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The influence of different ways of alfalfa (*Medicago sativa* L.) stands cultivation on its yield capacity

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ABSTRACT

The influence of different terms (spring, after the 1st, 2nd or 3rd cuts) and ways of cultivation (spike harrow, vibration harrow) on yield capacity of alfalfa (*Medicago sativa* L.) cv. Zuzana was studied in a plot polyfactorial trial in Červený Újezd 405 m above the sea level, on clay loam orthic luvisol in the years 1997–1999. Alfalfa was sown in the spring without a companion crop to rows of a space 125 mm, sowing rate 16 kg.ha^{-1} germination seeds. The trial had 10 replications and it was cut 3 times per year. The total yield of dry above-ground mass in the 2nd year of vegetation was not significantly influenced by harrow cultivation. The efficient cultivation of the stands by vibration harrow to the depth 70 mm after the 2nd or 3rd cuts resulted in significant decrease of dry mass yield in the 3rd year of vegetation by 7.2–21.1%, (in average by 11%) in comparison with the control variant. The amount of stems per 1 m^2 in the cultivated plots was mostly lower than in the control in average by 1.2–8.8% in the 2nd year of vegetation. Sporadic higher amount of stems in the cultivated plots in individual cuts did not result in their higher total amount in the 3rd year. The amount of plants per 1 m^2 was not significantly influenced by cultivation during 3 years.

Keywords: alfalfa; soil compaction; cultivation; stand density; yield

The negative influence of soil compaction on growth and development of alfalfa is generally known and it was studied in various trials that also clarified some principals of its influencing – soil air deficiency that affects water and nutrients uptake (Rechel et al. 1991b), worse conditions for molecular N fixation by rhizobial bacteria (Grath and Hakansson 1992), worse conditions for alfalfa overgrowing from underground buds (Velich 1971). It follows from other aspects that result from the reasons of soil compaction during the years of alfalfa vegetation. It means harvest machinery crossing and damaging of roots, crowns and stems (Rechel et al. 1991b; Šantrůček and Svobodová 1992), that cause higher consumption of reserve substances, lower effect of water uptake (Rechel et al. 1991a) and higher root and head diseases (Šantrůček and Svobodová 1992). The influence of different cultivation treatments on aeration of the soil top layer in the zone of heads and on alfalfa sprouting from underground buds has been studied in the Czech Republic many years. Especially the harrowing in the spring or after the 1st cut is regularly done in most of farms in the Czech Republic. In various trials of the Department of Forage Crops Production (Šantrůček 1989a, b, 1990; Šantrůček and Svobodová 1996; Šantrůček et al. 1997) it was found out that especially the harrowing of dense and complete stands by spike harrow results neither in effective aeration of the soil top layer, nor in yields increasing, for all that in some cases the number of stems was increased. The reason was their lower weight or higher share (by 4–22%) of very small stems that does not make the yield (Svobodová et al. 1997). Besides it, the harrowing and other ways of cultivation mean additional crossing over the plants and their mechanical injury with all the consequences mentioned above.

MATERIAL AND METHODS

A polyfactorial field trial with alfalfa (*Medicago sativa* L.) cultivar Zuzana with higher resistance against root and head diseases was conducted in the Research Station of the Czech University of Agriculture in Prague in Červený Újezd 405 m above the sea level, on clay loam orthic luvisol in the years 1997–1999. The alfalfa was sown in the spring without a companion crop to rows of a space 125 mm, sowing rate 16 kg.ha^{-1} germination seeds. The trial had 10 replications and it was cut 3 times per year by a hand mower MF 70. A part of the field was left without a treatment, the rest of the area was cultivated by a spike or vibration harrow to the depth 70 mm before the beginning of vegetation period, after the 1st, 2nd or 3rd cut (a control and 8 variants). The stand was cut 3 times per year by a hand mower MF 70.

Soil bulk density after drying (Or) by physical cylinders (25 samples per 1 variant), number of plants and stems per 1 m^2 on randomly chosen areas (8 times 0.125 m^2) and dry mass yields (10 m^2 in each plot) were measured.

