. for measuring materials with drying pauses (phase drying), drying with cyclic changes in the medium flow rate, for new representation of weight and thermal flows in drying, for constructing the modified i-x-w diagram of moist air, and designing draft specifications of and additions to technical standards. This makes the range of use of the lab system widely broad.

Material and methods

Fig. 1 shows the drying laboratory equipment. It consists of a primary heat exchanger (1) in which water is heated via a heating element. Water circulation is provided by a centrifugal pump (2). Proper functioning is ensured by an expansion vessel (3).

Fig. 1 Drying lab apparatus, Brno, CR, 2014



Heated in the primary heat exchanger to 90 °C, the water is transported to the secondary heat exchanger (5) using the pump. The fan (4) sucks the ambient air that flows through the secondary heat exchanger, while the heat from the water passes through the heat-exchange surface of the secondary heat exchanger into the air (60 °C), which then flows upward with the velocity being 0.3 metres per second to pass through the cartridges filled with samples designed for drying (6). After passing through the system of cartridges, the air is discharged back into the environment; its temperature is lower than that at the input and its relative humidity is higher. This is the mode in which the apparatus was working. The unit includes an air-relief valve (7) and a safety valve (8). Exact doses of dried samples were put into each of the cartridges, wheat first, followed by barley. After the start of the measurements, each of the cartridges was removed and weighed, this followed by measuring the temperature of the grains which was firstly done every 30 minutes and subsequently every 60 minutes. The relative humidity of the input material was determined using three cups filled with the material measured by weighing and subsequently dried in an oven at 105 °C. The temperature and relative humidity of the hot air were measured behind the cooler straight prior the inlet into the cartridges.

Measurement apparatus

-Thermometer (to measure grain temperature) - VOLTCRAFT DUAL-LASER LR-SCAN-350 RH, temperature range: -50 to 350°C
- Thermometer/hygrometer (measuring the temperature and relative humidity of hot air) - COMMETER D 3121 Temperature range: -30 to 105 °C, resolution of 0.1 °C, accuracy of 0.4°C. Relative humidity: range 0-100%, resolution 0.1%, accuracy 2.5%.
-Scale RADWAG WAS 220/C/2, range 10-220 g, resolution 1 mg, accuracy 0.1 mg
-Weight JADEVER LPW-1260, range up to 6,000 g, resolution 0.5 g.
Used formulas
Relative humidity

$$w = \frac{Mv}{Mm} = \frac{Mm - Mms}{Mm} \cdot 100 \quad [\%]$$

Mv – water weight [kg]
Mm – the weight of the moist material [kg]
Mms – the weight of dry matter [kg]

Results and discussion

Drying of the material in the high layer at a constant flow direction of the drying medium was unevenly, with the highest rate of drying occurring at the bottom layer; subsequently, drying continued in other layers. This is seen in a way that the area of the dried material gradually increases in the direction of airflow. Drying curves (decrease in relative humidity over time) of barley and wheat are shown in Figs. 2 and 3 (the top section). Sample numbering is from I to VI depending upon the place the cartridge with samples was located (see Fig. 1). As the air passes through the lower, moist layers, its relative humidity increases until the equilibrium relative humidity of the initial moisture content of the wet material. Thus, drying occurs in the higher layers (II-VI) with a gradually increasing time delay, which reaches the maximum for the uppermost layer, hence the uneven final moisture of the dried material in continuous drying when it is over dried in the bottom layer, while drying is not complete in the top layer. For these reasons, drying should take place with low layers of the material (1 metre is the maximum). This adverse effect can be eliminated by cyclic changes in the flow direction of the drying medium.

The drying speed of barley and wheat is obvious from Figs. 1 and 2 at the bottom. The first phase reveals a clear increase in the drying rate (0-0.5 h); it is the initial interval in which the dried material adapts to the new conditions of thermal and material equilibrium during drying. Extreme increase in time (0.5-1 h) is affected by the use of grain harvested after the rain. It is obvious that in the areas between the grains there remained surface water, which quickly evaporated at the beginning of the experiment due to the hot air. The following section (1.5-2.5h) is known as one of constant speed, in which prevailing is the evaporation of surface water, moisture loss is directly proportional to time. This section ends with a critical point (2.5 hours). The last part of the graphs shows a decreasing rate of drying. The loss of moisture is no longer proportional to the time; there is the evaporation of inner moisture inside the material. This section ends with an equilibrium point - the material reaches equilibrium with the drying medium.

Making a comparison of the measured data with the results of measurements at the Slovak Agricultural University in Nitra can lead to a conclusion that we managed to reach a consensus [4], [5], [6]. The difference can be seen only in the drying rate curve that reaches large extremes; the reason why this is so is described in the previous paragraph.

Fig. 2 Drying barley, Brno, CR, 2014

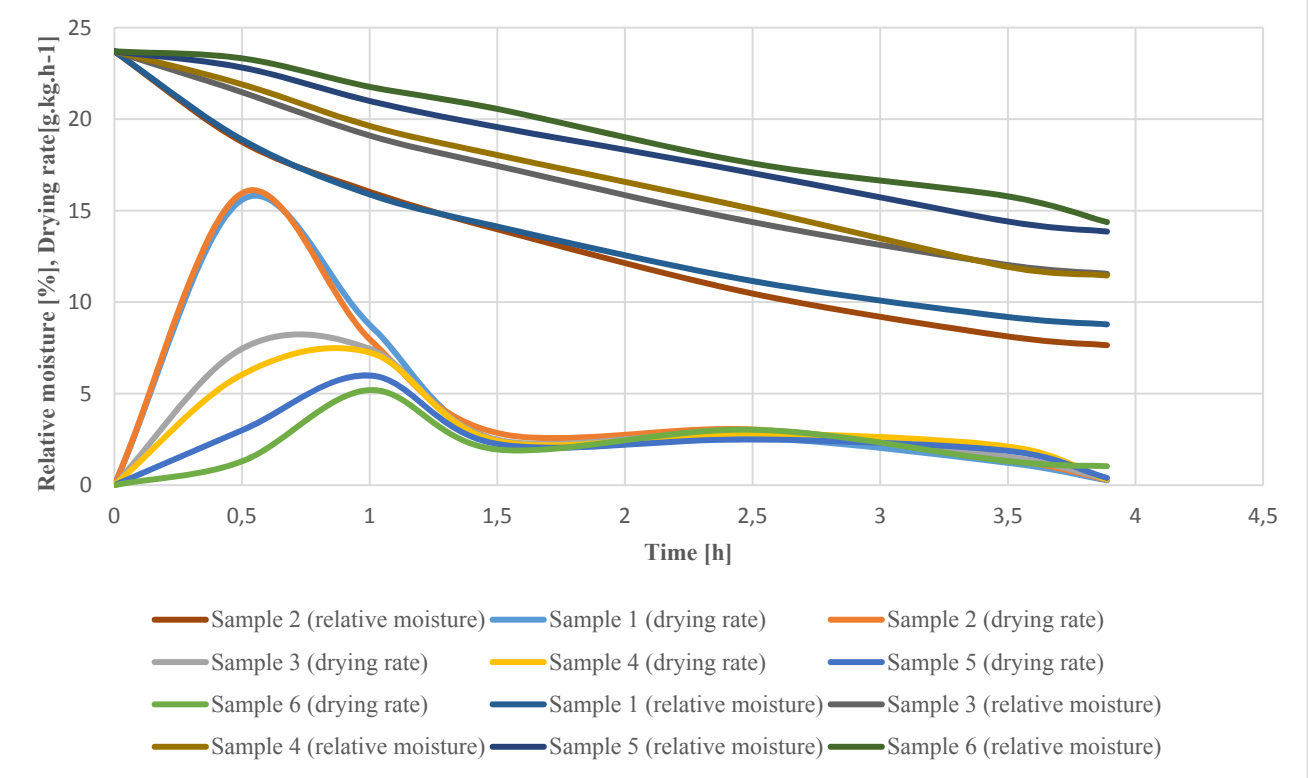
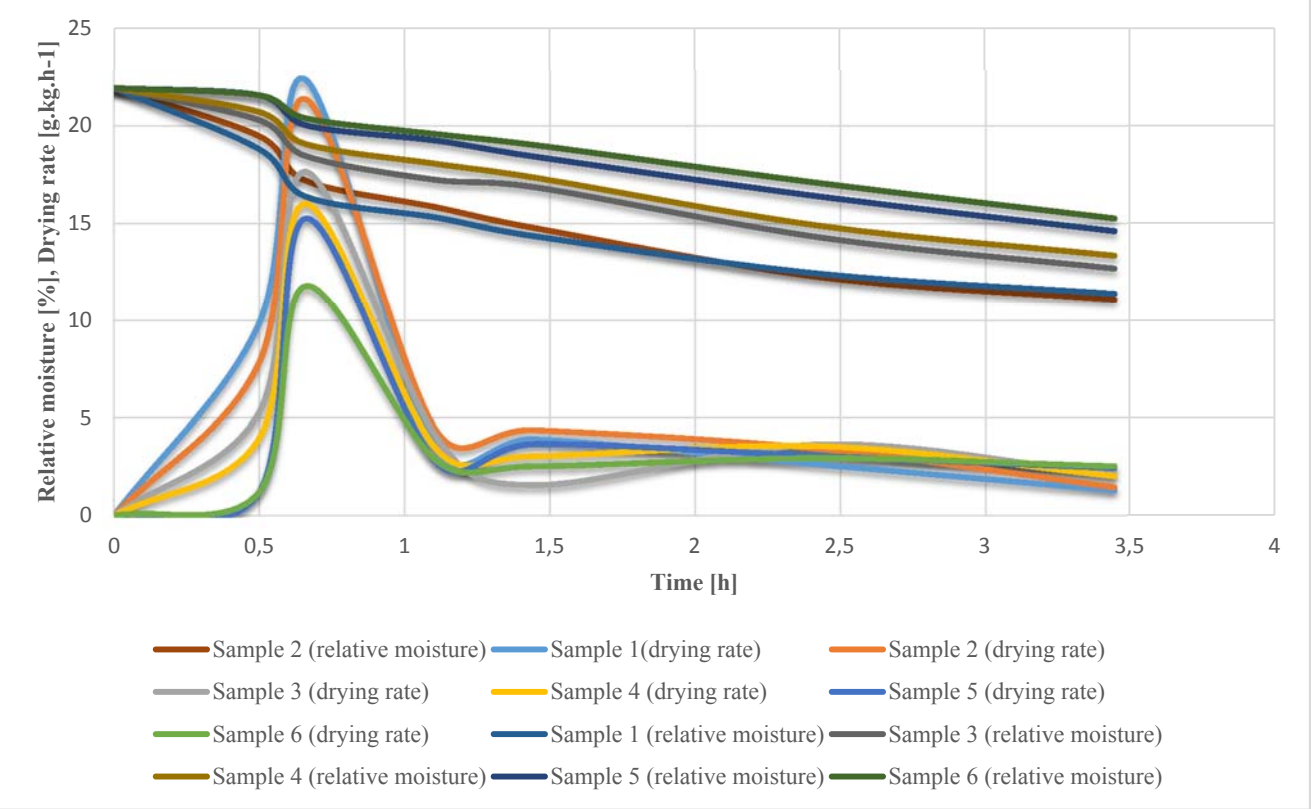


Fig. 3 Drying wheat, Brno, CR, 2014



Conclusion

The measurements showed that the laboratory apparatus constructed at Mendel University is capable of creating the proper conditions for measuring a cereal drying process; certain improvements of existing equipment are possible. The measurements showed the need to increase air flow rate. This can be controlled by a fan with adjustable speed. Due to the large layer of material (large flow resistance), this regulation is little effective and air velocity can be changed only slightly. The solution is to connect the suction pump via the piping and remove the fan. The air then would not be injected into the cartridge, but sucked with a vacuum pump and the system would turn from a high-pressure type into a vacuum type [7]. Once these adjustments are implemented, the device makes it possible to solve even rather challenging scientific problems of drying.

Acknowledgement

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The energy performance of the drying process according to maize harvest time

VLASTIMIL NEJTEK[1], JOSEF LOS[1], JIRI FRYC[1], IVAN VITAZEK[2]
[1]Department of Agricultural, Food and Environmental Engineering
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Department of Transport and Handling
Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2949 76 Nitra
SLOVAKIA

vlastimil.nejtek@mendelu.cz

Abstract: This paper deals with the topic of drying maize grain and potential energy savings. The measurements were carried out at LIPONOVA, a. s. (the farm established in Lipoltice in the Pardubice region) in the period from 6th October to 31st December 2012, with a total sown area of 655.7 hectares of agricultural land. The average yield was 12.832 kg per hectare with the moisture content of 29.86%. Drying was under way in two phases: the grain was first pre-dried to reduce moisture from the average of 29.9% to 19.6%. Then there was final drying to reach storage moisture of 13.7%. The dried samples of maize were subjected to the measurement of (1) relative grain moisture using a hygrometer and the measurement of (2) temperature using a digital thermometer with a measurement probe. A review testing was done for several samples in a laboratory at Mendel University in Brno. The values of relative humidity and temperature of ambient air were also recorded. In addition, notes were taken of grain-dryer parameter settings, i.e. drying medium temperature and dryer emptying delay. The data acquired make it apparent that ambient air temperature is a major factor for the energy demand of drying; the higher the temperature, the less energy is needed. Other important parameters include the target temperature of the heated air in the dryer and dryer outlet air humidity. It is possible to achieve the energy savings in the drying process if the parameters above are set properly and their level is considerable with regard to the quantity of the dried material.

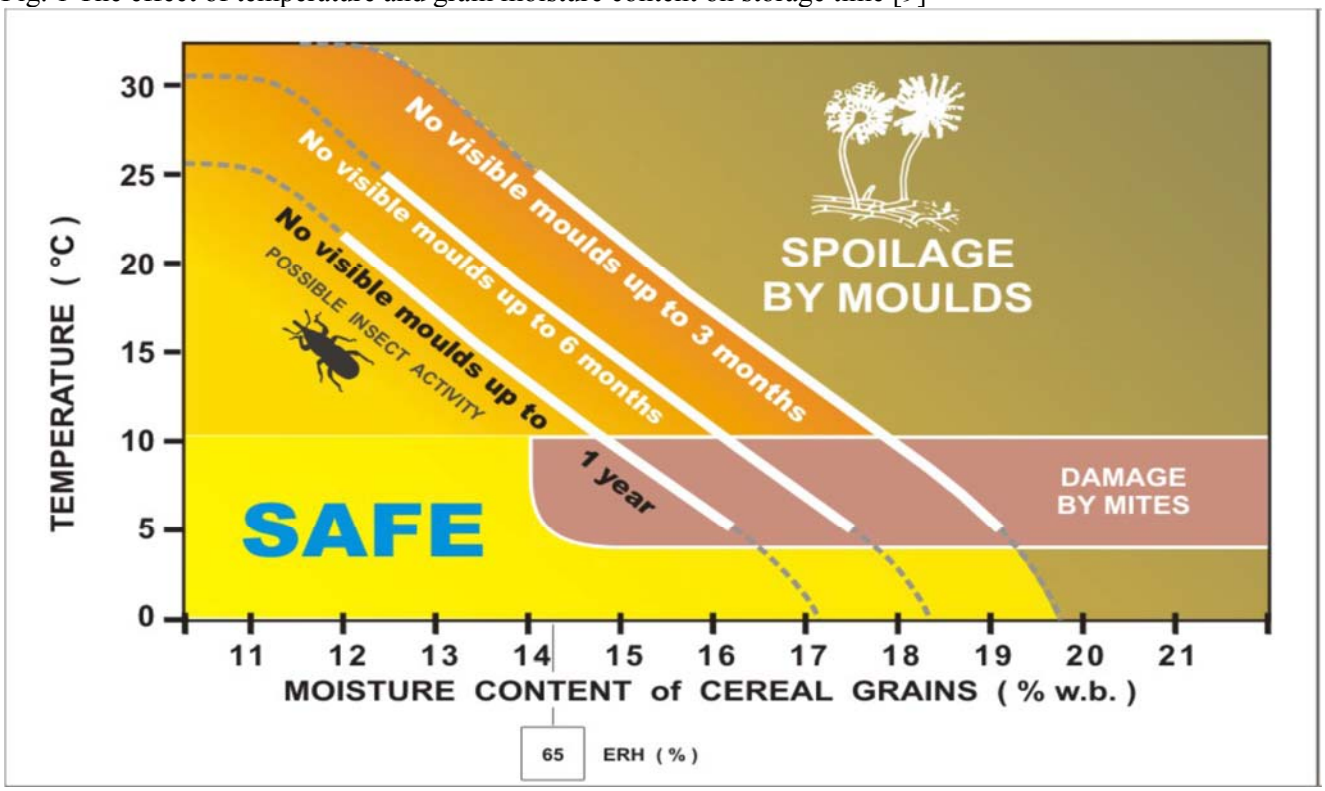
Key-Words: drying, maize, quality, energy intensiveness

Introduction

Grain crops possess the important position in the national economy as they are grown on more than half of the total area of arable land and cover about 40 % of calories intake in the population's diet [1]. Grain crops include maize. Since maize is a seasonal product, it must be stored once harvested until it is used for processing into final products or directly consumed. After harvesting, maize is a living matter and its life processes must not be restricted by the crop being treated, stored and managed improperly to ensure the required shelf life with minimised storage losses. When storing pre-conditioned maize, i.e. with removed excess water, impurities and pests, the storing requirements are not extensive; protection from additional moisture, pest infestation and undesirable micro flora are sufficient. [2] The most important variables influencing the storage period can be seen in (Fig. 1) and involve temperature and grain moisture content. Drying is the very action to reduce the water content of maize to the value at which storing the crop is possible over the long-term. It is a physical process, in which the water content of the dried product is reduced through the effect of heat, and any change in the chemical composition of the product is desirable. Since moisture (i.e. water) is removed by evaporation, drying involves a change in the water phase, from liquid to gas (vapour) [3]. This makes the drying fundamentally different from other means of reducing moisture of products, which particularly involves mechanical methods such as centrifuging, pressing, etc. With the grain shape and higher relative moisture (over 30 %), maize drying is much more complex than with other grain crops. If grain is heated to a high temperature and then suddenly cooled, it exhibits a higher susceptibility to mechanical damage [4, 5]. In the Czech Republic,

the issue of drying maize grain is particularly up to date because of new hybrids, as well as new maize drying plants [6, 7]. New post-harvest lines include that in Lipoltice, the Pardubice region, which became the site for measuring energy requirements of drying and evaluating installation's operating parameters.

Fig. 1 The effect of temperature and grain moisture content on storage time [9]



Material and methods

The measurements were carried out at LIPONOVA, a. s. - the farm established in Lipoltice in the Pardubice region. Grain maize was harvested from 6th October to 31st December 2012. The post-harvest line consists of a system of conveyors (screw and bucket conveyors), the grain cleaner Schmidt-Seeger TAS 154A-4, the mobile drying plant Schmidt-Seeger EcoDryFlex18, natural gas powered, and three grain storage tanks. Maize was first pre-dried to reduce moisture from the average of 29.9% to 19.6%. Then there was final drying to reach storage moisture of 13.7%. During the drying period, hourly consumption of electricity and the quantity of natural gas were being deducted using a sub-meter and gas flow meter, respectively. The dried samples of maize were subject to the measurement of (1) relative grain moisture using the hygrometer Pfeuffer HE 50 and (2) temperature using a digital thermometer with a measurement probe. A review testing was done for several samples in a laboratory at Mendel University in Brno. Values were also recorded of relative humidity and temperature of ambient air. In addition, notes were taken of grain-dryer parameter settings, i.e. drying medium temperature and dryer emptying delay. The plot areas, yields and computed values are shown on Tab. 1.

Establishing the average relative humidity of all harvested grain (TARH) by using the method of weighted average.

$$TARH = \frac{\sum TYwg_i \cdot RHh_i}{\sum TYwg_i}, [\%] \quad (1)$$

Where:

TYwg – Total yield per field of wet grain [kg]

RHh – Relative humidity of grain at field at harvest time. [%]

Average grain yields per hectare (TAGYH) were calculated from sum of wet (at harvest RH) grain or dry (storage RH) divided by total sown area A.

$$TAGYHwg = \frac{\sum TYwg_i}{\sum A_i},$$

$$TAGYHdg = \frac{\sum TYdg_i}{\sum A_i}. [kg.ha^{-1}] \quad (2)$$

Calculation of total dry matter, relative humidity of grain (RH), total water content at harvest and during storage describe for example Kováč (2012).

$$RHg = \frac{Mg - MDMg}{Mg} \cdot 100 = \frac{Mw}{Mg} \cdot 100 \text{ , [\%]} \quad (3)$$

where:
Mg – Total mass of grain at given humidity [kg]
MDMg – Mass of dry matter of the grain [kg]
Mw – Amount of water in grain at given RH.
Non ordinary used symbol is t%, which mean amount of percent of water, have to be remove from 1 ton of grain. For example drying 100 ton of grain from RH 30% to 14% is (30–14)* 100 = 1600 t%. It determines the fee for drying during buying or selling of grain.
If the RH of grain is known, the mass of dry matter (MDM) of the grain can be calculated by

$$MDMg = \frac{Mg \cdot (100 - RHh)}{100} \text{ .[kg]} \quad (4)$$

If we need calculate mass of water at corn at known RH, we use this equation (Ružbarský *et al*., 2004)

$$Mw = MDMg \cdot \frac{RH}{(100 - RH)} \text{ . [kg]} \quad (5)$$

Hourly energy consumption of dryer was calculated from volumes of burned natural gas and electricity energy.

$$q = \frac{VNG \cdot HNG \cdot 3600}{Mwrem} \text{ [kJ.kg}^{-1}\text{]and}$$

$$q_{el} = \frac{ELE \cdot 3600}{Mwrem} \text{ , [kJ.kg}^{-1}\text{]} \quad (6)$$

where:
VNG – Volume of burned natural gas [m^3]
HNG – Energy content of natural gas 10.55 kWh.m^{-3}
3600 – is constant for convert kWh to kJ
Mwrem – mass of water removed during measurement [kg].