The results were evaluated by analysis of variance by the Statgraphics programme, version 4.2, by Tukey.

RESULTS AND DISCUSSION

The average number of plants in the autumn of the 1st year of vegetation was 273 pieces per 1 m^2 on all the experimental area (average of 30 measuring, ±2%). The dry mass yield of 2 cuts in the same year was in average 5.31 t.ha^{-1}.

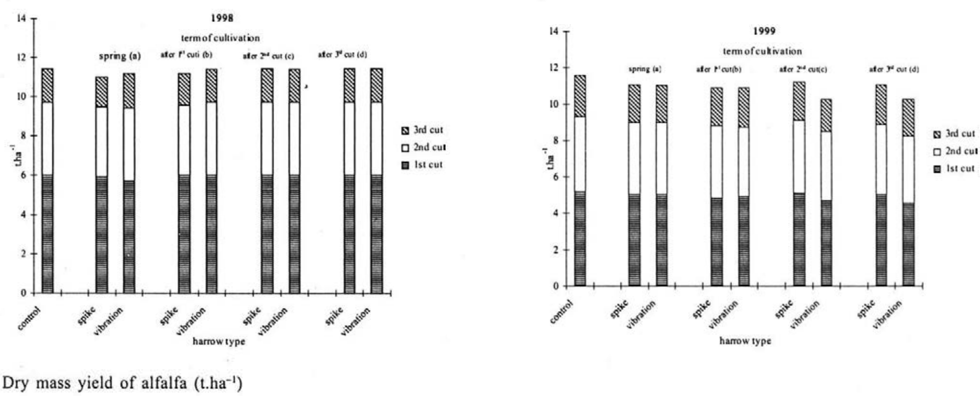
The soil bulk density was 1.29 g.cm^{-3} in the 2nd year of vegetation before cultivation by the spike or vibration harrow, what corresponds to the usual soil compaction in practice in similar conditions (soil type, year of vegetation, season of year, etc.)

Immediately after the cultivation by the vibration harrow the soil bulk density in the level of alfalfa heads (10–60 mm above the soil surface) decreased to 1.11 g.cm^{-3}, or 1.25 g.cm^{-3} (by the spike harrow), resp. The insufficient effect of spike harrow for cultivation of alfalfa stands was also found in our earlier trials (Šantrůček 1989a). In the autumn and in the early spring of the following year no significant differences were found between the soil bulk density of harrowed plots (spike harrow 1.47 g.cm^{-3}) and control variant (1.45 g.cm^{-3}). There was a lower soil bulk density (in average by 0.05 g.cm^{-3}) in comparison with the control in the plots cultivated by vibration harrow without regard to the term of harrowing, but the differences were not significant. It has been shown that during cuts (crossing by machines) and during the time the soil bulk density of the surface layer was equalised (natural compression) (Šantrůček 1989b) and the effect of the soil surface cultivation is only short.

The total above-ground dry mass yield in the second year of vegetation was not significantly influenced by the cultivation (Table 1, Fig. 1), but it was always (at all ways and terms of cultivation) lower by 1.5–5.7% than in the control plots. This negative influence of the cultivation manifested especially after spring harrowing by spike harrow with a time delay, it means the yield de-

Table 1. Yield of alfalfa dry mass – relatively (control = 100%)