Table 1 Plot areas and yields

Plot	Area [ha]	Variety	Total yield per field of wet grain [kg]	Wet grain yield per hectare [kg.ha^{-1}]	Harvest moisture Content [%]	t% actually dried	Total maize DM [kg]	Amount of water in grain at 14% [kg]	Dry maize stored at 14 % [kg]	Dry (RH 14 %) grain yield per hectare [kg.ha^{-1}]
	A		TYwg	GYHw	RHh	t%d	TYDM	GWCs	TYdg	GYHd
Za Peckova	46	MERIDIEN	582930	12770	31	8123	405136	65952	471089	10320
Zlá paměť	30	MERIDIEN	430510	14495	30	5784	301357	49058	350415	11798
V oklíku	18	MERIDIEN	129440	7272	30	1739	90608	14750	105358	5919
U letiště	35	BEATUS	309860	8778	29	3853	220001	35814	255815	7247
Černá skála	7	ATLETIKO	81920	12412	31	1182	56525	9202	65727	9959
Dolce	9	ATLETIKO	132000	15529	31	1905	91080	14827	105907	12460
Za Petrusem	25	ATLETIKO	319770	12740	31	4616	220641	35918	256560	10222
Před cyklosem	12	KAIFUS	190760	15384	30	2563	133532	21738	155270	12522
U hájovny	25	KAIFUS	433380	17546	30	5822	303366	49385	352751	14281
Mokř	15	KAIFUS	212400	14351	30	2854	148680	24204	172884	11681
Smejtenec	19	MERIDIEN	309340	16196	30	4156	216538	35250	251788	13183
Kadavovo	7	KAIFUS	117420	16538	30	1577	82194	13380	95574	13461
Kouty	4	KAIFUS	67140	16376	30	902	46998	7651	54649	13329
Za drůbežárnou	9	KAIFUS	141080	16216	30	1895	98756	16077	114833	13199
Za Nalezinkem	19	KAIFUS	263060	13845	30	3534	184142	29977	214119	11269
Za kůlnou	4	SYMBOL	52600	13150	32	812	35768	5823	41591	10398
Za Slavíkova	11	KAIFUS	140160	12627	27	1463	102317	16656	118973	10718
Ohrada	34	KAIFUS	407410	12161	30	5473	285187	46426	331613	9899
Na horách	31	KAIFUS	478120	15676	24	3555	363371	59153	422525	13853
Jedousov	3	BEATUS	33780	11260	30	454	23646	3849	27495	9165
Padesátka	34	DELITOP	363960	10705	22	1978	283889	46214	330103	9709
Křemena	11	BEATUS	98560	8895	28	1127	70963	11552	82515	7447
U Starkoče I	11	MERIDIEN	144400	13115	30	1940	101080	16455	117535	10675
Brložská	29	BEATUS	574540	19609	27	5995	419414	68277	487691	16645
U Lovčic	41	MERIDIEN	492930	12052	30	6622	345051	56171	401222	9810
Výborná	49	BEATUS	725010	14766	28	8290	522007	84978	606985	12362
Na černé	40	DELITOP	586020	14614	26	5529	433655	70595	504250	12575
Pod Borkem	17	DELITOP	183880	10816	22	999	143426	23348	166775	9810
Ryntířovo	21	MERIDIEN	238650	11641	30	3206	167055	27195	194250	9476
U hráze	41	MERID/BEAT	544020	13400	30	7309	380814	61993	442807	10907
U májovky	2	mix	17180	11453	30	231	12026	1958	13984	9322
Celkem	**656**		**8802230**	**12832**		**105489**	**6289224**	**1023827**	**7313051**	**11152**

Results and discussion

A total sown area was 655.7 hectares of agricultural land (Tab. 1). The yield of maize ranged from 7.274 to 16.645 kg per ha; the variance was generally caused by the different time of harvest and over-reproduced wild boars. Grain losses on late-harvested plots in combination with losses from lodging of whole plants are estimated at 20% by the farm management. The average yield was 12.832 kg per ha with the moisture content of 29.86%. Stored after drying was a total of 7.313 tonnes of grain with a moisture content of 14%. which is an average yield of 11.152 kg per ha. The drying was under way in two phases. with ambient air being the drying medium. heated in a heat exchanger through burning natural gas.

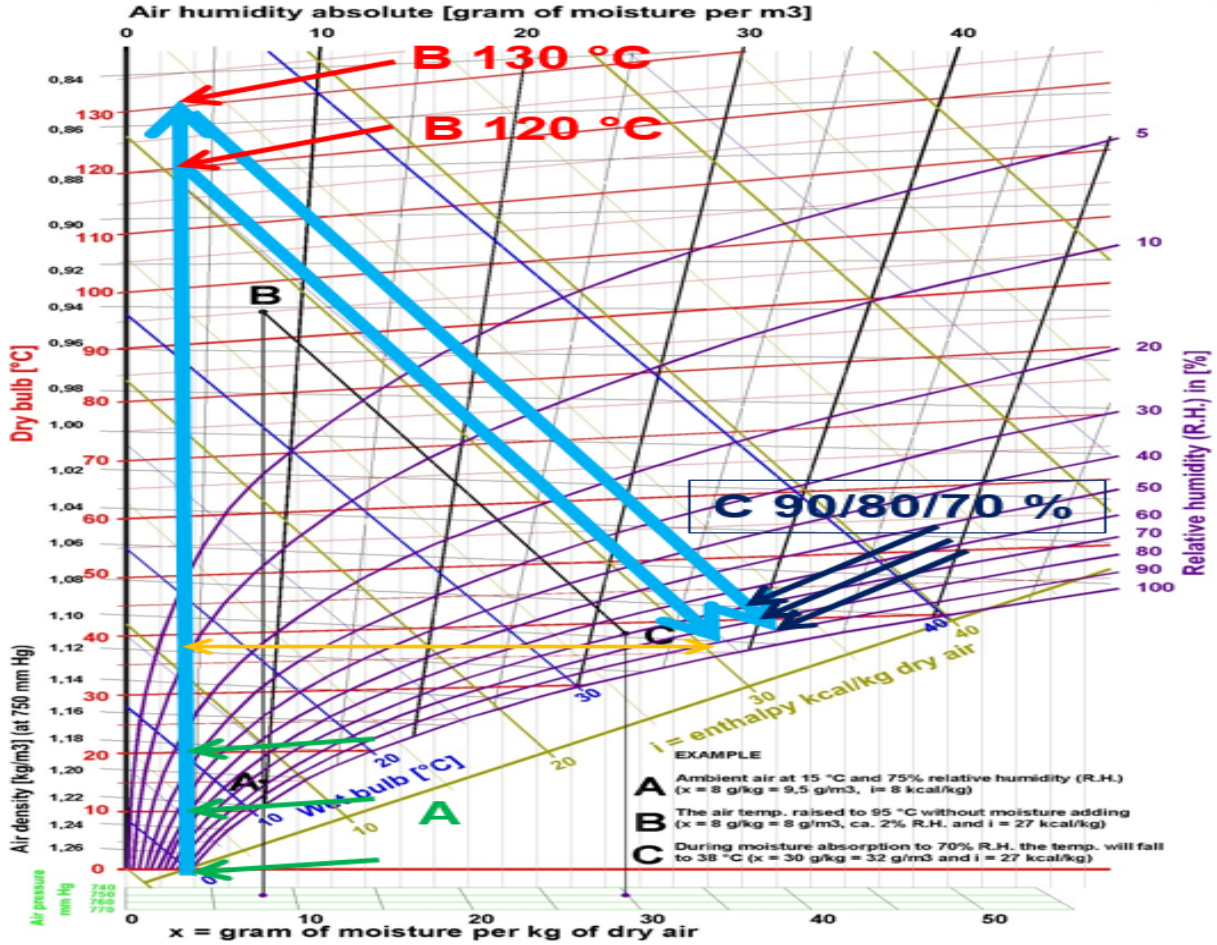
Measurements within drying phase 1 took place on 22nd and 23rd November 2012 with the outdoor air temperature ranging from 6 to 12°C. the temperature of the drying medium being 120°C and the dryer performance amounting to 4.850 kg per hour. The average grain moisture reduced from 31.6% to 19.6%. In the afternoon. the achieved average specific heat consumption was 3.603 kJ per kg. while at night with the outdoor temperature dropping to 6°C it was 4.049 kJ per kg.

For drying phase 2 measurements were carried out on 14th January 2013 at outdoor air temperature of -2°C. The temperature of the drying media: 90°C. the dryer facility performance: 8.023 kg per hour. The average grain moisture decreased from 16.7% to 13.7%. Specific heat consumption: 5.380 kJ per kg.

The measured and computed values of specific heat consumption fall within the range commonly reported by other manufacturers of dryer plants. [6]

As can be noticed. grain moisture decreased by 2.8% between the phases. which is due to forced ventilation and thermal inertia of the grain. This phenomenon brings savings in the energy required for drying and is a common feature of two-stage drying technologies. In terms of average consumption per 1 t%, the quantity of natural gas needed was 1.275 cubic metres for phase 1 and 1.563 cubic metres for phase 2.

Fig. 2 The drying process in the form of Mollier diagram, Brno, CR, 2014



Conclusion

It results from the data acquired by measurements that ambient air temperature is a considerable factor for the energy demand of drying [8]. The energy demand of the heating of the drying medium is determined by the selected temperature of the same at the inlet into the drying compartment (point B: 120/130°C) and by the temperature of ambient air (point A: 0 to 20°C), more specifically, by their different enthalpy. To improve the drying economy, choosing a higher temperature of the drying medium is preferable. As it can be seen in Fig. 2, when the temperature reaches 130°C instead of 120°C, the air can hold more water (point C), which is specified through the yellow line segment (the difference between the levels of specific humidity), which is extending while the saturation of the moist air exiting the dryer does not have such an effect. As can be seen in Fig. 2, point C, the yellow line segment shortens only slightly at the humidity of 90%, 8% and 70%. The combined two-stage drying method applied in Lipoltice and the measurements also showed some risks. High grain moisture (over 30%) causes problems with clogging of transportation routes and vault effects in silos, which was manifest in the measurements as well. There was also an incorrect (low) final cooling of grain at the dryer outlet when the grain temperature at the dryer outlet was 33°C during the first measurement, and 42°C during final drying, which definitely does not comply with technological requirements (max. 5°C difference compared with the ambient temperature). As a result, there was locally a rise in grain temperature inside the storage bin (to as much as 50°C), which was resolved by aeration fans being operated on a continuous manner and the grain being transferred between the silos, which however leads to greater mechanical damage to the grain. When combined with relative grain moisture around 20%, such a level of high temperature also forms an ideal setting for mould to develop and storage pests to propagate.

Acknowledgement

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Mathematical foundations of the optimalisation of the collecting munincipal waste route

MICHAL PETRIK, STANISLAV BARTON
Department of Technology and Automobile Transport
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

michal.petrik@mendelu.cz

Abstract: The aim of this paper is to present way how can be the optimalisation of the waste route handled by using of the program Maple13. This is the first time issue has been handled by using of the program and its universality enables to follow other calculations.
Waste is produced everyday all over the world. Therefore, it is necessary to collect waste to its end technologies and take actions needed for its treatment and disposal. Waste is collected on daily bases. Any change of the collection system (eg. separation directly by the early producer) or gaining new customer (eg. new village) requires a change within collection logistic.
Optimalisation is reasonable in case of current logistics systems, which can bring considerable financial savings and reduce its environmental burden. Issue of the waste collection can be compared to the issues of the commercial salesman or Chinese postman. A number of mathematicians dealt with this subject but its solution has not been solved yet, neither proof of its unsolvability.
Program Maple is used for all computations and graph visualizations. Solution to the minimal matching problem in the Maple is based on the set theory. To reduce exponential complexity of the problem two premises are used. Additional edges are introduced only if they connect adjacent points. Non-adjacent points are connected if only one point with even adjacency lies between them. Points with the odd adjacency are in ascending order with respect to adjacency. Minimal matching begins with the point of the lowest adjacency. This assumption enables to optimise trajectory of the municipal waste pick up trailer in much greater villages as it is shown in the following article.
Source code of the Maple13 program which has been used for calculations is not mentioned in the article due to its size but will be presented at the conference web page.

Key-Words: logistics, waste disposal, travelling salesman, optimalisation, minimum matching, graph theory, adjacency matrix, Maple13

Introduction

Waste is produced everyday all over the world. It is necessary to collect waste to its end technologies and take actions needed for its treatment and disposal. Waste is collected on daily bases. Any change of the collection system (eg. separation directly by the early producer) or gaining new customer (eg. new village) requires a change within collection logistic. Optimalisation is reasonable in case of current logistics systems, which can bring considerable financial savings and reduce its environmental burden.

Issue of the waste collection can be compared to the issues of the commercial salesman or Chinese postman, see [2]. A number of mathematicians dealt with this subject (from the Czech environment e.g. Jaroslav Nešetřil, Václav Chvátal). Solution of this problem has not been solved yet. Also, nobody has found a proof of its unsolvability so far.

This is the first time issue has been handled by using of the Maple13 program. Its universality enables to follow other calculations.

Material and Methods

As an input data for this case study were used maps provided by server www.mapy.cz/ and GPS locations of points and waste containers. Chosen village has approx.1.300 inhabitants and by one collection is meant 8.000 kg of waste. Considering power severity of the collection vehicle, total distance (distance driven to collect all waste containers in the village) has been used for the optimalisation as a key value.

Methodology of the theory of graphs, including Eulerian line, Hamilton circle, and minimum pairing, was applied in our case. The necessary calculations were performed by using Maple13 program, see [1].

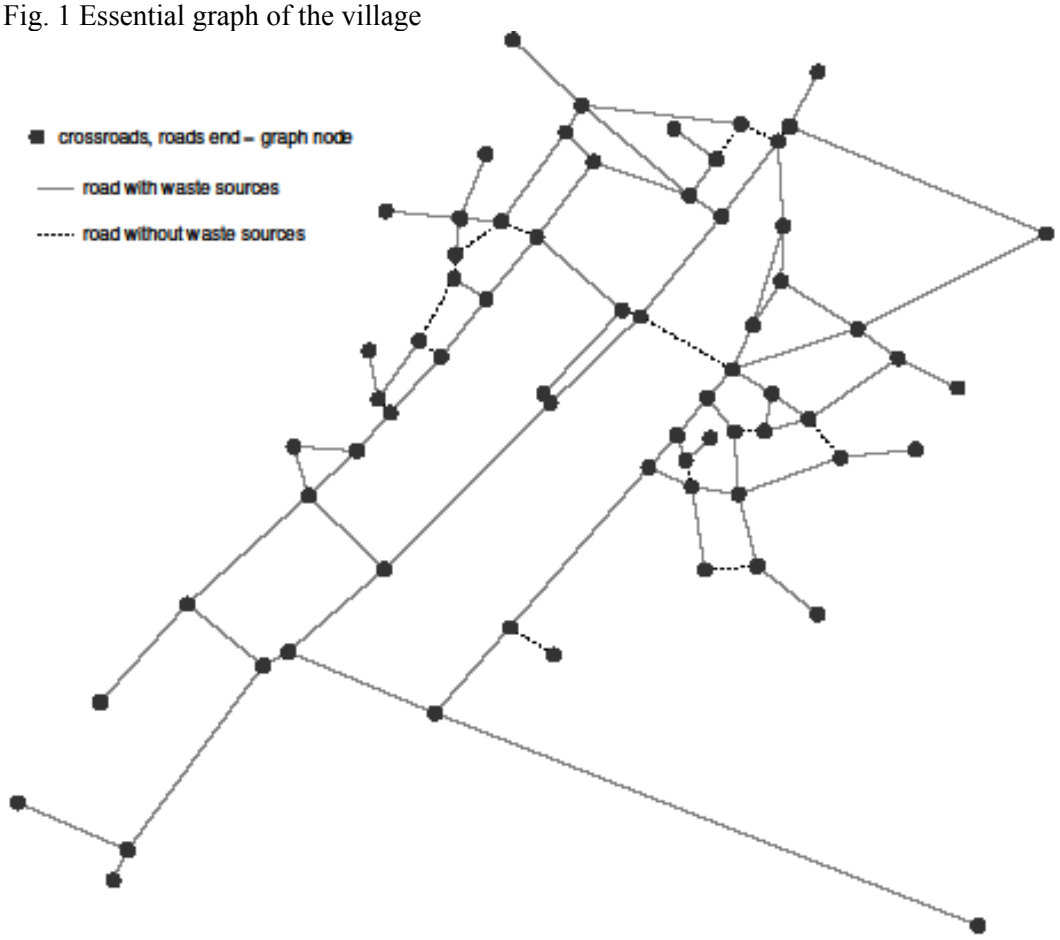
Results and Discussion

At the beginning it was necessary to convert map of the certain village into the graph - GPS location coordinates of the cross roads in the village were used as particular nodal points. Therefore GPS coordinates were converted by Reference Ellipsoid WGS84 (World Geodetic System 84) into the Cartesian Coordinate System.

To create the graph edges was needed to construct an adjacency matrix which contains distances between particular cross roads - graph nodes - connected by roads. For those cross roads which are not connected by roads is distance rated by value -1 (for easier handling). In our case study the adjacency matrix is symmetric along the main diagonal. Difference might appear in case of larger cities where are one-way communications.

Another difference may be a broader communication where the waste collectors are on its both sides therefore it is needed to drive the road twice (two ways along one edge). These steps created an undirected rated graph which serves as the basis for another solution. It was necessary to distinguish between communications with (Fig.1, solid line) and without (Fig.1, dotted line) waste containers in the first graph based on the topographical background.

Fig. 1 Essential graph of the village



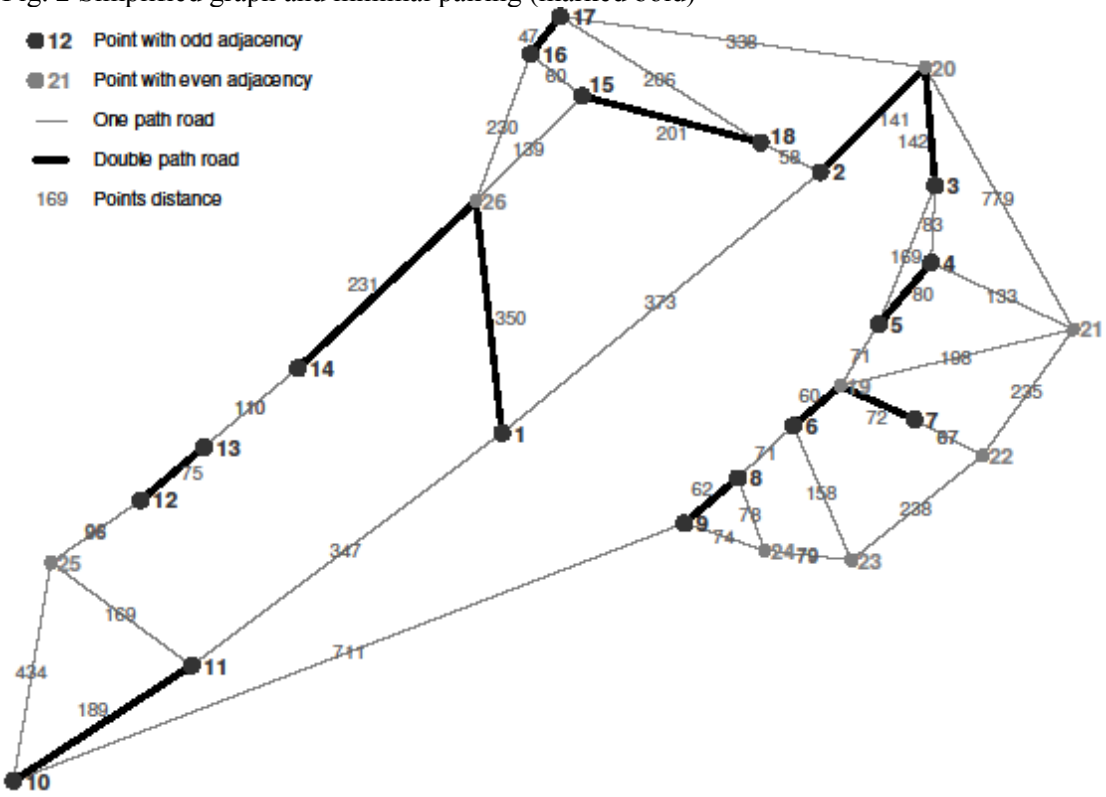
It is obvious that collection vehicles must go through the communications with waste containers. No action is needed for communications without containers. But these still appear in the graph as potential secondary paths with minimal pairing.

Primary graph consists of 68 points, which is in terms of computational complexity (number of points increases exponentially) infeasible by “brute force” (by comparing all possibilities; it would mean 68!). Therefore it is needed to find way how to simplified current graph with respect to the optimalisation function. In the first step, nodes, in which leads only one edge, can be removed from the graph, see [4].

Thus, the points which can be reached both in and out only by one communication - 52 points left after this generalisation. New nodes arose by removing of edges. These fulfil condition that only one communication leads in them. This step can be applied repeatedly until nodes with two or more edges left. Now there are 51 nodes in the graph.

We need to go through the set of edges without waste containers for further generalisation and find out whether they are helpful to our process or not and in that case we can omit them. There is one condition for this decision - we have to think of potency - number of ways leading to particular nodes, which connecting these edges without waste containers. The goal of this step is to get nodes of even potency. If we get two nodes of even potency by removing of the edge without waste, this edge is removed and not used for the further calculations. On the other hand, edges that make their end-nodes even remain. We do not consider the difference between edges with and without waste containers now. This step simplified our case study to 49 nodes and there are 6 edges less.

Fig. 2 Simplified graph and minimal pairing (marked bold)



The use of minimal pairing in the next step is needed - linking odd points by auxiliary edge which would go along the existing edge (communication). The sum of these edges will be lowest possible, see [5]. All nodes with odd potency are not adjacent therefore we will have to consider pairing with nodes lying next to these ones. Auxiliary edge, linking two nodes with odd potency, never intersect third node with odd value that would be inefficient.

Total amount of possible combinations of 18 nodes is 34 459 425 and that is why considering two simplifying conditions are needed.

1. Only edges directly connecting adjacent nodes will be considered as potential interjacent edges.

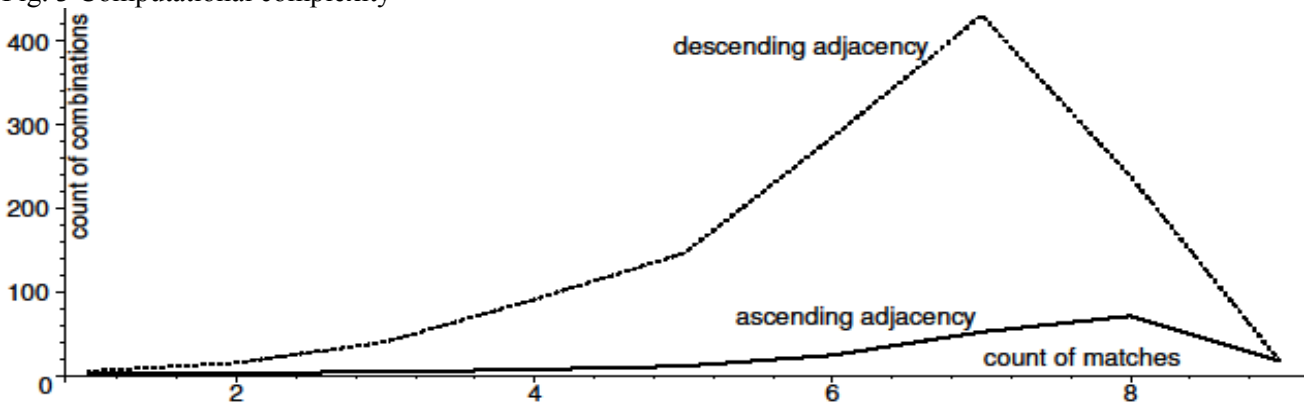
2. All nodes with odd potency are not adjacent. Therefore, we need to consider connection of points which are not directly in the line (means, there is one point in between which is not counted).

Adjacency matrix, created only for nodes with odd potency, following conditions mentioned above, predicates distance between particular nodes and mainly shows number of possible connections for each node. Each of these nodes has according to the adjacency matrix certain number of possible connections with other odd node. These possibilities represent irregular pairs {start point, end point}, which means that to each node are assigned irregular pairs according to their connection

possibilities. By sorting vector´s 18 nodes in an ascending order we reduce computational complexity as can be seen in Fig. 3. Now, we take from this vector first node, for example "4" which has two connection possibilities {3, 4} and {4,5}. Two options of the first choice appear - two solution lines. We have to consider each line separately. First of all, from the set of remaining aligned pairs we take off those which contain used points (means firstly for line "3" and "4", and then for "4" and "5"). Another irregular pair is added to each line for the consequent node and the whole process is repeated. If the second node has also two possible connections, there are 4 lines in the end. We are getting more lines. But with removal of used nodes lots of lines are becoming blind (without possibility of completion; reduction of line amount). Whole process is repeated until we use all unused nodes. Whole algorithm, written in Maple13 is presented in [6].

There are 17 possibilities of minimal pairing as a result of our case study (considering all possibilities by means of adjacency matrix does not represent difficult task). During the calculations we examined set of maximum 75 independent lines unlike the reverse procedure where the vector of nodes is arrayed from the one with the highest amount of connections. In this case we observed set of maximum 475 independent lines see Fig.3 (solid line - sorted from the minimum, dotted line - sorted from the maximum). Minimum pairing result is shown in Fig.2.

Fig. 3 Computational complexity



Conclusion

This article presented way how can be the optimalisation of the waste route handled by the use of program Maple. Source code of the Maple program which has been used for calculations is not mentioned in the article due to its size but will be presented at the conference web page.

For the waste route optimalisation it is necessary to convert topographic data into the digital form at the beginning of the calculation process. GPS coordinates and system WGS-84 were used and through these data the map base was converted into undirected rated graph. This step was followed by graph simplifying and creation of the Eulerian line via minimum pairing. Use of simplified premises let to the significant reduction in computational complexity of the problem.

Acknowledgment

The research has been supported by the project TP 4/2014 “Analysis of degradation processes of modern materials used in agricultural technology“ financed by IGA AF MENDELU.

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Modern approaches to identification of collected WEEE

LUKAS PLOSEK[1, 2], MARTIN FISER[1]
[1]Science and Research
ASEKOL a. s.
Ceskoslovenskeho exilu 2062, 143 00 Prague - Modrany
CZECH REPUBLIC
[2]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

lukas.plosek@mendelu.cz

Abstract: Producers and importers of EEE (electrical and electronic equipment) have a legal obligation to collect and recycling WEEE (waste electrical and electronic equipment). The most frequently this happens by nations-wide take-back systems. ASEKOL is a non-profit company that organizes, on behalf of the producers and importers of EEE, a nation-wide take-back system for WEEE, i.e. collection, transport and recycling of WEEE, including financing of the entire system.
Since ASEKOL wanted to trace and identify the WEEE flow and to make the system even more effective, it decided to design a device that could be able to identify taken back equipment (TVs and PC monitors which create almost 80 weight % in the stream primarily) in order to get to know as much information about the waste stream as possible.
These requirements have resulted in the design of the prototype - WEEE Identification Tunnel. The proposed system introduces a completely new approach to identify both the producer and the model of taken back equipment. The only current way how to identify models is to read the information from the labels placed on the device.
Current system efficiency of the prototype unit is higher than 90 % in case of model recognition and in case of producer recognition and the average processing time takes less than 5 seconds.
The main benefits that ASEKOL sees in the development of this device are environmental, economic and administrative, consisting in a significant improvement in data acquisition and subsequent recording.

Key-Words: WEEE, Identification, LCD, Database

Introduction

The production of electric and electronic equipment (EEE) is increasing worldwide. One of these EEE products is the liquid crystal displays (LCD). Both technological innovation and market expansion continue to accelerate the replacement of equipment leading to a significant increase of waste electric and electronic equipment (WEEE) [1, 3, 6].

EU strategies for waste management have long recognized the key role of recycling to move towards sustainable consumption and production. This resulted in a range regulatory measures, among which the WEEE directive, which sets weight-based targets for recovery, preparation for re-use and recycling [4]. In Europe, a number of different recycling systems for WEEE have been put in place, motivated by the EU directive on WEEE [5]. In Czech Republic is the most frequently WEEE is collected by nations-wide take-back systems.

ASEKOL is a non-profit company that organizes, on behalf of the producers and importers of EEE, a nation-wide take-back system for WEEE, i.e. collection, transport and recycling of WEEE, including financing of the entire system. Its services may be utilized by producers and importers of EEE on the basis of an agreement. Within the operation of the take-back system, ASEKOL co-operates closely with cities and municipalities, the final dealers and service shops, collection companies and processors of WEEE.

One of the main missions of ASEKOL is to provide for the collection and environmentally sound treatment of discarded electrical appliances on behalf of the producers and importers, to be a credible partner for governmental authorities and local

governments, to strictly comply with the quality of environmentally sound management of waste electrical and electronic equipment (WEEE) and as well as to ensure effective expending of costs.

Since ASEKOL wanted to trace and identify the WEEE flow and to make the system even more effective, it decided to design a device that could be able to identify taken back equipment (TVs and PC monitors which create almost 80 weight % in the stream primarily) in order to get to know as much information about the waste stream as possible.

Material and Methods

WEEE Identifications Tunnel

The proposed system introduces a completely new approach to identify both the producer and the model of taken back equipment. There is not implemented any uniform mandatory labelling of produced and marketed equipment. Automated recognition therefore couldn't be possible so far. The only current way how to identify models is to read the information from the labels placed on the device. Since this information is not standardized, it is a nontrivial task in the field of computer vision and artificial intelligence. Any standard commercially available technology does not have such a possibility.

The uniqueness of the solution was also demonstrated in a feasibility study on the device (carried out in 2011) which in practice could not find a similar project solution. In this study was described that there exist various partial solutions which are completely unsuitable for the technological purposes of the project plan.

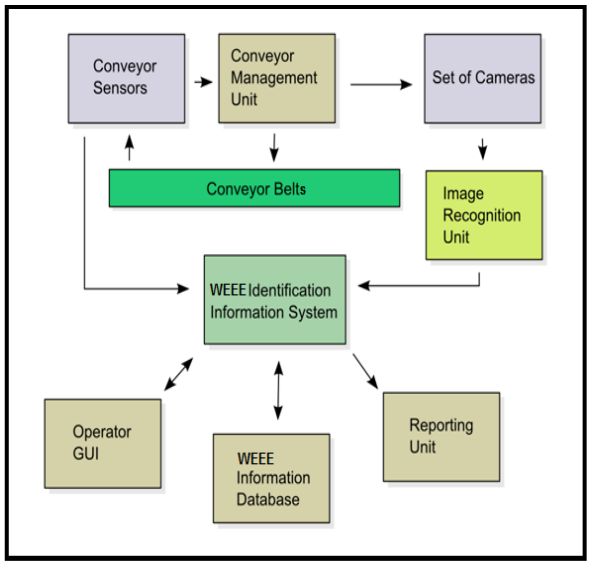
However this task is solved completely newly, its successful resolution was enabled due to the discovery of new algorithms for computer vision within last few years. Thanks to them it is possible to identify the position of general labels and manufacturers' logos at first and subsequently to determine the producer and to relate unstructured information from labels with a specific model of electrical equipment.

The suitability of using labels to obtain information about the appliances mentioned for example Dos Santos *et al*. [2],

Test procedure

Testing was carried out during the September 2014 in the premises of the company Enviropol in the village Lety. It was tested the success identifications of WEEE (especially identifications of LCD and CRT TVs).

Fig. 1 Scheme of the technical solution



Test methodology

It is necessary to establish rules for verifying the success of identifications:

- Appliances - experimental solution done so far has focused mainly on the screen devices (CRT televisions and monitors, LCDs, plasma). However proposed technology of this device enables to identify automatically a wide range of products bearing adequate readable information (logos, labels).
- Databases - the current database structure consisting of information about brand, model, dimensions, weight, completeness and technology used can be moreover extended for other relevant information related to the appliances involved in the database.
- Measurement - measurement consists in inserting the appliance to the conveyor belt is aligned to the upper side of the board of conveyor. Orientation of appliances can affect the result measurement.
- Successful identifications - State of the system report with the relevant identification model number is considered as a successful identification. Wrong model number or model change are considered to be mistake.

There were tested 1001 appliances (LCD TV or monitors). Before the measurements were excluded 101 appliances because they did not have identification label.

Results and Discussion

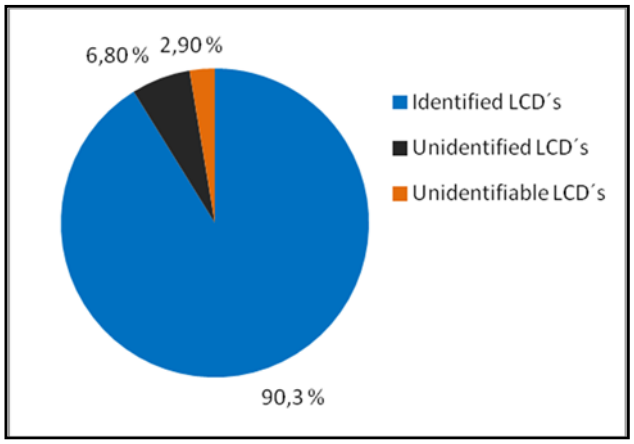
Total number of measurements was 1001 pieces of LCD displays (TV and monitors). Identification test results are displayed clearly in the Table 1.

Tab. 1 Results of the identification

Total LCD´s	1001
NEW in database	183
Identified LCD´s	721
Unidentified LCD´s	68
Unidentifiable LCD´s	29
Total success rate of identification	90,31%
Success identification on identifiable LCD´s	93,21%

The ratio between identified and unidentified is higher than 9:1. Unidentified can be divided into 2 groups (Unidentified and Unidentifiable). Unidentifiable LCD´s are appliances with missing or damaged labels, labels placed in the unreadable site for camera vision (see Figure 2).

Fig. 2 The percentage of identified and unidentified LCD´s



Reasons for failure identification:

1) Wrongly chosen height of the camera. Systems focusing on the wrong competitive surface on the LCD´s displays.
2) Lighting of the labels. For camera vision is problematic to identify too dark or bright labels with featureless descriptions or labels with reflections.
3) Errors of reading algorithm. Incorrectly identified clearly identifiable scanned text (substitution of LCD´s models)
4) Other causes, for example timeout for the identification.

In the pilot testing process is scheduled additional testing of the success identification by the end of 2014.

Conclusion

Operational testing of prototype „WEEE identification Tunnel" confirmed success from the pilot test results in identifications of WEEE (LCD TVs and monitors).

Total success of identifications LCD´s is more than 90 % of the prototype.

On the other hand, analyses of the unidentified appliances shows that is necessary to focus on ways to improve methods of determining the appliances position (3D model, point cloud) or any adaptation in algorithms reading.

Acknowledgement

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Electromotor and vacuum pump set up and measurement of basic parameters by frequency converter Siemens G-120 for milking equipment

ROBERT ROUS, JAN KUDELKA, JIRI FRYC, RADOVAN KUKLA, JAN MARECEK
Department of Agriculture, Food and Environmental Engineering, Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

robert.rous@mendelu.cz

Abstract: Usage of rotary vane vacuum pumps is currently prevailing to ensure the desired vacuum pressure in the milking equipment. This vacuum pump is driven by a squirrel-cage induction motor. Vacuum pressure regulation was until recently done by a main mechanical regulator set to a desired vacuum pressure and sucking the ambient air into the system during the milking process itself and during other activities. With this type of vacuum pressure regulation the electric motor and vacuum pump worked at full power and that leads to big electric energy consumption. The technical solution of energy saving was achieved by a development of frequency converters and their comparatively cheap investment purchase costs. The frequency converters control the operation of the asynchronous induction motor in the way that the actual vacuum pump delivery is in proportion to a volume of the air sucked into the vacuum pipe system. Frequency converters supplied by the manufacturers have a universal set of input parameters that do not respect the individual requirements of the milking system. This paper is dedicated to a correct setting of input parameters of frequency converter control unit using a Siemens STARTER 4.04 software. This paper describes determination and setting of asynchronous induction motor basic parameters using frequency converter. This paper also shows flow characteristics for different vacuum pump speeds.

Key-Words: Rotary vane vacuum pump, vacuum pressure, milking equipment, asynchronous induction motor, frequency converter, Siemens G-120.

Introduction

Development and research on the field of milking technology was in the past mainly aimed at problems of technological parameters of milking machines. That led to the effort to adapt the operation of milking machines to individual requirements of dairy cows. [1] On one hand it is important to accept the priority of this problem because suction device of the milking machine directly affect the milk gland of the cow during the milking process. On the other hand it is important to pay attention to the technical execution of the whole milking machine and mainly the efficiency of the vacuum pump, regulation of vacuum pressure and energy requirements.

Usage of rotary vane vacuum pumps is currently prevailing to ensure the desired vacuum pressure. Vacuum pumps suck in the air with pressure lower than atmospheric and compress it to the pressure that is slightly higher than atmospheric. [2]. Vacuum pump is driven by a squirrel-cage induction motor. Vacuum pump has to be able to cover operational requirements for desired vacuum pressure. The efficiency of vacuum pump during the milking process is not limiting, the efficiency during the disinfection and cleaning is.

Regulation of vacuum pressure is based on vacuum pump characteristic with constant rotation speed. If we want to achieve constant vacuum pressure, there has to be a constant amount of air flowing through the vacuum pump. Whereas the amount of air sucked in the milking machine is variant in time, there would be significant fluctuation of vacuum pressure without using any regulation device. Vacuum pressure regulation was until recently done by a main automatic mechanical regulation valve. It sucks in such amount of the air into the system that the sum of the air sucked in per unit of time by regulation valve and milking machine is constant. Because of the way the milking units work (they almost never work in the same time) and the fact that asynchronous motor works at full power, the immediate power consumption of milking

machine with this solution is significantly lower than efficiency of vacuum pumps. It is said that energetic loses caused by this inconvenient form of regulation is up to 60% of the whole power consumption. [6]

From the technical point of view, there is a need to achieve a stable vacuum pressure with optimal working parameters of asynchronous induction motor. Those are motor rotation speed, torque, power consumption. [3] For this purpose we can use frequency converter that allows us to regulate motor speed to desired values. In this way the total power consumption drops. [4] The power consumption drop using the control system with frequency converter is caused by a fact that the vacuum pump sucks in only the air that was created by the milking machine with optimal working parameters of asynchronous motor. [5]

The main goal of this paper is to describe assembled reference milking machine that is located in the laboratory in Mendel's University in Brno. Then to describe the methodology of measurement and to show the real parameters of vacuum pump and electromotor set. Then to calibrate and compare measured parameters with theoretical values of the device. Equally important goal is to inform the reader about the setup of the Siemens Sinamics G120P BT frequency converter with the SINAMICS PM 230 power unit in the Siemens STARTER 4.4 software.

Material and Methods

Characterization of laboratory milking machine

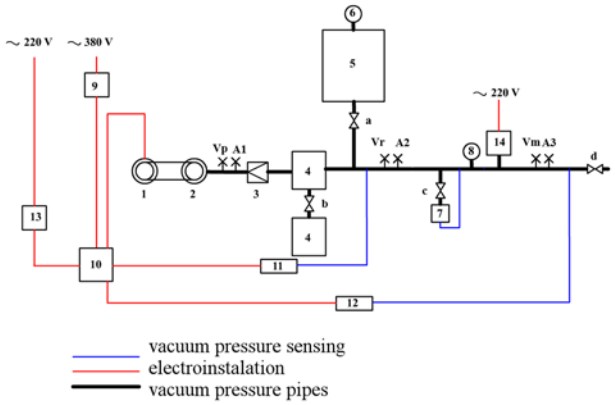
The reference milking machine was assembled in Mendel's University in Brno for finding the solutions for the problems of stability of vacuum pressure and power needs of vacuum pump and electromotor sets. This machine allows to simulate milking process and rinsing with various vacuum pressure regulation setups. The main block schema is in Fig. 1.

The main functional part of milking machine consists of rotary vane vacuum pump SACCO 1600 with theoretical power of 26.6 $dm^3 \cdot s^{-1}$, that is driven by asynchronous induction motor Siemens with theoretical mechanical power of 4.1 kW.

Stability of vacuum pressure in the system pipes can be set up and controlled by:

- To set input parameters in control unit of the frequency converter (Siemens SINAMICS G120)

Fig. 1 Main block schema of reference milking machine



1. electromotor; 2. vacuum pump; 3. check valve; 4. small air eliminator; 5. large air eliminator; 6. vacuum gauge; 7. membrane regulatory servo valve; 8. vacuum gauge; 9. electrometer; 10 frequency converter with PID regulator and control unit; 11 control pressure sensor; 12. check pressure sensor; 13. PC; 14. cycler (simulation of atmospheric presssure suction)

- Mechanically with automatic regulation diaphragm valve (Fullwood servac 3500)
- Combination of the above.

Parameter setup in STARTER 4.4 program was done in online mode when the converter was connected to a computer running the STARTER program. Needed parameters were inputted with setup wizard or they were accessible through *Expert list*. Expert list is a list of all the variables in the system. Basic motor setup includes input of rated voltage, (P0304), rated frequency (P0310), rated power (P0307), rated power factor (P0308), rated speed (P0311). Motor rotation speed limit was set up in P1001 [13].

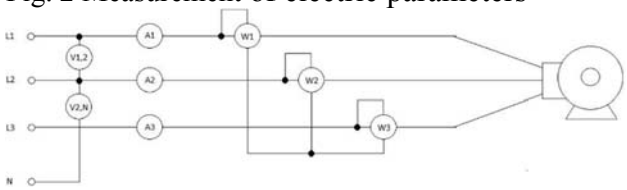
Vacuum was measured with a linear pressure sensor BD-Sensors with range from -100 kPa to 0 kPa and with 4-20 mA output connected into the analogue input 1 of the Siemens converter. Data from pressure sensor were stored in variable r7521. Power consumption was stored in variable R39.

The indirect method was used for measurement of three phase asynchronous motor Siemens with theoretical power of 4.1 kW that was used to drive rotation vane vacuum pump. This method allows to measure only electrical parameters (current, voltage, power input) and to calculate other parameters such as mechanical shaft power, slip, speed or effectivity. Whole measurement was conduct in compliance with technical standard CSN 350301 [11]. Motor was connected in the star configuration.

The multifunction clamp ammeter Chauvin Arnoux F27 with 1% accuracy in range 1000 V was used for the measurement of current, power and power factor. Measurement was done with the circuit in Fig. 2. There were two states measured – with no load and with blocked rotor. For every state the

current, power and power factor in every phase was measured. Result of the measurement were used to create circle diagram of induction motor that was made in custom made graphical program. [12]

Fig. 2 Measurement of electric parameters



Technical standards ISO 6690 [7] and ISO 5707 [8] were used for the measurement of the actual performance of vacuum pump for various rotation speeds and calculations about the reference milking machine. Actual flow amount of the air was recorded with flowmeter SAC FLOWING METER with accuracy 5 $dm^3 \cdot min^{-1}$ in the place V_p. Measured values of air flow were calibrated with gas meter for measurement of actual air flow with accuracy 0,001 m^3. Air flows measured with gas meter were converted with correlation computation for atmospheric pressure (1) that was measured in the laboratory with digital manometer Kimo MP55 with accuracy 0.2 kPa. Vacuum pressure in the system was measured in the point A_1 with a vacuum gauge RAMSES EN 837-1 with accuracy 0.5 kPa. All the measured values were calibrated by the mercury column in the U tube.

$$Q_{SV} = \frac{p_a - p_n}{p_a \cdot t} \cdot 1000 \ [dm^3 \cdot s^{-1}] \qquad (1)$$

Where:
p_a – atmospheric pressure [kPa]
p_n – vacuum in the system [kPa]
t – time for amount of 1 m^3 of pressured air to flow through gas meter. [s]

Rotation speed of the vacuum pump were set up in the control unit of the frequency converter in the STARTER 4.4 program and then checked with the rotations of the rotor of the vacuum pump with infrared Tachometer Model CA 27 with accuracy 0.1 rpm. Electromotor rotations were set up in range from 1450 rpm to 800 rpm. The actual input power was measured in the STARTER 4.4 program during changing flow rate of the air through the vacuum pump and the total power consumption for the nominal value of vacuum pressure of 50 kPa. This value is common in milking machines. Measured values of input power, total power consumption, actual air flow through vacuum pump and additional parameters were evaluated in the MS Excel. Statistical method – regression analysis. Determination coefficients (R^2) explains the percentage of variability (reliability) of the graphs and they are calculated by the equation (2).