Term of cultivation	Harrow type	Cut/Year								Σ 1998 + 1999
		1./1998	2./1998	3./1998	Σ 1998	1./1999	2./1999	3./1999	Σ 1999	
Control	(t.ha^{-1})	(6.01)	(3.71)	(1.69)	(11.41)	(5.19)	(4.12)	(2.24)	(11.55)	(22.96)
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Spring	spike	98.3	95.4	91.5	96.3	97.8	94.5	92.5	95.6	96.0
	vibration	95.1	99.9	102.5	97.7	97.7	94.9	90.2	95.2	96.5
After 1st cut	spike	100.0	95.6	94.0	97.7	93.5	96.0	92.0	94.1	95.9
	vibration	100.0	99.5	99.6	99.8	95.1	**92.1**	95.7	94.2	97.0
After 2nd cut	spike	100.0	100.0	99.9	100.0	98.5	96.1	95.2	97.0	98.5
	vibration	100.0	100.0	98.0	99.7	**90.3**	**92.8**	**78.9**	**89.0**	94.3
After 3rd cut	spike	100.0	100.0	100.0	100.0	97.0	**92.4**	98.8	95.7	97.8
	vibration	100.0	100.0	100.0	100.0	**87.6**	**89.3**	90.5	**88.8**	94.4
D_{min} (α 0.05)	t.ha^{-1}	0.51	0.31	0.31	0.64	0.51	0.31	0.39	0.95	3.84
	% of control	8.49	8.36	18.05	5.60	9.76	7.54	17.32	8.21	16.70
Average	spike	99.6	97.7	96.3	98.5	96.7	94.7	94.6	95.6	97.0
	vibration	98.8	99.8	100.0	99.3	92.7	92.3	88.8	91.8	95.5
Spring	average	99.6	97.6	96.4	98.5	96.8	94.6	94.6	95.6	97.0
After 1st cut		98.8	99.7	100.1	99.3	92.7	92.2	88.8	91.8	95.5
After 2nd cut		100.0	98.2	97.7	99.1	96.5	94.7	95.1	95.6	97.3
After 3rd cut		99.8	99.7	99.5	99.7	91.5	91.5	88.5	90.9	95.3



1. Dry mass yield of alfalfa (t.ha^{-1})

creased more in the 2nd and 3rd cut so in the 2nd as in the 3rd year of vegetation. A similar tendency, it means a negative influence of this treatment in next cut but with the influence till the next year is noticeable also at the other terms of cultivation. The cultivation by vibration harrow is more efficient treatment. It caused more intensive cultivation of the top level of the soil, but the yield was not increased neither by it. On the contrary, its in average negative influence on the stand can be observed (also with a time delay) especially at the cultivation after the 2nd and 3rd cut, that resulted in significant yield decreasing of the individual cuts in the following year of vegetation by 7.2–21.1% (in average of the 3rd year of vegetation by 11%). The stems formation (number per m^2) was more influenced by cultivation in the 2nd year of vegetation (Table 2, Fig. 2) than the yield.

With respect to this time shift we can expect that one of the possible reasons can be the injury of plant organs and following development of infestation especially by the diseases of heads and roots (Šantrůček and Svobodová 1992).

The number of stems, like the dry mass yield, was not significantly influenced by cultivation during 3 years (Table 2). At the end of the 3rd year of vegetation the number of plants in the cultivated plots decreased in average by 40% in comparison with the 1st year equally like in the control variant.

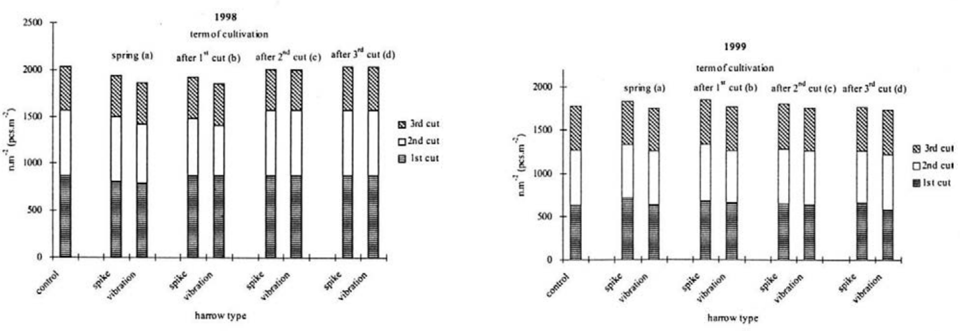
In the 2nd year of vegetation the stems formation (number per m^2) was influenced by cultivation more than the dry mass yield (Table 3, Fig. 2). The number of stems was always reduced immediately after each cut (using the spike harrow in average by 8%).