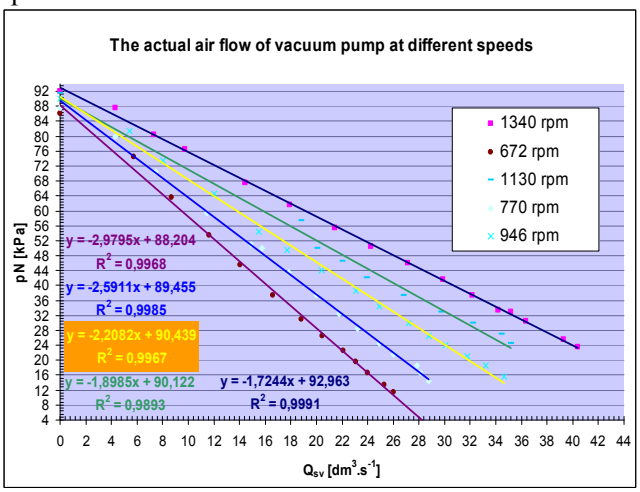
$$R^2 = 1 - \frac{\sum_{i=1}^{N}(y_i - y(x_i))^2}{\sum_{i=1}^{N}(y_i - m(y))^2} \qquad (2)$$

$y(x_i)$ – curve smoothing function
$m(y)$ – average of y

Results and Discussion

Dependency between the amount of air flow coming into the vacuum pump SACCO 1600 and the value of vacuum pressure for different rotation speed of vacuum pump (Fig. 1) was based on the methodology for the measurement of actual performance of vacuum pump above and was in compliance with the technical standard ISO 6690 [8]. Atmospheric pressure was 99.2 kPa therefore the correction of measured values according to (1) was needed. This measurement was done with varying vacuum pressure therefore the flow meter with calibration curve for different pressure levels had to be used.

Fig. 3 The actual flow of vacuum pump at different speeds



There is evident fact in Fig. 3 that the actual air flow of vacuum pump (performance) decreases with decreasing rotations of vacuum pump rotor. The lower performance vacuum pump is sufficient to achieve desired vacuum pressure (40-50 kPa), that is needed for the milking process and other associated activities. This will cause the lowering of the total power consumption. [9] When we look in Fig. 3, we can say the used vacuum pump SACCO 1600 with theoretical power of 26.6 $dm^3 \cdot s^{-1}$ is in good technical condition because its actual power with rated rotation speed is 25.1 $dm^3 \cdot s^{-1}$.

Actual input power was measured during varying air flow of vacuum pump and varying motor rotation speeds with the SINAMICS PM 230 and Siemens STARTER 4.4 software. In Fig. 4 the actual power consumption drops with the growing performance of the vacuum pump. Measured values of power consumption were fitted with quadratic equation and we can say that equations are statistically significant. Coefficients of determination R^2 are in range from 0.91 to 0.96. The actual input power of electromotor drops down from 4.41 kW (for 1450 rpm) to 1.89 kW (for 718 rpm) when we are comparing actual input power with various rotation speeds and nominal vacuum pressure (p_n=50 kPa). Actual power saving is therefore 58%. This confirms that energetic losses consequent to oversized design of milking machine can be up to 60% as was described in the beginning. [6] Because of this it is important to design input parameters in the control unit of frequency converter in the way that rotation speed is as low as it is possible with stable vacuum pressure in the system. [10]

Next measurement was focused on total power consumption in the system. Milking machine was set up for the 50 kPa vacuum pressure with the regulation valve Fullwood servac 3500. Frequency converter Siemens SINAMICS G120 can save up to 2.64 kW·h per an hour of work (Fig. 5). Measurements were done for rotation speed from 1450 rpm to 718 rpm.

Fig. 4 Actual power consumption at different motor speeds

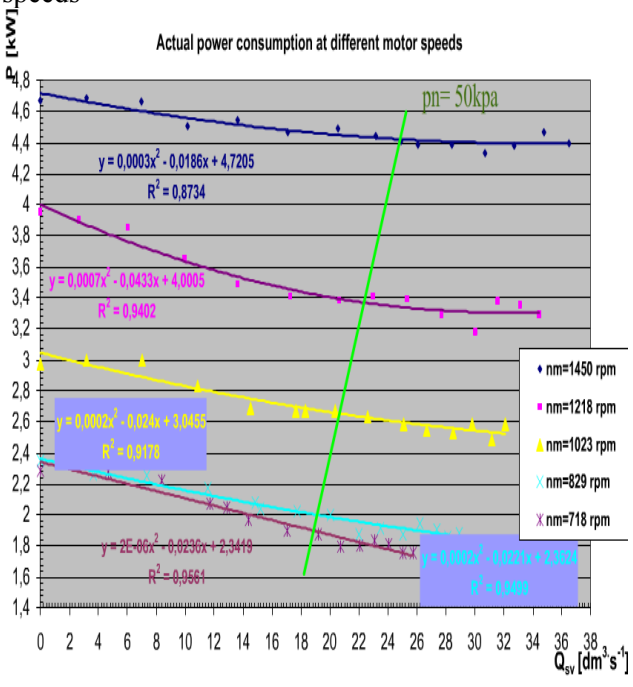
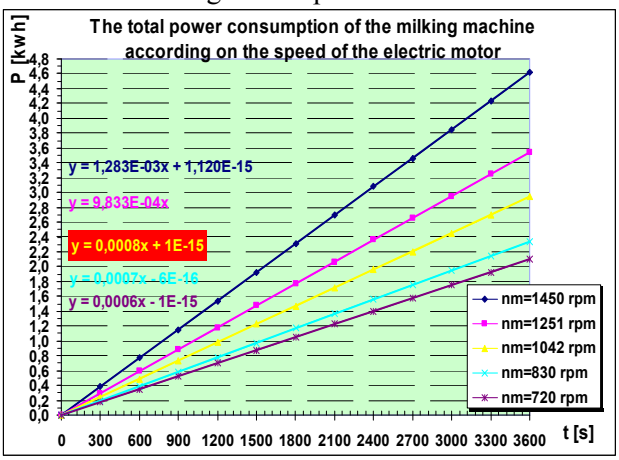


Fig. 5 The total power consumption of the milking machine according to the speed of the electric motor



The circle diagram of induction motor (Fig. 6) was created from measured electrical values. Measured values are in Table. 1 and Table. 2.

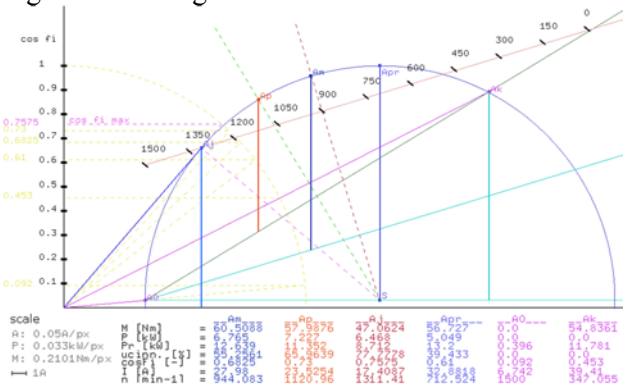
Table. 1 No load

I_0	cosφ	P	S	Q
A	-	W	VA	VAr
6.742	0.092	412.000	4470.167	4437.500

Table. 2 Blocked rotor

I_k	cosφ	P	S	Q
A	-	W	VA	VAr
39.410	0.453	549.667	1224.667	1088.667

Fig. 6 Circle diagram of measured motor



Motor has rated values: $\cos\varphi_j$=0.79; P_m=4.1 kW; I_j=8.9 A and rotation speed 1410 rpm. Point A_k in the circle diagram is situated in the right part of the circle and lies behind the point A_{pr}. That means the motor is in a good operational state. The maximal power factor in A_j is lower than rated power factor by 0.0325. This value should be in theory bigger or equal but for the common working conditions it is an acceptable value.

Conclusion

Siemens STARTER 4.4 showed as a good tool for measurement, setting and recording of input and output parameters of used asynchronous induction motor.

Measurements allowed us to describe the main characteristics of described milking machine. These characteristics will be used in future to design better control of vacuum pressure stability. Values from the circle diagram can be used to create torque, current and power characteristic of motor for its identification.

Values of actual air flow of vacuum pump SACCO 1500 and values from the circle diagram shows the vacuum pump and electromotor set is in good working condition.

Up to 58% of input electrical energy can be saved on the reference laboratory milking machine with correctly selected method of regulation of vacuum pressure (with frequency converter) while maintaining the vacuum pressure stability.

Acknowledgement

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Usage of multibody system in tractor hitch force effect analysis

DUSAN SLIMARIK, FRANTISEK BAUER, PAVEL SEDLAK
Department of engineering and automobile transport
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

dusan.slimarik@mendelu.cz

Abstract: The work is aimed on possibilities in usage of multibody systems in tractor hitch force effect analysis. Modern approach in tractor hitch force simulation is shown. First part of text describes the main principle of MBS. Then rear hitch of Zetor Fortera 140 HSX model creation is mentioned. Subsequently load is applied on computational model with usage of force load results obtained from previous measurement. Conclusion shows achieved results from hitch force effect analysis which were used as a source of force load on draft sensors.

Key-Words: MBS, tractor hitch, force load

Introduction

Three point hitch of the tractor consists of several links which are used as a coupling between tractors and implements. During field operations forces are generated in linkage. For force analysis, a measurement of the forces in each link is important. For monitoring of the forces, three point hitch dynamometer is required. Simply it's an ordinary tractor hitch fitted by strain gauges on each. From this kind of hitch forces output could be achieved for an analysis. Consequently, the force analysis could be realized with a usage of several methods. One of the most progressive and nowadays method is an application of a multibody system. This system is defined as a system consisting of a finite number of bodies and joints between them [1]. Analogous model contains the real mechanical system. Simulations are held under the real load conditions with the possibility of oscillations inclusion. For the mechanism creation, dimensions of real mechanism are necessary. Among the other necessary parameters force load belongs. Thus assembled model is ready for application. The analysis output gives a force and momentum load which in this case defines load resultant which acts directly on tractor.

Complete model of virtual three point hitch can be used for searching of proper adjustment of the hitch or for finding of tractor force load. Model described in this text serves as a computational model for force analysis of lower arms including draft sensors and adjustable spacer. Dimensions of three point hitch were obtained from Zetor Company. The outcome of this work is a force load located on to draft sensors for its calibration and sensor output examination.

Material and Methods

Three point hitch dynamometer

There are several designs of tractor hitch dynamometers. One of them is equipment which uses ordinary linkage mechanism called tractor hitch. Each link is fitted by calibrated load cells for a proper measurement of force during the field operations. There are at least five load cells on that kind of hitch. One is located on upper link, others ale placed on lower arms and rest of them on lift arms. This composition allows monitoring of draught forces and momentums generated from implement. Disadvantage of this approach is utilization only on one testing specimen of a tractor. There is a possibility of device reinstallation but only on tractors of the same brand and hitch category.

Another option is to develop central T-shaped box which can be telescopically adjusted and installed between tractor and implement. This approach brings the opportunity for universal solution. Still there is one major disadvantage. Geometric configuration of the rig is amended due to diameters of T-shaped box. Original connection is not respected [6].

Then there is an idea of usage of draft sensors as strain gauges for monitoring of draft force in lower arms. With a combination of upper link fitted with strain gauge and this kind of solution idea of universal tractor hitch dynamometer arises. There is necessity of draft sensors suitability investigation. Aim of this paper is draft sensor output examination with usage of multibody system application.

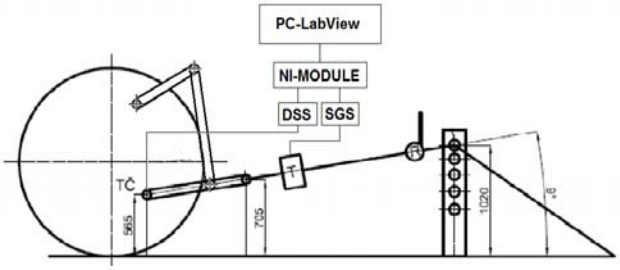
Draft sensor definition

Draft sensors takes the form of bearing pins located between tractor body and lower arms. Magneto elastic effect is used when the shear stresses occur at the bearings. Draft sensor operating principle consists of primary and secondary coil, primary and secondary pole face and steel sleeve. In case of non-load condition, a symmetrical magnetic field is formed between the poles by means of the primary coil. When the draft forces are introduced, there is a change in the originally isotropic magnetic properties. Subsequently, the magnetic field becomes asymmetrical. Magnetic potential difference occurs between the secondary poles. This causes a magnetic flux through the secondary circuit. Voltage is induced in the secondary coils. This voltage is proportional to the influencing force. Signal is amplified and rectified in an integrated evaluation circuit. [5]

Obtaining of force input

Measurement of force input was held on Zetor 140 HSX tractor which is equipped by three point hitch of III N category. Measurement chain composed of tractor itself, connection to the tractor hitch via adjustable spacer located between left and right lover arm, chain and ratchet mechanism with a strain gauge sensor for force generating and monitoring and National Instrument modular sensing control unit for monitoring of draft sensor signal and signal output from load cell. LabView evaluating program was used and proper program was designed. Measurement chain is plotted in figure 1. Ratchet mechanism was anchored to the top of fixed beam. Also supportive chain was connected to the same part of the beam and other end of the chain was anchored to the anchor embedded in the ground.

Fig. 1 Scheme of measurement chain

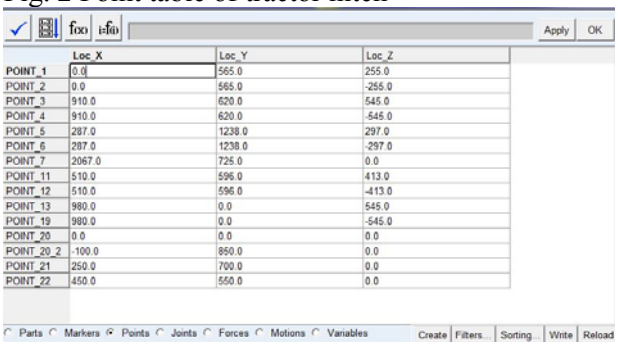


This measurement successfully provided input data for multibody system model. As can be seen from figure 1, specific geometric configuration was achieved. This was reflected in formation of the computational model.

MBS hitch preparation

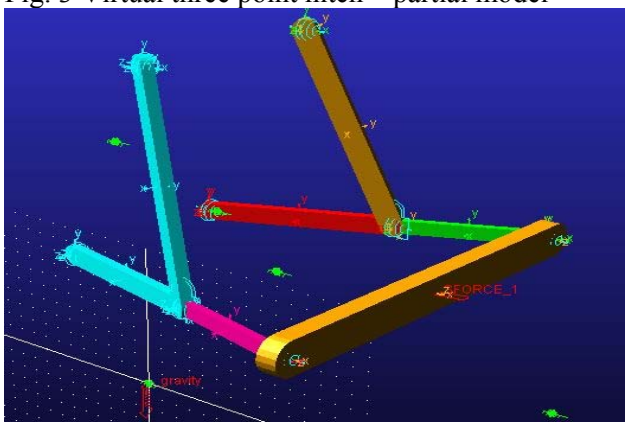
Formation of a computational model was based on diameters and dimensions obtained from Zetor Company. Firstly points in space were defined. Point table was used for brief and fast definition and it's plotted in figure 2. This approach provided the possibility of geometric configuration change. Spatial points system was obtained and it was prepared for placement of bodies.

Fig. 2 Point table of tractor hitch



Each point represents beginning and ending position of link of tractor hitch. Subsequently connection between each point is defined. Body consists of a mass properties and type of used material. In this case construction steel defined by density, Young´s modulus and Poisson´s Ratio was specified. Next step in design of virtual three point hitch was clarification of constraints between each of the bodies. In connection between lower arms fixed and spherical joint was used. Lift arms are connected via spherical joints in the middle of lower arms. Subsequently adjustable spacer is connected via spherical joints at the end of each lower arm. Lower arms are fixed to the ground. Adjustable spacer is connected to the ends of lower arms via spherical joints. Due to spherical joint several degrees of freedom are available. Figure 3 represents the virtual three point hitch (partial model) in MB system.

Fig. 3 Virtual three point hitch – partial model



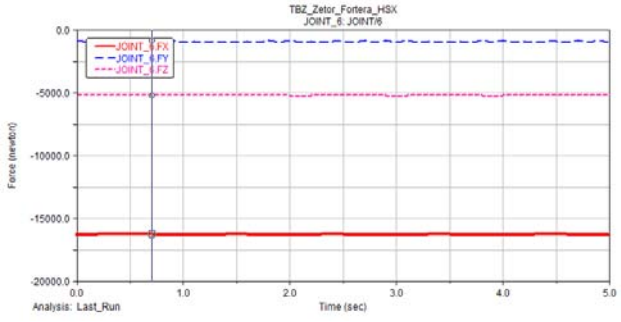
Another part of the virtual three point hitch completion was definition of force load. As can be seen from figure 3 force was applied in the middle of adjustable spacer and direction was specified by points which correspond with measurement chain from laboratory measurement plotted on figure 1. Ending point for a force direction was located on the top of fixed beam. This definition determined the direction of the force. As a source of force load previous draft measurement was used. From the output of strain gauge equivalent force was obtained. Correct value was placed into the computational model. Last part of preparation of the model was to define measure function. This function is a basic feature of mutlibody system software and the purpose of this is to clarify the examined variable. Measure function was located in left pin of lower arm where draft sensor is located. After this step force analysis could be successfully realized.

Results and Discussion

Force analysis of three point hitch

After the model creation and measure function placement on draft sensor location force analysis was realized. Results are shown in graph in figure 4. As can be seen force resultant is divided to X,Y and Z axis. Major draft force is located in X axis direction of measure function. Coordinate system of measure function is orientated with it X axis longitudinally with tractor axis with direction pointing forward from the tractor. Resultant force magnitude reached mean value of -16235 N. Force analysis is shown with maximum force obtained from strain gauge. Various load steps were implemented and results were evaluated for all load cases.

Fig. 4 Results obtained from measure function

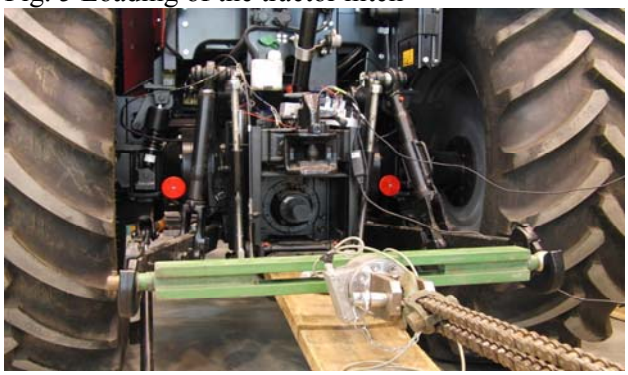


Determination of the resultant force is possible in any location of the virtual hitch. This can be used in case of largest load searching or in optimization of upper link connecting position to the lower arms in design of tractor hitch. In this case the resultant force was used for definition of load for draft sensor.

Force resultant on draft sensor

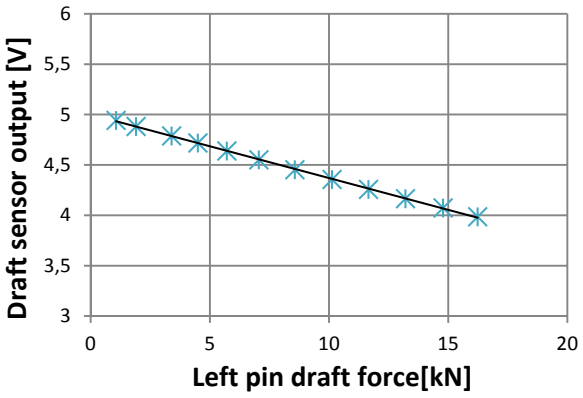
Force load generated by ratchet mechanism was gradually increased from 0 to 32,587 kN. Main goal of the measurement was to observe the output from draft sensor. Due to load character, force load analysis was necessary to run. With the maintaining of symmetry initial assumption was to divide the main force obtained from strain gauge in half. With respect of lift arms connection, force analysis was realized with usage of MB system approach. Figure 5 shows the principle of tractor hitch force loading. Chain is connected to strain gauge which represents load force. Adjustable spacer is located between connecting points of lower arms. As can be seen lift arms are connected to the lower arms. This approach has been taken into account during the computational model design.

Fig. 5 Loading of the tractor hitch



From measured values of draft sensor voltage output and draft force computed with usage of force analysis in multibody system computational model dependency was obtained and it is plotted in figure 6. Dependency between left pin draft force and draft sensor output shows linearity.

Fig. 6 Dependency between force and voltage output of draft sensor



This brief review shows possibility of usage of modern approach in force resultant analysis.

Conclusion

The work is aimed on possibility of multibody system usage in force resultant analysis. With force input obtained from previous measurement the force load on draft sensor was calculated. This allowed finding out dependency between voltage output and force load of draft sensor. These results will be used in design of three point hitch dynamometer.

This is one of the possibilities in usage of multibody system. Another option is to search for largest load in this kind of linkage mechanism or in optimization of upper link connecting position to the lower arms in design of tractor hitch. Also complete model of virtual three point hitch have got lot of opportunities like searching for path generation of hitch points [4]. During the measurement modern opportunities were interconnected. This approach indicates brief and fast solution for solving problems of a similar type.

Acknowledgement

This study was supported and financed by the internal grant agency of the Mendel University in Brno – Faculty of Agronomy, No. IP 15/2014.

The research has been supported by the project TP 4/2014; Analysis of degradation processes of modern materials used in agricultural technology; financed by IGA AF MENDELU.

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Influence of exhaust gas temperature on treatment of harmful pollutants

DUSAN SLIMARIK, ADAM POLCAR, MICHAL JUKL
Department of engineering and automobile transport
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

dusan.slimarik@mendelu.cz

Abstract: This paper describes the influence of exhaust gas temperature on treatment of harmful pollutants on truck engine equipped with 2 generation SCR technology. First part devotes to the clarification of second generation of SCR technology. Advantages and disadvantages are mentioned. Subsequently the experiment in truck transport is mentioned. With usage of CAN-BUS data reading, specific parameters of the engine and exhaust gases were obtained during the duty cycle in truck transport. Dependencies between AdBlue injection and temperature of exhaust gases with taking account the engine load were observed. Text devotes to obtaining of the parameters and subsequent evaluation of results. Activation parameters of SCR technology were declared. The influence of engine load and exhaust gases temperature on quantity of AdBlue injection were confirmed.

Key-Words: - SCR, nitrogen oxides, exhaust gas temperature

Introduction

With an increase of emission limit standards after treatment systems of exhaust gases are evolving. For removal of the harmful pollutants from exhaust gases selective catalytic reduction was developed for about 24 years [1]. Problematic of fundamental problems and challenges of urea-SCR utilization in automobile industry was handled by many authors. Urea-SCR used as reducing agent is a well-established technique for $DeNO_x$ of stationary diesel engines [2]. It is presently considered as the most promising way to reduce NO_x emissions from trucks and tractors [3]. This solution brings advantages but also risks in form of secondary emission ammonia slip. This is a major problem when ammonia is used directly as a reducing agent [4].

With EURO 6 engines comes second generation of selective catalytic reduction. Main goals of this solution are efficiency boost in NOx reduction and fuel savings with high level of engine performance. With a usage of NOx sensors located in front of SCR catalyst and behind of it, ECU monitors NOx level during the harmful pollutants treatment. Main goal of SCR is to remove the harmful pollutants of the exhaust gases in the exhaust system. This gives maximum performance and optimum fuel economy of the engine with fulfilling of strict emission limits. Exhaust gases are treated with a fluid called AdBlue.

$$4\,NO + 4\,NH_3 + O_2 = 2\,N_2 + 6\,H_2O \quad (1)$$

$$NO + NO_2 + 2\,NH_3 = 2\,N_2 + 3\,H_2O \quad (2)$$

As they passed through exhaust system a catalytic converter converts the harmful nitrogen oxides into harmless nitrogen and water vapor. These changes are at molecular level. Firstly the maximum performance and torque are gained by optimized fuel injection and timing with minimize of fuel consumption. Subsequently harmful exhaust gases are treated by the injection of AdBlue into exhaust system. Nowadays SCR systems dynamically control the quantity of AdBlue by monitoring of the NOx level and temperature before and after the treatment. This leads to maximizing of the efficiency and minimizing of absolute use of AdBlue. SCR system consists from several parts. AdBlue tank is located beside the main fuel tank for convenient filling and insulation against cold weather. Supply module pumps the AdBlue up to the AdBlue injectors. Dosing module injects the AdBlue behind the DOC. Reduction of NOx is achieved by injecting AdBlue into the exhaust gas flow before SCR catalyst. Adblue is decomposed to ammonia and carbon dioxide due to heat of the exhaust gases. Formed ammonia and exhaust gas flows to SCR catalyst where ammonia reacts with NO and NO2 molecules converting the NOx molecules to harmless nitrogen and water vapor. Catalytic reduction of NO runs according to the equation 1 and reduction of NO_2 runs according to equation 2.

Temperature and NOx sensors at the end of the exhaust system monitors the exhaust gases after

passing through the catalyst to ensure that the AdBlue injection is optimized at all engine loads. SCR system is electronically controlled by ECU which regulates amount of injected AdBlue based on these parameters: engine RPM, actual torque, temperature of exhaust gases, amount of NOx and intake air humidity. Injection is realized after fulfilling these specific conditions: coolant temperature above 45°C, temperature of SCR catalyst over 180°C, 1000 engine revolutions and 20% of torque load of the engine. Without fulfilling of these parameters, AdBlue is not injected due to inability of initialization of chemical reaction. These variables are classified as activation parameters.

Following text is devoted to reading of CAN-BUS data and obtaining of specific parameters of engine like exhaust gases temperature, fuel consumption and torque load for observing of AdBlue injection timing. Main goal is to point out on influence of exhaust gas temperature on treatment of harmful pollutants directly connected with AdBlue injection.

Material and Methods

Structure of measurement chain

Measurement chain for monitoring of observed parameters was designed especially for the road test on highway. Portable computer with data acquisition card with OBD connector placed on board of IVECO truck was used. OBD connector was connected directly on SAE J1962 16 pin connector. For online reading from CAN-BUS specific code was programmed with a usage of LabView software. This approach brought the possibility to read the data from already fitted sensors on the engine. Data logging was realized during the whole transport duty cycle for variety of engine loads.

Transport duty cycle

The main testing subject was an IVECO STRALIS 420 EEV truck with second generation of SCR technology exhaust gas treatment system. Truck was equipped with engine with 313,2 kW nominal power and 1900 Nm torque at 1050÷1550 RPM. Engine was also fitted with a variable geometry turbocharger for heavy duty transport. Engine was warmed up to operating temperature. Mean value of atmospheric pressure was 98,6 kPa. Ambient air temperature was in range between -3 and 11 °C. Automatic transmission was activated during whole measurement. For proper engine workload specific track was scheduled. Transport duty cycle was realized on transport road between Vyškov and Prostějov. Total distance was 28 km consisting of city traffic in the first and the last part and speed way in the middle. During the duty cycle different engine loads were achieved. This had a direct influence on fuel consumption and exhaust gas temperature. Also AdBlue injection was influenced due to exhaust gas temperature fluctuation.

Results and Discussion

Exhaust gas temperature and engine load course

First part of results is devoted to monitoring of dependencies between basic parameters like engine load, exhaust gas temperature and fuel consumption. Individual engine states were selected from entire transport duty cycle.

Fig. 1 Engine load, EG temperature and fuel consumption in 800 – 880 time range.

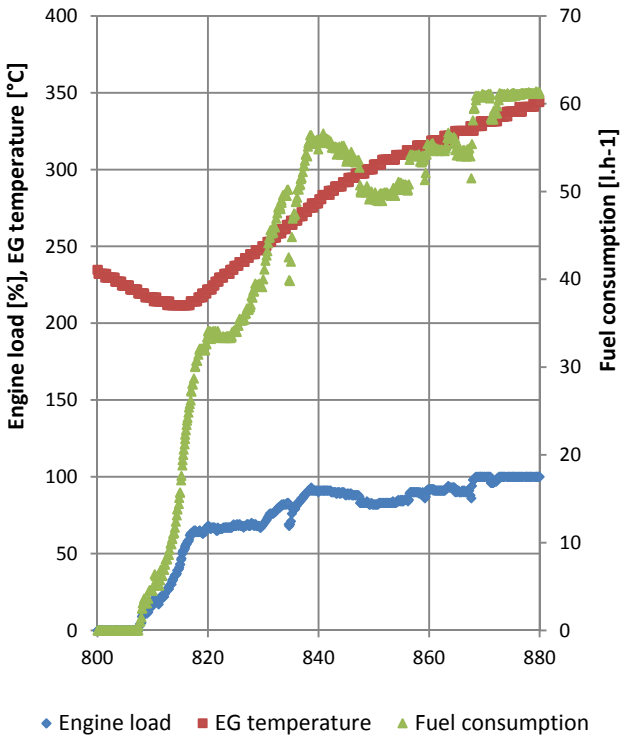
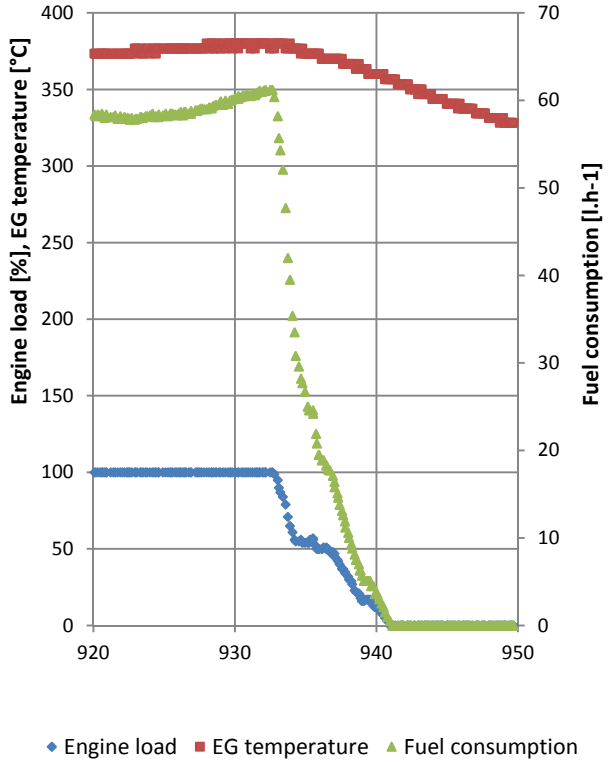


Figure 1 presents gradual increase in engine load up to 100%. This state represents hill climbing. As can be seen, increase of engine load goes hand in hand with increase of fuel consumption. These two states are interrelated with the temperature of exhaust gases. With more burned fuel, temperature of exhaust gases increase due to the combustion process. Another state describes deceleration process when engine load decrease. This brings the sharp drop in fuel consumption due to termination of fuel supply. Without fuel supply there is no combustion in the cylinder resulting in decrease of temperature of exhaust gases (see fig. 2).

Fig. 2 Engine load, EG temperature and fuel consumption in 920 –950 time range

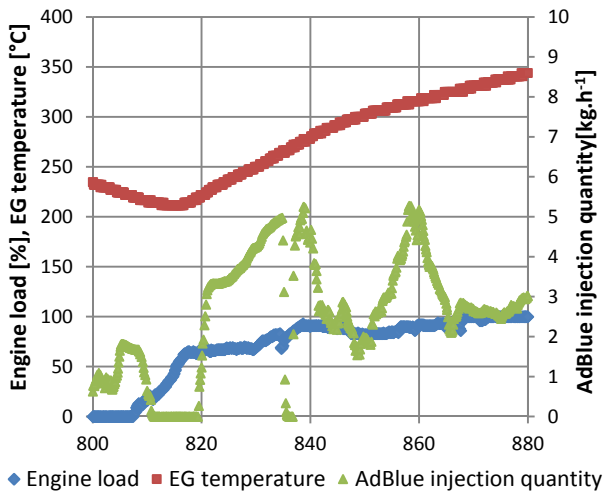


These two states represent behavior of engine basic parameters with regard to operating load caused by difficulty of the road, workload of the truck and weather conditions. Exhaust gas temperature has a major impact on AdBlue injection process. Also engine load plays a crucial role in treatment of harmful pollutants.

AdBlue injection process

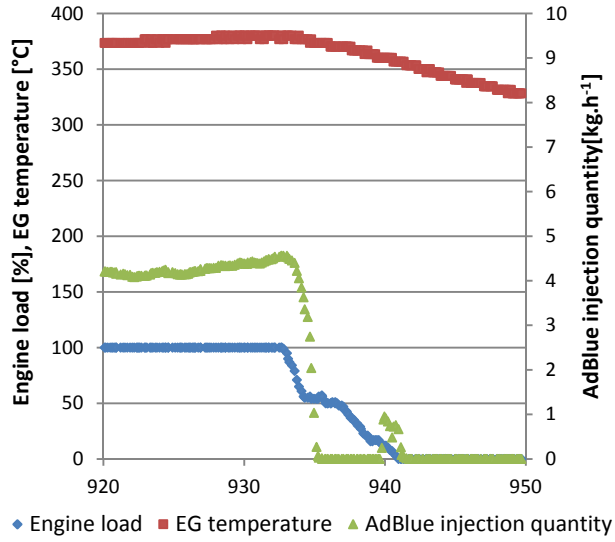
For a proper function of selective catalytic reduction, AdBlue injection process is very essential part. During the transport duty cycle different engine loads were achieved. Control unit of the SCR system evaluates input parameters required for proper function of NOx reduction. Figure 1 represents injection process of AdBlue during the engine load increment. As can be seen from figure 1 AdBlue liquid is not injected continuously. Reason is supply of SCR catalyst with ammonia and carbon dioxide. SCR catalyst stores specific amount of ammonia and chemical reaction needs time for NOx reduction. Ammonia level with exhaust gas flow through SCR catalyst decrease and additional dosage is required. Also excessive AdBlue injection leads to ammonia leakage directly into the atmosphere which causes undesirable and toxic pollution. At full and medium engine loads the most of NOx is formed.

Fig. 3 AdBlue injection process in 800 – 880 time range



During the deceleration, quantity of AdBlue injection is significantly restricted. This is caused by combustion process cessation due to fuel supply termination. During the stable and full engine load amount of AdBlue injection remains almost constant due to high exhaust gas flow through the SCR catalyst. These phenomena are presented on figure 4. The beginning of characteristics represents full engine load with high injection of AdBlue followed by eminent decrease of engine load accompanied by AdBlue injection shutdown.

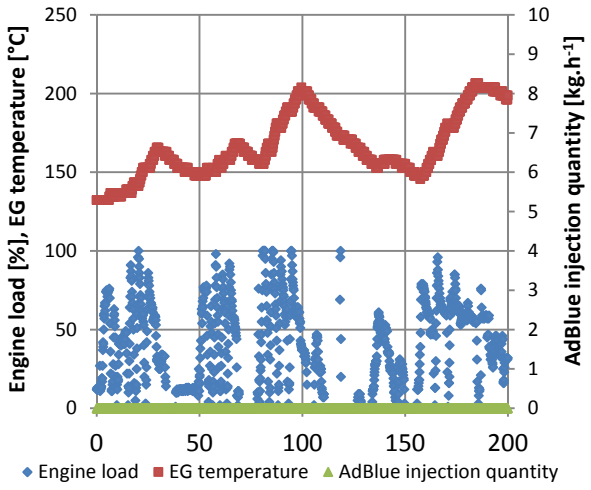
Fig. 4 AdBlue injection process in 920 – 950 time range



Demonstration of the importance of exhaust gas temperature is presented in figure 5. This diagram shows an injection process at the beginning of the measurement. Although all of the engine liquids achieved their operating temperature, due to variety in engine loads, temperature of exhaust gases

fluctuated between 130 – 205°C. This temperature is not in appropriate level for start of chemical reactions in SCR catalysts. If there would be an injection of AdBlue directly to the main flow of exhaust gases, decomposition of AdBlue to ammonia and carbon dioxide could not be realized. It would result in high content of urea in exhaust gases and subsequent environmental pollution.

Fig. 5 AdBlue injection process in 0 – 200 time range



Conclusion

Based on results with usage of the CAN-BUS as a source of data collection, temperature of exhaust gases is one of the major factors affecting the whole selective catalytic reduction process. Activation parameters must be fulfilled for a proper function of SCR technology. Temperature of exhaust gases inside of SCR catalyst must reach beyond 190°C with a torque load at least 20%. This research also confirms the basic dependencies between fuel consumption, engine load and temperature of exhaust gases.

The results also indicate that second generation of selective catalytic reduction taking account the NOx levels in front of and behind the SCR catalyst. This phenomenon was observed from achieved diagrams obtained from transport duty cycle.

During the truck engine deceleration quantity of AdBlue injection is significantly restricted due to lack of NOx levels contained in the exhaust gases. This is the moment when the output from NOx sensor informs the control unit. Subsequently the injection of AdBlue is suspended. Secondary emission of ammonia slip is taken account [4] due to NOx sensors located in front of and behind the SCR catalyst.

Acknowledgement

This study was supported and financed by the internal grant agency of the Mendel University in Brno – Faculty of Agronomy, No. IP 15/2014.

The research has been supported by the project TP 4/2014; Analysis of degradation processes of modern materials used in agricultural technology; financed by IGA AF MENDELU.

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EDIT: axis description was mismatched during copying of graph with fuel consumption. Units were not adjusted for AdBlue injection. Now are units of AdBlue shown correctly.

Anaerobic fermentation of maize (Zea mays) contaminated with cadmium

MARTIN SOTNAR, MILAN GERSL, TOMAS VITEZ
Department of Agricultural, Food and Environmental Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

martin.sotnar@mendelu.cz

Abstract: Maize (CE 220 hybrid) was chosen as a phytoremediation plant for Cd decontamination. A solution combining $CdCl_2$ and EDTA was added into the nutrient solution during the growing phase of the maize plants cultivated hydroponically. The contaminated maize plants were harvested and fed into model biogas plant reactors. Inoculum sourced from agricultural biogas plant operations was applied into these 3-litre reactors, with the addition of Cd-contaminated maize plants. Subsequently, there was an analysis of the quantity and quality of the biogas produced in the reactors. The addition of maize plants increased production of both biogas and methane compared with control samples without the addition. Cd-contaminated maize can therefore be biotechnologically processed in biogas plant reactors, in a mesophilic, anaerobic environment. Analyses of Cd concentration in fermentation residues and grown contaminated plants confirmed the presence of Cd while providing the evidence of the fact that at such concentrations Cd presence in maize plants does not form an element inhibiting biogas production. Fermentation residues may however be not suitable, given the content of Cd, for application to agricultural land. Recommended measures involve analysis of the cadmium concentration and then disposal of the fermentation residue in compliance with applicable legislation.

Key words: phytoremediation, anaerobic fermentation, contamination, methane

Introduction

The use of toxic metals by the society still represents a real threat to the environment. Attention has been paid very recently to the methods of environmental decontamination, these including phytoremediation. Maize is a commonly grown field crop. In addition to it being a phytoremediation plant, it is also the most widely used substrate today - in the form of silage - for biogas production in agricultural biogas plants, where it is typically applied in association with liquid manure from livestock farms. If processing is needed in phytoremediation plants, combustion is an option. For large areas with the possibility of utilising the contaminant, extraction of heavy metals from phytoremediation plants is another alternative. Local contaminations, however, do not provide sufficient quantities for economic plans of the extraction method. [6] Making use of one of the biotechnological plant processing methods is thus possible. Utilising phytoremediation plants to obtain biogas and energy released by the combustion of biogas is therefore another option.

Material and methods

Seeds of maize (CE 220 hybrid) were placed on a filter paper moistened with potable water, without any other chemical treatment. After six days at a temperature of 23-25°C, 216 pre-germinated plants were placed in plastic tanks subjected to a periodic, 12-hour light regimen. Maize plants were grown locally by hydroponic method on the Richter solution. The temperature inside the laboratory ranged between 25 and 26°C. After thirteen days of cultivating under these conditions, a contaminant ($CdCl_2$, the concentration being 10 μMl^{-1}) and a complexing agent (ethylenediaminetetraacetic acid, EDTA) were added into the nutrient solution. A total of four tanks were contaminated out of the six tanks containing maize plants, while two tanks were left uncontaminated as controls. The contamination phase was underway for 17 days. Subsequently, 107 plants contaminated with cadmium and 53 uncontaminated plants were harvested and cut manually cut to form 0.5 cm long sections for easier application into the fermenters. The cutting length roughly equals

the ensilaged maize particle size. Dry matter and loss on ignition was also determined in plants, as well as dry matter and loss on ignition of the inoculum as the basic input substrate. The inoculum was transported from the operations of the biogas plant in Čejč, Czech Republic. Subsequently, the inoculum was applied into model biogas plant reactors, the volume being 3 litres per each. The reactors were maintained in a water bath at a constant temperature of 42^0C. A total of eight model reactors were filled of which two units contained only the inoculum without the addition of maize and served as control samples of the process of anaerobic fermentation, two units contained the inoculum with the addition of uncontaminated maize plants (25 g and 50 g of plants) and two units contained 25 g of contaminated plants along with the inoculum. Finally, two reactors contained 50 g of contaminated plants along with the inoculum.

This was followed by analysis of the quantity and quality of the biogas produced. Biogas quantity measurements were using the BK G4 gas meter. The biogas composition was analysed by means of the device Combimas GA-m with columns for measuring CH_4, CO_2, O_2, H_2S and H_2. The average laboratory temperature was 20^0C, the humidity was 55% and pressure was 101,735 Pa.

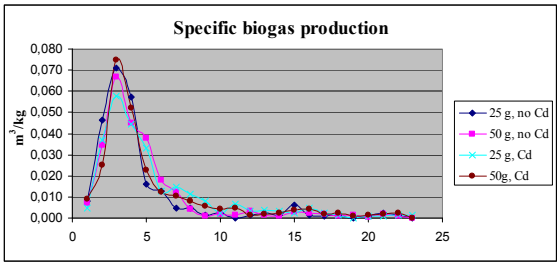
The determination of cadmic metal concentration in the maize plants and in fermentation residues was conducted electrochemically by differential pulse voltammetry with the conventional tri-electrode system. There was a working (mercury - HMDE) electrode, auxiliary (platinum - Pt) electrode, and reference (argentchloride - Ag/AgCl/3M/KCl) electrode. Measurements were carried out using the devices 813 Compact Autosampler + 797 VA Computrace (Metrohm, CH). Dosage consisted of 100 ml of sample and 1,900 ml of acetate buffer (pH 5.0). When carrying out the cadmic metal determination method, the potential range was (-1.3) to (+0.2) V, potential step was 0.005 V, the accumulation time was 120 seconds, the accumulation potential was -1.15 V, the bubbling of the sample with argon took place for 90 seconds and equilibration time was 5 seconds. Acetate buffer (pH 5.0) was used as electrolyte.

Results and discussion

Out of a total of 173 plants of full-grown maize plants (CE 220 hybrid), 107 plants contaminated with cadmium and 53 uncontaminated plants were harvested after 17 days of contamination. The total weight of the harvested contaminated plants was 609.68 g. The total weight of uncontaminated plants was 326.46 g.