Harrowing by the spike harrow after the 1st cut significantly reduced the number of stems by 12.5% in comparison with the control in the 2nd cut. The vibration harrow damaged the stand in the 2nd year of vegetation even more considerably – the number of stems was significantly lower by 8–22% in comparison with the control variant in the following cuts after the cultivation. The reduction of the number of stems was also manifested in the next cuts of the 2nd year of vegetation of the stand. In the 3rd year of vegetation the number of stems in the particular variants was more uniform, sometimes even higher than in the control (by 1.7–7% in the plots cultivated in the previous year, by 12.4% higher in the plots cultivated by the spike harrow before the beginning of vegetation). But the differences were not statistically significant. As it was proved also in this trial, the soil surface in alfalfa stands is very compacted in the 3rd year of vegetation and its aeration for better stems formation from heads often results in increasing number of stems, but it mostly does not result in a higher yield (Šantrůček and Svobodová 1996). The influence of the cultivation without regard to the term was not significant in the 2nd and 3rd cut.

It can be said, on the basis of the results of this trial, that they correspond with our many previous ones received from field small plot trials and trials in production fields, that all the efforts of the agricultural practice and special institutes try to find a suitable way and term of cultivation of dense and complete alfalfa stands without weeds during the vegetation period, that would result in

Table 2. Number of plants per m^2 in the years 1997–1999

Term of cultivation	Harrow type	1997	1999	
		pcs.m^{-2}	pcs.m^{-2}	%
Control		273	164.0	100.0
Spring	spike		168.0	102.4
	vibration		172.5	105.2
After 1st cut	spike		163.0	99.4
	vibration		161.5	98.4
After 2nd cut	spike		175.0	107.0
	vibration		143.5	87.5
After 3rd cut	spike		157.5	96.0
	vibration		162.0	98.8
D_{min} (α 0.05)			29.08	17.0
Average	spike		165.9	101.2
	vibration		159.9	97.5
Spring	average		170.3	103.8
After 1st cut			162.3	98.9
After 2nd cut			159.3	97.3
After 3rd cut			159.8	97.4



2. Number of stems per m^2

Table 3. Number of stems per m² – relatively (control = 100%)

Term of cultivation	Harrow type	Cut/Year								Σ 1998 + 1999
		1./1998	2./1998	3./1998	Σ 1998	1./1999	2./1999	3./1999	Σ 1999	
Control	($n.m^{-2}$)	(870)	(692)	(470)	(2032)	(633)	(636)	(505)	(1774)	(3806)
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Spring	spike	93.8	97.8	95.2	95.5	112.4	98.4	98.1	103.3	99.1
	vibration	**91.9**	89.0	93.8	91.3	101.6	97.3	98.7	99.2	95.0
After 1st cut	spike	100.0	**87.5**	94.5	94.5	107.0	103.7	102.0	104.4	99.1
	vibration	100.0	**77.9**	94.5	91.2	104.3	95.0	99.1	99.5	95.1
After 2nd cut	spike	100.0	100.0	94.8	98.8	102.0	99.7	103.2	101.5	100.1
	vibration	100.0	100.0	92.0	98.1	101.6	97.1	98.9	99.2	98.6
After 3rd cut	spike	100.0	100.0	100.0	100.0	104.1	95.2	100.2	99.8	99.9
	vibration	100.0	100.0	100.0	100.0	92.4	100.1	103.4	98.3	99.2
D_{min} (α 0.05)	% of control	67.5	81.0	50.9	180.8	91.2	111.0	77.5	218.2	510.0
		7.8	11.7	10.8	8.9	14.4	17.5	15.3	12.3	13.4
Average	spike	98.4	96.3	96.1	97.2	101.9	98.8	101.2	101.2	99.5
	vibration	98.0	91.7	95.1	95.2	95.0	95.6	99.0	97.6	97.0
Spring	average	92.8	93.3	94.4	93.4	107.1	97.8	98.4	101.3	97.1
After 1st cut		100.0	82.7	94.4	92.8	105.7	99.3	100.6	101.9	97.1
After 2nd cut		100.0	99.9	93.3	98.5	101.8	98.4	101.1	100.4	99.3
After 3rd cut		100.0	99.9	99.9	100.0	98.3	97.6	101.8	99.0	99.5