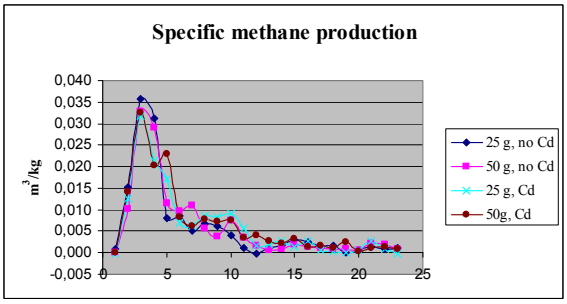
The total biogas production in the samples with applied plants was higher than in control samples without the addition of plants. Adding the biological material thus caused the biogas production to increase. The daily specific biogas production from the fermenters is shown in Fig. 1.

Fig. 1 Daily specific biogas production



The production of applied samples was obtained by deducting the average biogas production in controls from biogas production of test samples. The increased production in the first five days is determined by an increase in activity of microorganisms caused by the addition of the biodegradable material. The daily specific production of biogas does not show any noticeable difference between the samples with the cadmium content and those with no cadmium. Energy assessment of biogas however requires sufficient methane content in the substance. Methane production is thus critical as regards energy utilisation. Daily specific methane production is illustrated in Fig. 2.

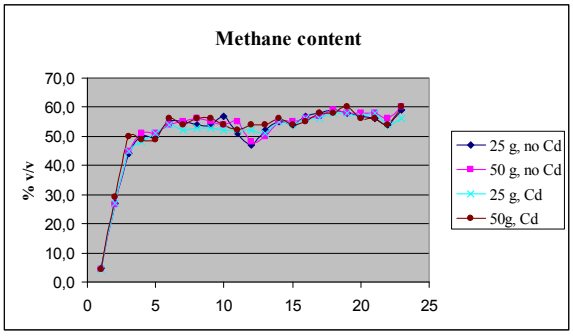
Fig. 2 Daily specific methane production



After feeding the system of model reactors, the air present is consumed by aerobic processes in the first two days to produce carbon dioxide.

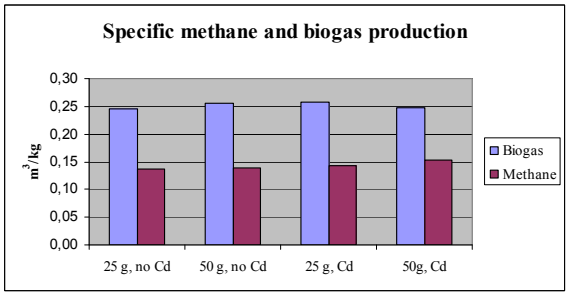
Subsequently, there is an increase in methane concentration, thus a higher daily specific production of methane. Daily specific methane production for each of the test samples is comparable. Production of methane, as the main combustible component of biogas, is essential for energy utilisation. Important, however, is its concentration in biogas. Cogeneration units burning biogas to produce electricity and heat require the methane concentration to be above 50%.

Fig. 3 Methane content in biogas



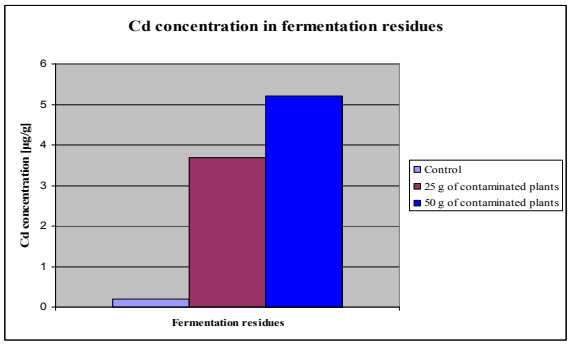
After balancing the methane concentration in the biogas at the beginning of the test, the methane content was above 50%. All tested samples thus show the methane concentration suitable for direct combustion of biogas in cogeneration units. Co-fermentation with other materials is not required in terms of methane content in biogas.

Fig. 4 Total specific production of biogas and methane



Ensilaged maize and liquid manure present the commonly used substrates for biogas production in agricultural biogas plants. Fresh maize reaches the production volume of 0.52 $m^3 \cdot kg^{-1}$. [1] The tests achieved the production to range between 0.229 and 0.254 $m^3 \cdot kg^{-1}$. The decreased production results from harvesting the maize plants before the growth of cobs and from the cultivation method. For ensilaged maize, the normal production of biogas is 0.55 $m^3 \cdot kg^{-1}$. The average methane content in the biogas made from the tested samples is around 55%. Typically, the methane concentration in the biogas produced from fresh maize is 65%. [1] This difference is due to the launch of the system and application of maize before the growth of cobs.

Fig. 5 Cadmium concentration in fermentation residues



In the real life, the fermentation residues are applied to agricultural land. Any cadmium concentration in the fermentation residues is therefore undesirable. At a concentration above 5 $\mu g \cdot g^{-1}$, the biological material is considered to be beyond the permitted concentration for category 3 pursuant to the Czech legislation (Regulation 341/2008 Coll.), and is therefore considered to be non-degradable waste intended for disposal. Group 2, class III can be used at a concentration to 4 $\mu g \cdot g^{-1}$ on the surface of a terrain being generated by reclamation layers of secured landfills according to ČSN 83 8035.

Conclusion

During the tests, a total of 107 plants of maize (CE 220 hybrid) contaminated by cadmium were grown and harvested, along with 53 plants without contamination. Subsequently, anaerobic fermentation tests were carried out. Analyses showed no difference as regards production of biogas and methane contained in the substance between the samples with the addition of contaminated plants and those containing uncontaminated plants. The total specific production during 23 days ranged between 0.229 and 0.254 $m^3 \cdot kg^{-1}$. The methane concentration (50-60 %) demonstrates the possibility of energy utilisation for the biogas generated by direct combustion in the cogeneration unit. Energy utilisation in phytoremediation plants of maize contaminated with cadmium is thus possible. The problem

consists of cadmium concentration in the fermentation residues. As the dosage of cadmium-contaminated plants increases, the concentration of cadmium in the fermentation residue grows. Subsequently, the fermentation residue becomes waste the concentration of which could also theoretically reach the limit values for hazardous waste.

Acknowledgement

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Application of acoustic emission for monitoring of plant root behaviour

PIYAPONG SRIWONGRAS, PETR DOSTAL
Department of Technology and Automobile Transport
Faculty of Agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xsriwong@node.mendelu.cz

Abstract: The plant root is the organ of a plant that typically lies below the surface of the soil. The four major functions of roots are absorption of water and inorganic nutrients, storage of food and nutrients, anchoring of the plant body to the ground and vegetative reproduction. The aim of this study is to monitor the plant root behaviour of studied plant which is grown in pot under the laboratory condition by means of acoustic emission testing (AE). In this study, AE waveguide developed to receive AE signals from plant root and installed AE sensor on the tip of AE waveguide is put in the soil contained in test pot. The experiment is divided into 2 parts; watering and unwatering the studied plant. From the results of this experiment, event accumulation number of AE signals can be parameter to describe the plant root behaviour of studied plant while they are encountering the different conditions. Consequently, AE can be new alternative appliance for monitoring the plant root behaviour in real-time as well as non-destructive method.

Key-Words: acoustic emission, plant root, non-destructive method

Introduction

Non-destructive testing (NDT) is a wide group of analysis techniques used in science and industry to evaluate the properties of a material, component or system without causing damage.

One of these methods is acoustic emission (AE). This inspection technique detects elastic waves generated within a test specimen by such mechanisms as corrosion, plastic deformation, fatigue, and fracture. It differs from ultrasonic inspection, which actively probes the structure; AE listens for emissions from active defects and is very sensitive to defect activity when a structure is loaded beyond its service load in a proof test. This process can detect flaw and imperfections such as the initiation and growth of fatigue crack [1].

Currently, there are a few researchers use AE method to be tool for detecting or evaluating the condition or quality of agricultural and forestry productions. For instance, Varner D. and et al. employed the AE method for monitoring of plant transpiration. His experiment report describes measuring of AE signals during a research task dedicated to monitoring of life behaviour of green plants using state-of-art NDT methods. In this particular pilot experiment, *a Dieffenbachia plant* was equipped with 3 AE sensors and subject to continuous monitoring for 2 weeks. The experiment proved the method to be applicable and outlined future research directions in this interesting and promising area of investigation [2]. Zweifle R. and et al. used Ultrasonic Acoustic Emission (UAE) to monitor the tree in drought-stressed condition. Their results found that UAE in trees is often related to collapsing water columns in the flow path as a result of tension that are too strong (cavitation). However, in a decibel (dB) range below that associated with cavitation, a close relationship was found between UAE intensities and stem radius changes [3].

From AE method used for monitoring the plant as mentioned above, there is no research study in behaviour of plant roots by using AE method. Thus, it is very interesting to apply the AE method to study the behaviour of plant root.

Normally, method to investigate root systems range from non-destructive techniques such as rhizotrons and X-ray-imaging with computed tomography (CT) to highly destructive approaches involving excavation in the field or washing roots from soil sample [4].

Therefore, the aim of this paper is to use the AE method to detect the AE signals generated from the roots of studied plant grown in laboratory condition in order to study the plant root behaviour. The AE parameters got from result of this experiment is discussed to find the relationship between those AE parameters and the plant root behaviour.

Material and Methods

Studied plant

Zamioculcas zamiifolia Engl. (ZZ) was used to be the specimen to study the behaviour of plant roots by means of acoustic emission method (AE). ZZ is herbaceous perennial plant native of eastern Africa, growing on tropical moist forest floor or stony ground [5], with large leaves, simply unequally pinnate and a thick horizontal rhizome (with tuber like formations). It has been documented as a "unique" indoor foliage plant because of its exquisite appearance, ability to grow under low light conditions, extremely high tolerance to drought stress, and lack of disease or pest problems [6].

Acoustic emission apparatus

A schematic diagram of the experimental set-up is shown in Fig.1. The important parts in this experiment were consisted of studied plant with soil in pot, AE waveguide, AE sensor, AE preamplifier, AE acquisition system, AE software and PC.

The AE waveguide which was made from stainless steel had dimension of 10 mm in width, 1 mm in thick and 125 mm in height. It was employed to receive the AE signals generated from root movement in soil. Therefore, AE waveguide would be partly put into soil and nearly located around the root zone of studied plant. An AE sensor which was a wideband transducer (25-600 kHz) from Dakel company (Czech Republic) was installed on the tip of AE waveguide as shown in Fig.2 in order to transform the mechanical signals into electrical signal. The AE preamplifier which had amplifier value of 35 dB was used for increasing received signals from AE sensor in order to get the higher electrical signals. The electrical signals were converted from analog signals to digital signals by using AE acquisition system after that all digital signals would be analyzed and displayed the results of signals by using software which was Daemon and Daeshow that were developed by Dakel company respectively.

In order to get the AE signals from roots of studied plant better, a studied plant was grown in a proper size of pot and installed AE waveguide in the proper position of pot as shown in Fig.2.

Experimental procedures

The intention of this experiment was that using AE application studied the behaviour of plant roots in different conditions affecting to the studied plant.

Fig. 1 Experimental set-up for the real-time monitoring the behaviour of plant roots using AE method.

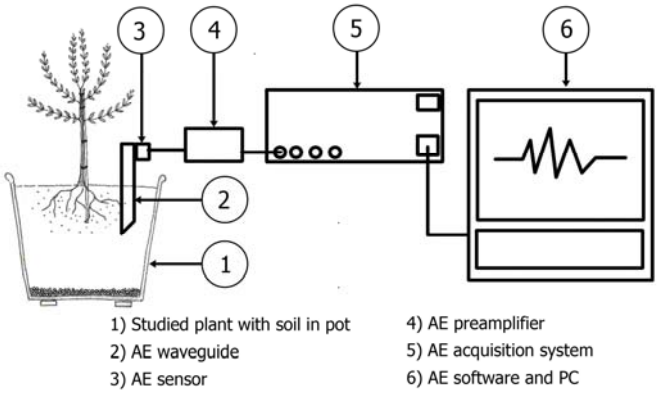


Fig.2 The position of an AE sensor installed on tip of AE waveguide (top)

Thus, the procedures of this experiment could be separated into 2 parts as following:

1. Monitoring the plant root behaviour of studied plant by using AE method without watering the plant for 3 days.
2. Monitoring the plant root behaviour of studied plant by using AE method with watering the plant an everyday for 3 days.

In both procedures, they were conducted in the laboratory condition in order to eliminate background noise such as traffic noise, environmental noise, electronic device noise, people talking and etc. For interesting parameters from AE signals generated from plant root, Root Mean Square (RMS), count and event accumulation number of AE signals were considered to be parameters to show the plant root behaviour of studied plant due to movement of plant root in soil.

Results and Discussion

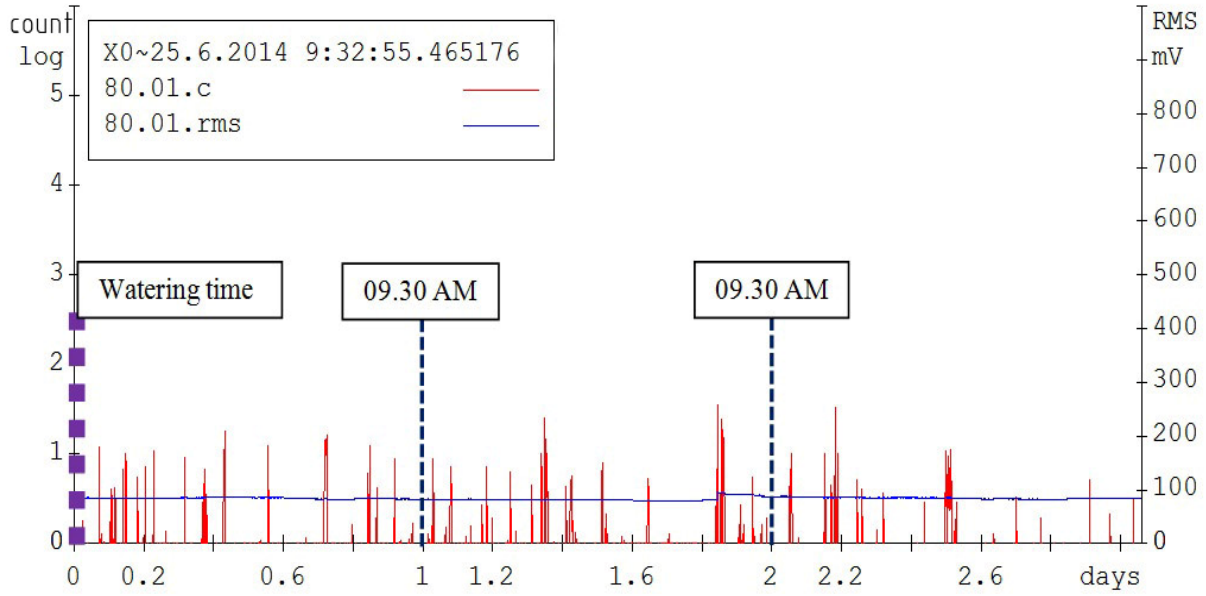
In first experiment, the studied plant was watered only one time at first day of this experiment. Fig. 3A

shows the AE parameters, which are count number and RMS, detected from first experiment throughout three days and also Fig. 3C shows the event accumulation number of detected AE signals from first experiment for three days. From the result shown in Fig. 3A and Fig. 3C, there were a lot of AE signals generated from the roots of studied plant in test pot in the first experiment.

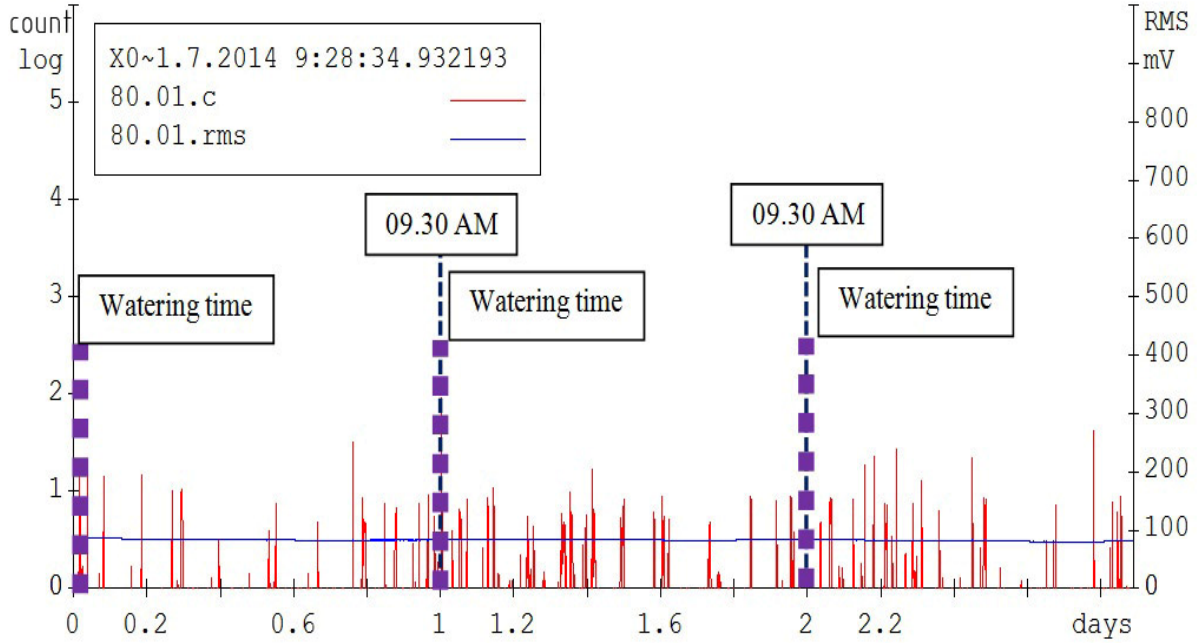
In second experiment, the studied plant was watered one time an everyday for three days. Fig. 3B shows the AE parameters, which are count number and RMS, detected from second experiment throughout three days. Fig. 3D shows the event accumulation number of detected AE signals from second experiment. From the results shown in Fig. 3B and Fig. 3D, there were many AE signals generated from the roots of studied plant in test pot in the second experiment.

Fig. 3 Result plots from both experiments

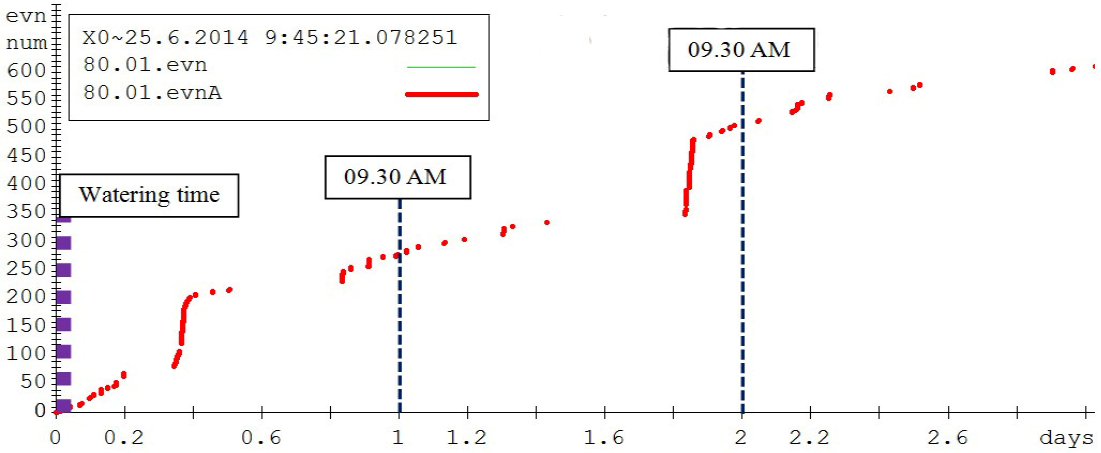
A) The plot of AE parameters (count number and RMS) versus time of studied plant without watering for 3 days



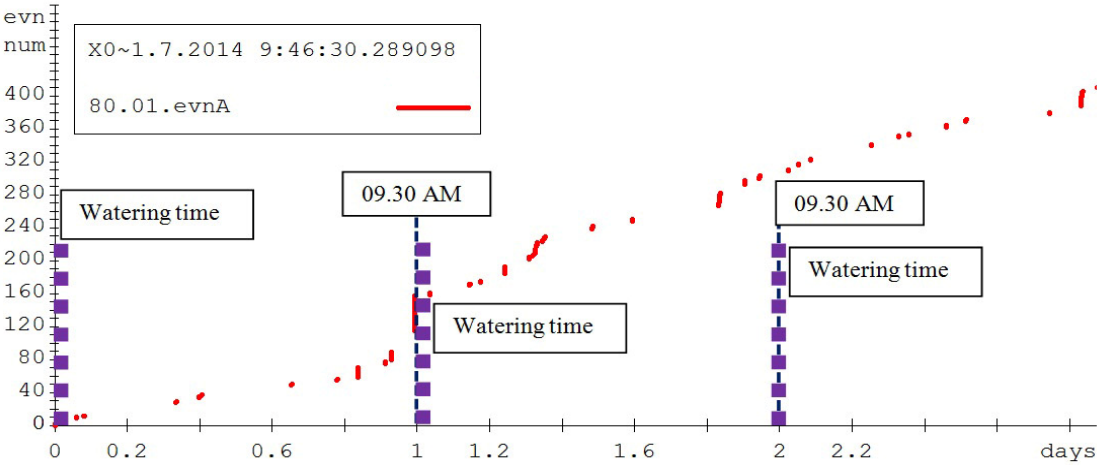
B) The plot of AE parameters (count number and RMS) versus time of studied plant with watering an everyday for 3 days



C) The plot of event accumulation number versus time of studied plant without watering for 3 days



D) The plot of event accumulation number versus time of studied plant with watering an everyday for 3 days



According to the results of both experiments, it was possible to mention that the event accumulation number of AE signals as shown in Fig. 3C and Fig. 3D could be brought to describe the behaviour of plant roots in soil when the studied plant was grown in different condition due to having the different event accumulation number occurred in the each experiment. From this experiment, the maximum event accumulation numbers of first experiment and second experiment were approximately equal to 600 and 400 respectively. Therefore, there are more AE signals in first experiment than second experiment due to more change of behaviour of plant roots and soil structure which were affected from water contained in soil.

Moreover, there have been no researchers using AE method to monitor the plant roots. Therefore, this experiment was a pilot study in order to present the possibility of monitoring the behaviour of plant roots by using AE method.

Conclusion

AE method is used to monitor the behaviour of plant roots in laboratory condition. From the results of experiments, the event accumulation number of AE signals can be parameter to describe the behaviour of plant roots of studied plant while the studied plant are encountering the different conditions. Therefore, AE method is probably used for monitoring the plant root behaviour in real-time and non-invasion method

For further study in this research, the authors will consider some parameters which directly have effect to the root of studied plant such as air temperature, air moisture and light intensity in order to get more new information of plant root behaviour monitored using AE method.

Acknowledgement

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Common rail injector for the diesel engines and different fuels

PETR STARY
Technical University of Liberec
Studentska 2, 461 17 Liberec
CZECH REPUBLIC

petr.stary2@tul.cz

Abstract: The paper deals with the problems of the injector for high pressure Common Rail fuel system. The test results on the experimental stand for the Common Rail injectors are discussed. The monitoring of the needle movement course is of great importance to the future research which will study the relationship between the movement of the solenoid lifting and dynamics of the input pressure.

Key-Words: Common Rail, Injector, Experimental Stand

Introduction

Thanks to relentless development of injection systems and application of turbochargers with intercoolers for air intake the diesel engines have been keeping pace with continuously higher and higher requirements for rated power, low fuel consumption and reduction of harmful emissions. The Common Rail fuel injection system adequately meets such requirements because it features high injection pressure ensuring accurate fuel metering and its efficient mixing with air.

The high-pressure part of the Common Rail system ensures generating the controlled fuel pressure in the high-pressure pump, maintaining the high pressure in the Rail and metering accurately the amount of the fuel by the injector.

The primary task of the Rail is to deliver the high-pressure injectors with fuel having the specified pressure. The fuel pressure is measured by a common rail sensor and its specified level is maintained by a pressure control valve located either on the rail or on the high-pressure pump. The fuel is delivered from the rail to the injector by a high-pressure fuel delivery pipe. Before entering the injector, the fuel flows through a flowlimiter whose safety function is to interrupt the fuel flow if the flow rate increases due to a common rail injector leak. The common rail injector nozzle leaks permanently and the amount of fuel exceeds the specified level. Moreover, the flowlimiter should absorb the amount of fuel delivered during the
injection when the delivered fuel pressure drop occurs.

Solenoid operated injectors control the needle movement by means of changes in pressure applied to the needle body which are caused by the fuel flow back to the tank through a solenoid operated ball valve.

The values specifying in particular the nozzle opening start and velocity, the duration of opening and the nozzle closing velocity are specific for the needle movement curve pattern. The values affect the injected fuel amount pattern.

Table 1 Engine Specifications

ESC Test Point	Engine Speed (rpm)	Rail Pressure (bar)	Torque (Nm)	Fuel Mass Flow Rate (g/cycle)	Peak Cylinder Pressure (bar)	Duration of Injection (Deg CA)
8	1900	1400	670	0.122	164	28
9	1900	1025	177	0.034	104	9

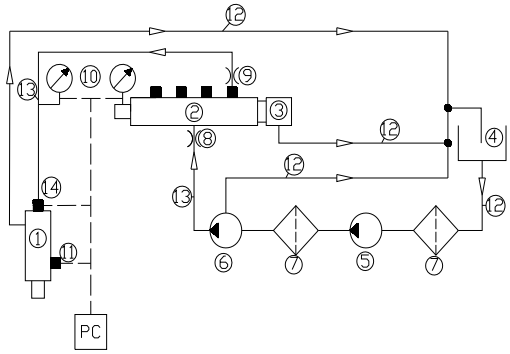
Measurement Description and Methods

For model testing at the experimental stand, initial parameters of two operating points (point 8 and point 9 of a 13-point ESC test, See Table 1) were defined which were based on extensive experiments conducted in the past on a Cummins ISBe4 engine with the Common Rail fuel system with injectors controlled by electromagnetic actuator [3] and [4]. The engine in question is a supercharged engine featuring the intake air intercooling and having an

engine displacement of 4 dm^3 and rated power of 140 kW at 2400 rpm. The measurements were performed in accordance with the methodology of the 13-point stationary ESC test.

Fig. 2 Location of the piezoresistive pressure sensors at the test station

Fig. 3 Layout of pressure sensors at the test station



Injector, 2- Rail, 3- Pressure regulation, 4- Tank, 5- Low-pressure pump,6- High pressure pump, 7- Fuel filter, 8- Throttling the inlet to Rail, 9- Throttling the output from Rail, 10- Pressure sensors, 11- Needle lift sensor12- Low-pressure pipe, 13- High-pressure pipe.14- Lifting armature sensor

The experimental stand (See Fig. 2 and 3) contains the identical Common Rail fuel system elements as the internal combustion engine Cummins ISBe4 (a high-pressure pump, a Rail, a solenoid operated injector, a high-pressure pipe, piezoresistive sensors), it means the elements identical to the system used in the engine. Sensors Kistler 4618 A2 were used to measure the dynamic fuel pressure in the Rail and before the injector. The duration of the solenoid activation (the injected amount) was controlled by the ADCIS unit which enabled the realization of only one injection per cycle. The piezoresistive sensor signals were saved by means of our own software using the increment of 0.2°CA.

Measuring and its Evaluation

The work focused on the experimental verification of how the dynamic changes in fuel pressure pattern before entering the injector affect the injector parameters when the controlling voltage setting and electromagnetic coil current values remain the same. Besides, the work examines if the armature movement can be sensed. The effect of a biocomponent in the fuel (in concentrations of B0, B30, B50 and B100) on the injector parameters (the nozzle opening duration, the course of armature lifting, fuel amount and the dynamic pressure patterns in the Rail and at the injector input) at the fuel temperature ranging from 38-40 °C was examined.

Fig. 4 The dynamic pressure pattern before the injector at 9°CA,1025 bar, 1900 rpm

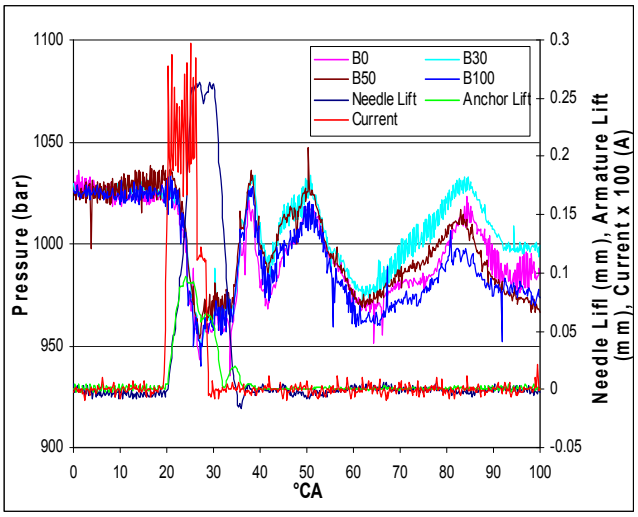
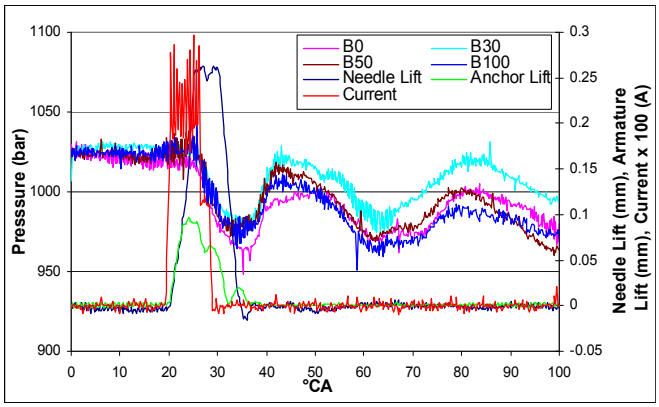


Fig. 4 illustrates the dynamic pressure patterns at the input to the high-pressure injector for the biocomponent concentrations from B0 to B100 and the needle lifting pattern, armature lifting pattern and electromagnetic coil activation current. The Figure 4 shows clearly the dramatic drop after opening the injector nozzle and the consequent pressure fluctuation. The nozzle opening duration is 9°CA at 1025 bars and 1900 rpm.

The following Figure (5) shows the fluctuation of pressures in the Rail for the identical injection parameter setting i.e. 9°CA, 1025 bars, 1900 rpm. The pressures will become stable, i.e. they will return to their initial values of 1025 bars after approximately 180 °CA.

Fig. 5 The dynamic pressure patterns in the Rail at 9°CA, 1025 bars, 1900 rpm



The full load of the engine at 1900 rpm was another mode which was examined within the 13-point test. This corresponds to the injector nozzle opening duration 28°CA and injection pressure of 1400 bars in the Rail. The following Figures show the dynamic pressure patterns and other measured quantities in the mode of full load for the biocomponent concentrations ranging from B0 to B100.

Fig. 6 The dynamic pressure pattern before the injector at 28°CA,1400 bars, 1900 rpm

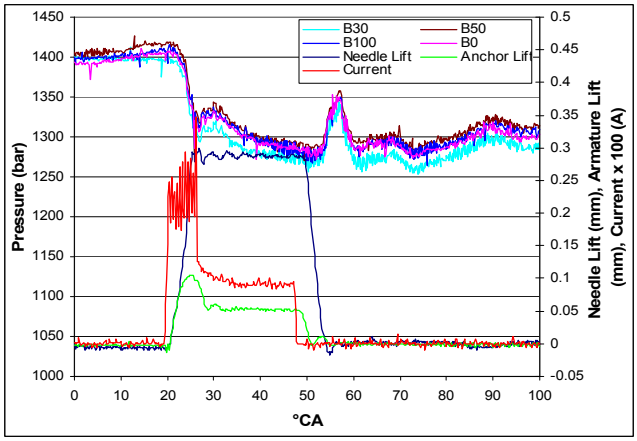
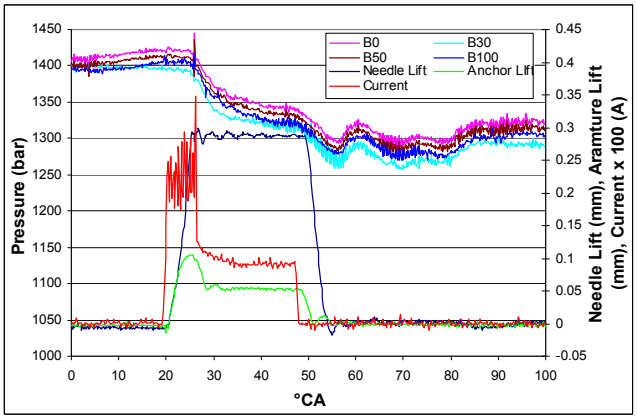


Fig. 7 The dynamic pressure pattern in the Rail at 28°CA,1400 bars, 1900 rpm



Figures 6 and 7 illustrate behaviour of the dynamic pressures in the Rail and before the injector for the set injector nozzle duration, 28°KH and pressure of 1400 bars. The setting shows clearly the time delay between the current supply start to the electromagnet coil and the subsequent response of the armature, which corresponds roughly to 1°CA. The time delay between the armature response and the injector nozzle needle is almost insignificant.

Effect of the Biocomponent on the Fuel Amount per Cycle

Other examined parameters were the fuel amount per cycle and the waste quantity of the high-pressure injector. The amount of fuel which flowed through the injector nozzle and the solenoid valve ball is measured gravimetrically. The measurement can help us assess the condition of the injector from the view point of stability of supplied amount of fuel per cycle as well as express the overal injector efficiency and characteristics.The ratio between pure diesel (B0) and pure biocomponent (B100) was used for comparison. The following two graphs show the amount of fuel supplied through the nozzle per cycle (Fig.) and waste fuel quantity which flowed through electromagnetic coil valve back to the fuel system waste pipe (Fig.9).

Fig. 8 Comparison between B0 and B100 amounts per cycle

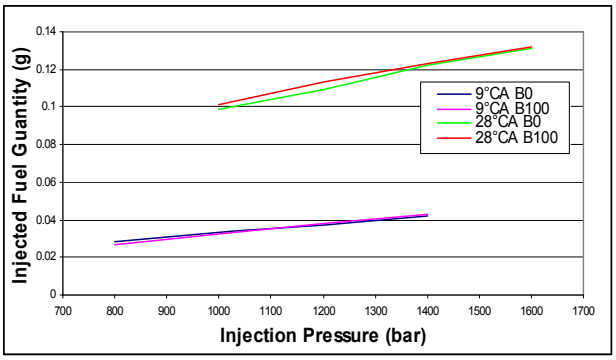
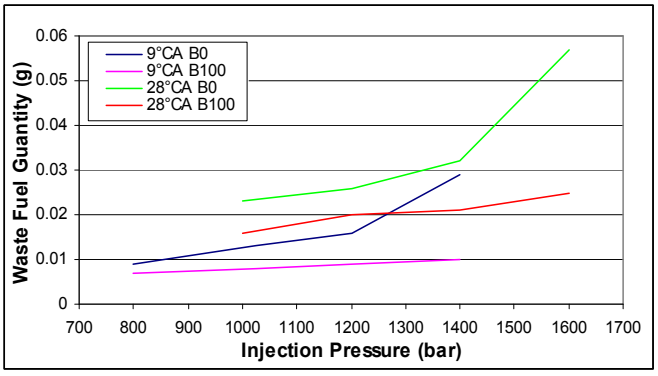


Fig. 9 Waste fuel quantity comparison (for B0 and B100)



Figures 8 and 9 indicated that there is a minumum effect of the biocomponent on the fuel amount per cycle, both for the injector nozzle opening duuration of 9°CA and 28°CA within the whole range of measured pressures from 800 to 1600 bars. More significant differences relate to the waste fuel quantity, for the same setting of pressures and injector nozzle opening duration. Considering that no time difference for individual pressures was recorded from the detailed measurements of the armature lifting and needle lifting patterns, the small flow rate through the electromagnetic coil ball cannot be attached to a different position of the armature and injector nozzle needle closing. A potential explanation can be connected with different density for B0 and B100. When the ball valve is opening, the flow rate is massively throttled by the input cataract which, in the ratio of areas considered, is significantly smaller than the whole area of the injector nozzle outlet orifice. This could cause redistribution of flows between the nozzle and the ball valve so that the higher percentage of the total input fuel amount flowing to the injector can flow through the injector nozzle orifices. These effects will be examined in detail.

Conclusion

The work focused on verification of how the FAME biocomponent in diesel fuel in concentrations of B0,B30,B50 and B100 affects the high-pressure injector behaviour. The measured data indicate that the biocomponent content neither affects the time setting of the Common Rail high-pressure injector nor has an effect on characteristic dynamic pressure patterns corresponding to diesel fuel. The measurement of the monitored quantities was carried out at the fuel temperature ranging from 38 to 40°C and therefore it cannot be expected that any higher temperatures would significantly change the system behaviour from the viewpoint of the dynamic pressure patterns and injector timing setting. Another goal of the work was to verify how sensing of the electromagnetic coil armature movement can be made and consequently, how the delays of the individual parts of the injector could be specified both in the course of the solenoid activation itself and the high-pressure injector nozzle closing. The measurement did not demonstrate any difference in characteristics between the pure diesel fuel and any biocomponent concentration which had been used. However, some partial design shortcomings related to the sensor installation were seen. These can be noticed from the armature lifting pattern itself. In particular, at the beginning and at the end of the armature movement the pattern is accompanied by plate bouncing from the armature itself in limit positions. This happens because of inertial masses of the plate and insufficient thrust between the sensor plate and the electromagnet armature. Consequently, as far as the measured values are concerned, only the beginning of the armature movement and its velocity (slope) can be taken into account. The other values cannot be considered as accurate. To measure a real armature lifting pattern, a fixed attachment between the sensor plate and the electromagnetic coil armature is a must. This will necessitate new design solutions for the needle movement sensor installation**.**

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Technical and economic indicators in the handling of mowed grass

MICHAL SUSTR, PETR DOSTAL
Department of Technology and Automobile Transport
Faculty of agronomy
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xsustr2@node.mendelu.cz
petr.dostal@mendelu.cz

Abstract: The thesis monitors the metering and evaluates the acceptable technical economic indicators of reapers and harvesters. The target of the practical part is to obtain data in order to compare the effectiveness of different horticultural tools. The comparison of these instruments is included.

Key-Words: green vegetation, reapers and harvesters, founding of garths, mulching, maintenance of green vegetation

Introduction

The principle of the right foundation of garth in parks is not only the preparing of ground before seeding and using of quality grass mixture but also the maintenance of grass during several weeks and months since its foundation. If there is any failure (often caused by saving of finances) it necessary to make an effort to establish the greenery according of investor´s request. [1, 2, 3]

The regularly mowing of grass areas supports tillering and contributes to plant density. [4, 5]

The mulching is a kind of a mowing when the grass is repeatedly cut by rotating knife. This type of mowing create grass sheet which isn't collected inside of rear grass catcher but it stays under the instrument in the garth. [6, 7]

The mulching is the process of recycling of grass and leafs which serves also as a fertilizer. The mulching declines the need of fertilizing in value of ¼. The humidity which is contained in the grass sheet declines the need of irrigation. [6, 7]

Material and Methods

Characteristic of maintained area

The monitored area of 11 353 m2 is situated in the business area in the centre of Brno neighbouring with the river Svratka. The main point of the area is an artificially contracted lake which is composed of three separated parts. The lake disposes of several basic functions (visualization of aesthetic element of the area and formation of ecosystem of the area, in the summer days it declines the temperature of the area and has a function of retention of rainwater. The main function is the ability of irrigation for green vegetation in the all area.

Tools

- Garden reaper LT 2223 CMA 2
- Trailer
- Garden rider FR 2218 FA
- Measuring cylinder
- Stopwatch MASTER JUNSO JS-6618
- Protective means

It is important to make sure that rotating knife is grinded every 40 motor hours (every 14 days in fully workload)

Both of those mowing instruments were tested in 12 acts of mowing. The cutter mechanism was set up in the high of 35 mm.

The high of garth cannot reach over than 150 mm. The degree of humidity has to be lower when the degree of wet grass. It is important to make sure that in the area of mowing there is not any object witch would intercept the mowing (for example parking cars).

The methods of measuring

To be able to repeat the measuring the following condition has to be satisfied:

- The size of flat surface is 0. 5 – 1. 5 ha
- Two mowers with vertical axis of rotation of power 10 – 20 KW
- Consumption of fuel in period of time

The evaluation will be determined by calculation of the power instrument.

- $\bar{x}$ - The arithmetic mean

$$\bar{x} = \frac{1}{n}(x_1 + x_2 \ldots + x_n) = \frac{1}{n}\sum_{i=1}^{n} x_i$$

- T_P – pulse width [h]
- S_P – the content of maintenance of the area [m^2]
- Q_{PHM} - the quantity of consumption of fuel [l]
- C_{PHM} – the price of fuel [CZK]
- N_{PHM} – the costs for fuel [CZK]

The cost of fuel is composed by product of the quantity of consumption and of the price of fuel.

$$N_{PHM} = Q_{PHM} \times C_{PHM} \quad [CZK]$$

Q_{Tp} - The quantity of consumption of fuel/one working hour [l]

The quantity of consumption of fuel/one working hour is the quotient of quantity of consumption of fuel/working time of the instrument.

$$Q_{Tp} = \frac{Q_{PHM}}{T_P} \quad [l]$$

NJ_{PHM}- the costs for 1 m^2 [CZK]

The cost for fuel for 1 m^2 is the quantity of the costs for fuel/the content of maintenance of the area.

$$NJ_{PHM} = \frac{N_{PHM}}{S_P} \quad [CZK]$$

QJ_{PHM}- the quantity of consumption of fuel for 1 m^2 [l]

The quantity of consumption of fuel for 1 m^2 is quotient of the quantity of consumption of fuel/the content of maintenance of the area.

$$QJ_{PHM} = \frac{Q_{PHM}}{S_P} \quad [l]$$

TJ_P- the time necessary for mowing 1 m^2 [s]

The time necessary for mowing 1m^2 is quotient of the working time of instrument/ the content of maintenance of the area.

$$TJ_P = \frac{T_P}{S_P} \quad [s]$$

Results and Discussion

The values which were measured and calculated from the process of mulching are demonstrated in the table 1.

Table 1 Measurement of mulching and its values

Order of mowing	T_p [h]	Q_{PHM} [l]	Q_{Tp} [l]	N_{PHM} [CZK]
1. moving	5.46	12.56	2.3	452.40
2. moving	5.57	12.25	2.2	441.45
3. moving	5.00	12.50	2.5	450.31
4. moving	5.33	12.79	2.4	460.83
5. moving	4.80	12.48	2.6	449.59
6. moving	5.40	12.96	2.4	466.88
$\bar{x}$	**5.26**	**12.6**	**2.4**	**453.58**

The values which were measured and calculated from the process of moving into the rear grass catcher are demonstrated in table 2.

Table 2 Total costs of mowing into the rear grass catcher (mowing + transport of mowed grass)

Order of mowing	T_p [h]	Q_{PHM} [l]	N_{PHM} [CZK]
1. moving	8.16	17.69	637.14
2. moving	8.14	18.15	654.00
3. moving	7.97	17.94	646.32
4. moving	8.05	16.91	609.00
5. moving	7.98	18.45	664.73
6. moving	8.02	17.91	645.06
$\bar{x}$	**8.05**	**17.84**	**642.71**

The difference of saved fuel in between of two different instruments FR 2218 FA (mulching system) and LT 2223 CMA 2 (mowing in to rear grass catcher) is 5.24 litres. According of the price of natural 95 (2013) 36.025 CZK the saved finances are 189.13 CZK. FR 2218 FA is able to mow the grass area during 5 hours and 16 minutes in comparison to LT 2223 CMA 2. This mowing followed by discharging of second instrument takes 8 hours and 3 minutes. The difference is 2 hours and 47 minutes. It is demonstrated in the following figure 1, 2 and 3.