dry mass yield increasing and that would be economically effective, will not be probably successful under this contemporary economic situation, with the assortment of cultivars and machines for cultivation. We find useful testing of other stand cultivation methods of new alfalfa cultivars with higher resistance against diseases of heads and roots. Harrowing or other ways of cultivation of alfalfa stands during the vegetation period are not studied in other countries with similar climate, soil and economic conditions. The research is aimed more at the field of the soil protection against excessive compaction or using of track rows and narrow wheels of agricultural machines with unified gauge for agricultural crops treatment during vegetation period (Hakansson 1990).

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ABSTRAKT

Vliv různých termínů a způsobů kultivace porostů vojtěšky seté (*Medicago sativa* L.) na její výnosovou schopnost

V maloparcelovém polyfaktoriálním pokusu v řepařské výrobní oblasti 405 m n. m., na jílovitohlinité hnědozemi byl v letech 1997 až 1999 sledován vliv různých termínů (jaro, po 1., 2. a 3. seči) a způsobů kultivace (hřebové brány nebo vibrační brány – zahloubení 70 mm) na výnosovou schopnost vojtěšky seté odrůdy Zuzana, se zvýšenou rezistencí proti chorobám kořene a kořenových krčků, vyseté na jaře bez krycí plodiny do řádků s roztečí 125 mm, s výsevkem 16 $kg.ha^{-1}$ klíčivých semen. Pokus měl 10 opakování a byl sklízen trojsečně ručním žacím strojem MF-70. Byla sledována objemová hmotnost půdy (Or) fyzikálními válečky (25 odběrů na jedné variantě), počet rostlin a lodyh na 1 m^2 na náhodně vybraných ploškách 8 × 0,125 m^2, výnos suché hmoty (z 10 m^2 na každé parcele). Průměrný počet rostlin na podzim v 1. roce vegetace byl na celé pokusné ploše 273 rostlin na 1 m^2 (průměr 30 měření, ±2 %). Výnos sušiny dvou sečí v témže roce činil v průměru 5,31 $t.ha^{-1}$. Ve 2. roce vegetace před provedením kultivace porostu hřebovými, resp. vibračními bránami byla objemová hmotnost půdy 1,29 $g.cm^{-3}$, což odpovídá zhutnění půdy obvyklému v praxi v obdobných podmínkách (půdní druh, rok vegetace, roční období aj.). Bezprostředně po kultivaci vibračními bránami se Or půdy v zóně kořenových krčků (10 až 60 mm pod povrchem půdy) snížila na 1,11 $g.cm^{-3}$, vlivem hřebových bran na 1,25 $g.cm^{-3}$. Na podzim a v předjaří následujícího roku již nebyly patrné žádné průkazné rozdíly mezi objemovou hmotností půdy redukovanou na parcelách vláčených hřebovými bránami (1,47 $g.cm^{-3}$) ve srovnání s kontrolou (1,45 $g.cm^{-3}$). Parcely vláčené vibračními bránami bez ohledu na termín provedení vykazovaly Or v průměru o 0,05 $g.cm^{-3}$ nižší ve srovnání s kontrolou (neprůkazné). Celkový výnos sušiny nadzemní hmoty ve 2. roce vegetace nebyl kultivačními zásahy průkazně ovlivněn (tab. 1, obr. 1). Ve všech termínech kultivace a u obou použitých typů kultivačního nářadí byl však nižší (o 1,5 až 5,7 %) než na nekypřené ploše. Tento negativní vliv kultivace se projevil především u jarního vláčení hřebovými bránami s určitým časovým skluzem, tj. výnos byl výrazněji snížen až u 2. a 3. seče, jak ve 2. tak ve 3. roce vegetace. Obdobná tendence, tj. negativní vliv tohoto zásahu ob jednu seč s účinkem do následujícího roku je patrný i u ostatních termínů vláčení. Kultivace vibračními bránami je intenzivnější zásah, způsobující větší prokypření povrchu půdy. Přesto ani jejím vlivem nedošlo ke zvýšení výnosu píce. Naopak lze pozorovat její v průměru větší negativní působení na porost rovněž s určitým časovým posunem, zejména při kultivacích porostů po 2. a 3. seči, které měly za následek průkazné snížení výnosů jednotlivých sečí v následujícím roce o 7,2 až 21,1 % (v průměru za 3. rok vegetace o 11 %). Tvorba lodyh (počet na 1 m^2) byla kultivací ovlivněna ve 2. roce vegetace výrazněji (tab. 2, obr. 2) než výnos hmoty. Docházelo bez výjimky ke snížení počtu lodyh bezprostředně v následující seči po kultivaci – při použití hřebových bran v průměru o 8%. Při vláčení porostu hřebovými bránami po 1. seči byl počet lodyh ve 2. seči průkazně snížen o 12,5 %. Vibrační brány poškozovaly ve 2. roce vegetace porost ještě výrazněji – počet lodyh v následujících sečích po zásahu byl průkazně nižší o 8 až 22,1 % ve srovnání s kontrolou. Snížení počtu lodyh se projevovalo i v dalších sečích 2. roku vegetace porostu. Ve 3. roce vegetace byl počet lodyh na jednotlivých variantách vyrovnanější a někdy dokonce vyšší než na kontrole (o 1,7 až 7 % u parcel kultivovaných v minulém roce, o 12,4 % vyšší u parcel vláčených hřebovými bránami před začátkem vegetace). Rozdíly však nebyly statisticky průkazné. Ve 2. a 3. seči 3. roku vegetace byl vliv kultivací (bez ohledu na jejich termíny) na počet lodyh zanedbatelný. Počet rostlin nebyl v průběhu 3 let kultivačními zásahy průkazně ovlivněn (tab. 3). Na konci 3. roku vegetace se snížil počet rostlin na kultivovaných plochách ve srovnání s 1. rokem v průměru o 40 % stejně jako na nevláčené kontrole. Výsledky byly vyhodnoceny analýzou rozptylu v programu Statgraphics, verze 4.2., metoda podle Tukeye.

Klíčová slova: vojtěška setá; zhutnění půdy; kultivace; hustota porostu; výnos

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Inserting red clover stands into different cover crops

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ABSTRACT

Experiments were established at the Research Station of the Faculty of Agronomy in Červený Újezd in 1992–1997. Yield ability of cover crop, portion of individual parts in the total yield, yields of stubble cuts and individual cuts in the second year of vegetation were observed. The cover crops SLL pea and SLL pea with spring wheat realize condition for quality and yielding ability for establishing of red clover stands. Higher yielding ability of legumes stands in cover crops of wheat + SLL pea, SLL pea and SLL pea + faba bean was based mainly on yield growth of first cut in the second year of vegetation for more than 1 t.ha^{-1} when compared to commonly used cover crops (oats or barley + pea). Stands complexity in the second vegetation year are favorable prerequisites for more persisting and higher yielding red clover stands.

Keywords: red clover; cover crops; dry matter yield

Biological yield capacity of leguminous is very high. It ranges from 14–24 t.ha^{-1} of dry matter according to different conditions. One of the main reasons why the yield ability of red clover crops is practically exploited only in 40–50% is the problem of inserting the clover crops in improper cover crops. If we establish red clover crops into the cereals harvested for grain, there are minimum presumptions for formation closure of stands and it results in low yields and necessity to ploughing in of undercrops (Klesnil et al.1980).

Early mature cereals are important for uncovering undersowing red clover crops, which can produce another significant productive cut in the year of establishing, after that (Lesák and Svěráková 1992).