Fig. 1 The total comparison of consumption of fuel in between of LT 2223 CMA 2 and FR 2218 FA (in litres)

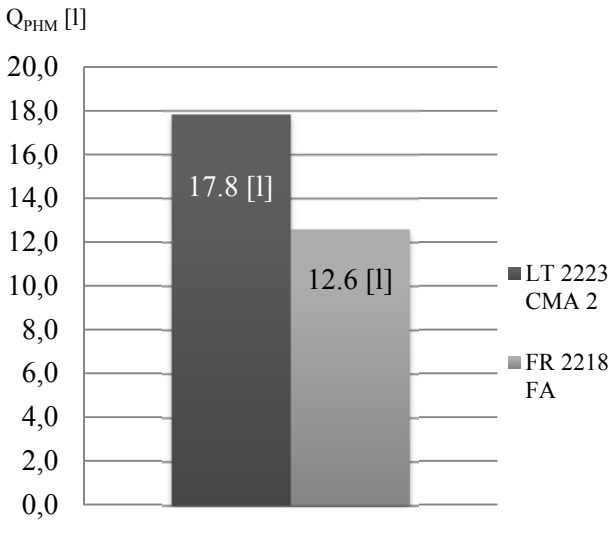


Fig. 2 The total comparison of time of completion between of LT 2223 CMA 2 and FR 2218 FA (in hours)

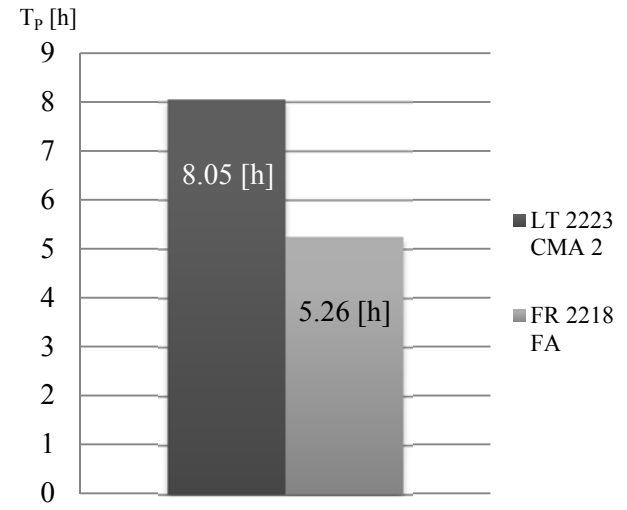
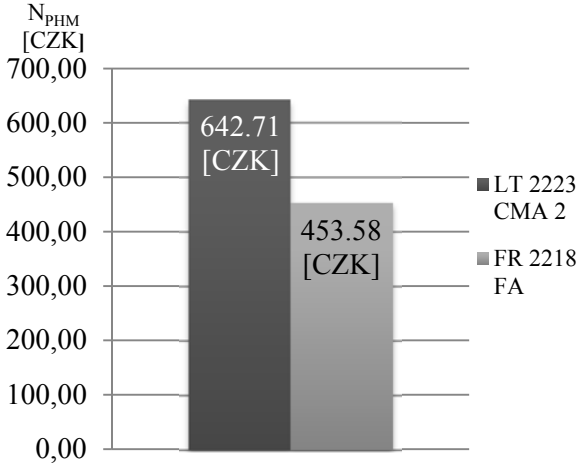


Fig. 3 The total comparison of total costs between of LT 2223 CMA 2 and FR 2218 FA (in CZK)



According of all results of measuring the mulching of greenery is technically-economic more profitable act. If the mulching should be used on different areas of greenery and should reach the same values of saving it is necessary to use an appropriately prepared area and keep the principles of mulching. The results say that using of rear grass catcher and following discharge of the catcher is more expensive than mulching.

According of the primary judgment was supposed that the act of mulching was energetic more exacting and its average of value of consumption was higher. It was caused by dividing of mass of the grass.

According of result it is perceptible that the average consumption of fuel increased. This growth is inconsiderable. During the mowing act into the rear grass catcher the total weight of the instrument changes and it causes the growth of consumption of fuel. The mowing act into the rear grass catcher causes the lost time (the time for discharge), the total working time of the instrument is about 35% longer than the time of using of the mulching instrument. The instrument using the rear grass catcher has to work on a wider distance. It is necessary for transport of mowed mass. The growth of consumption of fuel is about 20 % higher than using of the mulching instrument.

The basic problem which causes an abysmal difference in respect of finances between mowing into the rear grass catcher and mulching can be obvious on example of different recycling possibilities. If there is not any possible place for collecting of mowed grass in the neighbourhood area or if the capacity of this place is not satisfactory in comparison with the size of maintenance area it is necessary to ensure a container, its transport and following recycling of mowed grass. The price of one container is in between of 2 500 CZK and 5 000 CZK. The price contains recycling and transport of waist. This amount can be added to costs of mowing in to the rear grass catcher.

Conversion of units

To be able to apply the resulting values for different maintained grass areas as well, I accomplished the conversion of resulting values into the units. The resulting values are demonstrated in the table 3 and 4.

Table 3 Conversion of values (mowing in to the rear grass catcher and transport of mowed grass)

NJ_{PHM} [CZK]	QJ_{PHM} 1 m^2 [l]	TJ_p [s]
0.057	0.0016	2.55

Table 4 Conversion of values (mulching)

NJ_{PHM} [CZK]	QJ_{PHM} 1 m^2 [l]	TJ_p [s]
0.040	0.0011	1.67

These values can be used for the grass areas which have similar parameters as the monitored area.

Conclusion

The results of measuring confirmed that the act of mulching saves about 35% of time and 20% of consumption of fuel in comparison with the act of mowing into the **r**ear grass catcher.

If the mulching should be used on another grass areas and the mulching should reach the same amount of saved finances it is necessary to have prepared an

appropriately area and follow of instruction of mulching.

The results say that the mowing with the rear grass catcher with following discharge of mowed grass is more expensive than the mulching. In addition the mowing grass catcher with following discharge takes nutrients from the grass area off. It causes a requirement of fertilizer which takes the nutrients back to the grass.

Acknowledgement
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Construction development of school furniture for developing countries

JAROSLAV ZACAL, MILAN SIMEK, PETR DOSTAL
Department of Technology and Automobile Transport, Faculty of Agronomy
MendelUniversity in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

xzacal@mendelu.cz

Abstract: Aim of this work is construction development of school seats for developing countries from locally available resources using the local industry and low-end technology. Functional school furniture contributes to basic comfort of pupils and therefore facilitates the learning process. Aim of diploma thesis is experimental assessment of mechanical properties of joints flexion in angular plane. Furthermore thesis considers assessment of joint firmness and comparison between various joint types. Thesis encloses the review of various joint manufacturing designs (tenon and mortise), which were proposed for its suitable mechanical properties and simplicity of manufacturing. Designated joint type is easiest form of joint construction and technologically feasible for chair manufacturing. The joints were constructed from fir wood (*Abies alba Mill)*.

Key-Words: tenon, mortise, furniture construction, bending test, transversal plane, manufacturing technology

Introduction

School seat construction from locally available resources is a priority for developing countries [1]. Leading advantages of this approach are shortening of supply chains, creating the maximal value of raw material and employing the local workforce in proximity of demand. Theoretical basis of this technology was proposed by [2], who implies the possibilities of application without sophisticated technologies. Method was tested in practice by [3]. Leading objective of this project was the design and practical construction of seats and chairs for schools in Zambia from locally available materials using the local industry and low-end technology. Design of simple wooden chair construction was performed using the "Greenwood chair" technology with use of manual woodworking tools commonly available in Czech Republic [4] introduces five basic schemes for furniture design. He favours the approach solving the joint dimensioning to safely withstand the internal forces as well as external load that affects the furniture in course of use. For simplification it is possible to claim that regardless of large scale of shapes each piece of furniture is basically designed using frame construction. One of fundamental perks for chair construction is the joint design. These joints have to be easily producible and composable. Therefore furniture design has to adapt the simple construction scheme and has to be adapted for use of simple manufacturing technology [1]. Fixation system is supposed to facilitate the exchange of individual furniture components.

School seating furniture should be designed in most effective way considering the use of developing country resources and simultaneously to assure the optimal performance. Due to present economic status in education – not only in Zambia – one of leading aspects for consideration is the acquisition price of school furniture. That in certain levels affects both appearance and ergonomic characteristics and quality of school furniture.

It is expected that school furniture is designed for maximal lifespan, easy maintenance and repairs. It should also be easily composable from individual parts and designed as modular system with possibility to exchange the individual components in repair. Joints in the seating furniture could be manufactured as demountable without use of adhesives.

Another important factor for furniture construction are safety criteria, on whose basis the furniture has to achieve very high level of passive safety, considering both construction (stability, rounded edges, durability, joint sturdiness) and used material.

Material and Methods

Construction material could be obtained from conventional sources, e.g. lumber from sawmills. Many parts could be obtained from wooden scrap; other parts could be manufactured from reused profiles like pallets, crates, cabinets etc. Another

option is purposive cultivation of fast-rotation coppice, whose thin trunk could be used as chair legs without subsequent turning.

Abies alba Mill. – This material, although not typical for designated country, was chosen for manufacturing of experimental samples for its mechanical properties. Namely for low flexibility module and different mechanical properties of earlywood and latewood. Mentioned properties helped adequately dimension the joint parameters to achieve the sturdy and durable connection of furniture components. Mechanical properties of *Abies alba Mill* wood helped to stimulate the use of low-quality material from local sources in developing countries and occasional technological mistakes in processing by incompetent staff. Despite these adverse factors the sufficient technical characteristics were achieved for proposed joints. Material available in target countries demonstrates the better mechanical properties, therefore is considered suitable for proposed use.

Manufactured testing samples represent the central joint used in seating furniture. For convenient manufacturing of tenon the plug cutter was used – it is a tool for cutting the plugs from transversal profiles for repairs of knot holes after drilling out the knot. Mortise was manufactured with forstner drill Fig. 1 which is a peripheral drillbit with segmented head consisting of two main edges, centering spike and two separate pre-cutters, which are easy to sharpen. Large opening for splinters in the drillbit extracts the splinters from the manufactured piece. Both these drillbits are used for drilling of the compact wood and also to drill out the loose knots or construction of simple tenon joints.

Fig. 1 Joint production scheme: (left) forstner drill bit – mortise, (right) plug cutter bit – tenon

Sample description

Joint construction was performed in variants of round tenon/partial tenon and mortise as designated simplest and easy-to-manufacture type of chair component joint. The joints were manufactured from fir wood. Samples are constituted of two parts, which were joined in T-shape. Samples were conditioned in air temperature 24±2°C and 50±5% relative air humidity. Length of tenon should relate to counterpart thickness in the manner of slightly overreaching the edge of mortise. Too long tenon needs to be trimmed. Round tenon overreaching the counterpart doesn't have sufficient sturdiness, because after hammering the wedge into the groove the wedge isn't inserted in its full length.

Fig. 2 Through tenon (sample A)

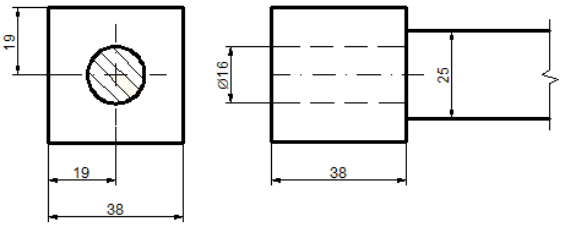


Fig. 3 Partial tenon (sample B)

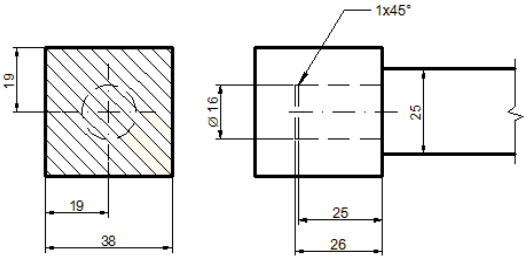
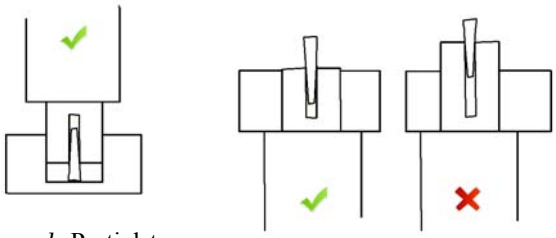


Fig. 4 Round tenon



Legend: Partial tenon
Joint performance (left) correct (right) incorrect

Testing method

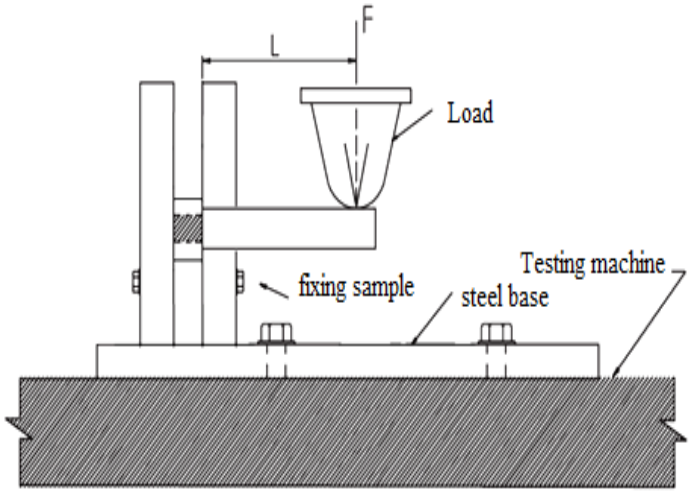
Experimental testing of furniture joints in industry was performed by [4] and [5] their research was focused on simple joints like pin, tenon and mortise for frame construction of massive wood. Experiment is designed to assess the joint behaviour in transversal plane, its sturdiness and shift under pressure with regard to parameters of designated material. Methodology of experiment was used according to [5] for assessment of joint sturdiness in transversal plane. Despite the lack of valid technical standard the method is commonly used for mutual comparison of testing results. Method was further used by numerous

authors e. g. [6, 7]. Testing was performed on Universal Testing Machine (SHIMADZU UH-300kNA). From previous experiments the optimal velocity of 10 mm/min was deduced to achieve the maximum load in 90 s ± 30 s. Method of experiment was used as described by [5]. Testing samples were divided into 8 groups. Groups no. 1 and 4 were manufactured with round tenon and groups no. 5 and 8 were constructed with partial tenon Tab. 1. For comparison of measured values the control samples were manufactured with use of adhesive and without the wedge, where final sturdiness correlates to D2 adhesive class. The joint was constructed as tenon and mortise, whose main feature is the time and cost efficiency of firm joint construction. Use of wedge for fixing the components appears as a rational alternative in conditions eliminating the possibility of the use of adhesives.

Table 1 Groups of tenon samples

Type of joint		dimension in mm	Numbers of samples
With wedge	D	25x38	12
	C	25x38	12
	B	25x25	12
	A	25x25	12
With glue	D	25x38	5
	C	25x38	5
	B	25x25	5
	A	25x25	5

Fig. 5 Scheme of fastening the samples in testing device



Results and Discussion

Table provides direct comparison of resulting firmness for individual sample types. Mentioned results and firmness division are described also by Angular coefficient 10–40%. In the experiment it is necessary to take into account the thickness of adhesive layer, material type, roughness of glued surface or component preparation previous to gluing. According to Tab. 2 there are obvious differences between glued and unglued samples. In second phase of the measurement the results indicated higher sturdiness for round tenon and round trestle.

Table 2 Angular coefficient 10–40%

Type of joint	Unglued (wedge)	Glued
A	y = 132,9x + 0,330	y = 242,5x + 0,446
B	y = 131,5x + 0,526	y = 152,9x + 1,419
C	y = 140,7x + 0,860	y = 166,8x + 0,859
D	y = 153,9x + 0,306	y = 266,8x + 0,323

For designing the optimal variant of joining the components the least favourable condition of stress should be taken into account, in our case the variant with least sturdiness and durability was achieved in impassable tenon. In my opinion it is best to discard these joint types for technical difficulty in manufacturing, therefore these variants are not further considered. For comparison data measured by [5] and [7] were used, who performed the same testing with application of adhesive and different dimensional parameters of joint stressed in angular plane. Result comparison is interpreted in Tab. 3.

When comparing results for carrying capacity in variants samples A glued / unglued – 31.78 / 28.35 N·m (difference 3.43 N·m) and samples C glued / unglued – 35.14 / 32.69 N·m (difference 2.45 N·m), the setup of joints is in this case very similar. In comparison with results published by [5] for dependency between carrying capacity in angular plane and tenon width, it is obvious that tested samples have higher carrying capacity compared to spruce wood samples. This claim ensues that used non-glued (wedged) and glued samples achieve the sufficient carrying capacity in angular plane and this setup is suitable for required parameters.

In comparison with results reviewed by [7] it is not possible to compare the data in full scale. Estimated experiment assessed the mechanical properties in angular plane using the PVAc adhesive N203, where final sturdiness values correspond to D3 class for populus and fagus wood.

Table 3 Measured values in angular plane according to various authors.

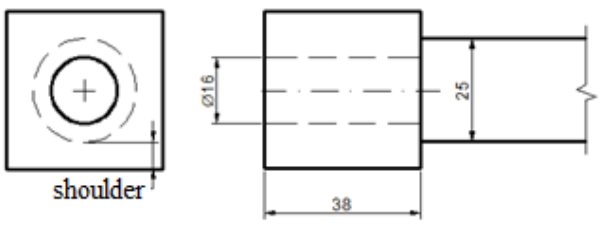
Author	Through tenon/ Variant	Wood	Length of tennon in mm	Bending moment in N·m	Glue / recommended values
Kamperidou [7]	Through tenon	Fagus	30	91.42	PVAc N203 (D3)
		Populus	30	44.38	
Joščák [5]	Through tenon	Fagus	40	48.76	recommended values
		Picea abies	40	23.07	
With glue	A	Abies alba	38	31.78	PVAcRakoll (D2)
	C	Abies alba	38	35.14	
With wedge	A	Abies alba	38	28.35	
	C	Abies alba	38	32.69	

The results also imply the necessity to take into account the effect of adjacent lever arm of sample when pressed against the counterpart Fig. 6. Previous experiments confirmed the effect of lever arm, where round tenon samples C, D with dimension 25 x 38 mm shows higher values of carrying capacity. The results show that adjacent lever arms could substantially enhance the joint sturdiness. This confirms the theory of "inner arm effect" in the sample on final sturdiness value.
If the inserted piece has sufficient area to lean against the counterpiece in course of stress, the tension shows directly in the joints, resulting in deforming the mortise and breaking / pulling out the tenon.

Construction of joints affects not only the final piece, but in construction and furniture manufacturing it is important to pay attention to manufacturability and most importantly the usability of finished piece. The sturdiness of joints depends on tenon diameter, inserting depth into the counterpiece and also on material used. Influence of variable joint length correlated with its constant diameter is significant for its sturdiness. In prevalent
cases there is an effort to achieve the maximal length and diameter of tenon to ensure the best sturdiness characteristics.

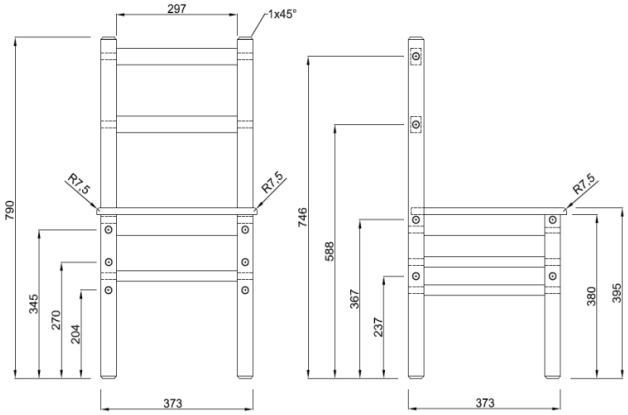
Joints manufactured in this experiment were used to dimension the simple construction method to achieve the required sturdiness.

Fig. 6 Representation clingy arm



Chairs construction
In real development of particular product it is necessary to first manufacture the functional prototype Fig. 7. Then the individual details are considered and altered, including the exact dimensions and proportions of final product, as well as its individual parts. Prototype is often manufactured with some compromises like financial budget. For these reasons it is necessary to consider the final documentation with tolerance. It is possible, that the final product will be further modified.

Fig. 7 Chair documentation



Conclusion

Aim of this work was the design of constructing solution for school furniture in Zambia, manufactured by local industry from locally available resources and employing the low-end technology. The hypotheses are following:

- Is it possible to manufacture the school furniture from locally available materials?
- Is technologically possible to simplify the manufacturing processes in scale allowing
- the local production of the furniture in target

- developing countries?

First I was looking for a simple way to produce furniture in our history. With advancing my knowledge of given issue I found out a possibility to manufacture the seating furniture with use of simple „Greenwood chair“ construction using only the simple woodworking tools.

Considering the material it is possible to produce the school furniture from wood scraps, old pallets or pickets from forest thinning. Parts required for construction could be cut from sawmill trimmings.

In realisation the main objective was to create the economically available and easy-to-produce seating furniture. Material and energy costs invested in furniture production should reflect its durability and lifespan. Therefore it is necessary to maximize the lifespan and utility value in design and production.

Possibility of replacement of individual construction elements decreases the amount of material needed for chair repairs and in a certain manner facilitates the manufacturing process. These advantages allow to repair the construction in case of turning the joint loose.

In chair construction the broad spectrum of tenon joints is used. This type is regarded as one of the oldest ways of seating furniture joints solution and simultaneously it is regarded as the most efficient due to its sturdiness characteristics. In spite of construction design diversity it is not possible to judge that one joint solution is inadequate or less sturdy than another, because the position of joint in construction is always a determining factor. Experiment was aimed on joint durability and carrying capacity measurement angular plane, as well as its resistance to turning. Length of tenon affects the joint durability. In performed tests better sturdiness characteristics in round tenon trestle, samples A, C were found, which show better sturdiness compared to partial tenon variant. Further the acquired data show that technological and temporal expenses of partial tenon construction variant are undesirable.

Use of these joints produced with low-end technology displays a great potential for developing countries; it is considered to be one of the easiest joints to produce, not requiring any qualification to assemble.

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