At investigation of influence of row span 125 mm, 250 mm and 375 mm in spring barley as fodder crop with seeding rate 2.5 mil. germinated seeds per one hectare. Fogl and Štráfelda (1992) mentioned, that there were not found any statistically significant differences in yield of dry matter in the first year of vegetation in undersowing legumes of alfalfa and red clover. Variants with wider row span of cover crop had higher density of undersowing legumes, what results in higher certainty of established productive undercrop red clover crops. Minimum yield of stubble cut was in barley crop with row span 125 mm with alfalfa as undercrop (1.87 t.ha^{-1}), maximum yield had the control seeded without cover crop (4.21 t.ha^{-1}). In average total yield of all cuts was 6.00–9.81 t.ha^{-1} of dry matter in the first year of vegetation. There are major differences after 21 days from coming of red clover as less sensitive species on influence of cover crop.

High yields of barley and spring wheat considerably reduced the yield of stubble cut of red clover. In comparison with lower yields of cover crops supported growth of stubble cut (Vrzal and Fogl 1995). Zajaç et al. (1997) mentioned, that immoderate overshadow of undersowing with cover crop had a negative impact on development of red clover.

MATERIAL AND METHODS

In the Research Station of the Faculty of Agronomy in Červený Újezd experiment with verifying of cover crops with regards to degree of lodging and overshadowing of undersowing red clover was established. The Research Station is based in beet growing area. Diversification of the field is simple with mostly southern exposition, 405 m above the sea level average (ranged from 390 to 420 m). The annual rainfall over 30 years was 493 mm, 333 mm during vegetation period. There was used red clover variety Start, seeded into 125 row span with seeding rate 8 mil. germinated seeds per 1 hectare. The plots size was 24 m^2, yield area 10 m^2. The experiment was established in randomized blocks in four replicates. Five different cover crops were observed.

1. control – sowing clover crop without any cover crop
2. peas with reduced leaf area (0.5 mil. germinating seeds further on g.s.ha^{-1})
3. barley + pea with reduced leaf area (1 mil. + 0.5 mil. g.s.ha^{-1})
4. wheat + SLL pea (1 mil. + 0.5 mil. g.s.ha^{-1})
5. faba bean + SSL pea (0.3 mil. + 0.5 mil. g.s.ha^{-1})

Yield ability of cover crop, portion of individual parts in the total yield, yields of stubble cuts and individual cuts in the second year of vegetation were observed in this experiment.

RESULTS AND DISCUSSION

In experiments of the Department of Fodder Crops Production influence of cover crops on red clover in last 20 years was studied. At the beginning red clover was established into cover crop harvested for seeds. Later on we started to use cover crops harvested for green matter or in silage ripeness, using different row span of cover crop. Mix of cereals with pod-bearing plants and clean seeded pod-bearing plants with undersowing was observed on the basis of results gained with cereals as cover crop.

Average yields of dry matter of cover crops and undercrops in the year of establishing are shown in Table 1. Results are completed by yield of weed for individual cover crops. Yields of cover crops, undersowings and weeds can suggest a lot about quality of red clover establishing. Generally, it is mentioned that high yield of cover crop and its long vegetation period has negative influence on undercrop of red clover crops (Fogl and Štráfelda 1992).

The obtained results show that cereals and SSL pea used as cover crops gave the highest yields in the year of their establishment. The yields of cereals are usually higher by 33% in companion with barley and by 40% in wheat yields. In the mixture consisted of cereals and leguminous crops the yield increase was 32–38% compared with the homogenous leguminous stands. The yield of cover crops was influenced by different indices in individual years – mainly by climatic conditions. Therefore, significant evaluation has to be given to the yield of undersowings together with the cover crops and their mutual yield relations. It is evident (Table 1) that the higher yields of the undersown crops were reached in the cover crop established in mixtures consisted of cereals and leguminous crops.

If we compare long term results of cover crops pod-bearing plants and their mixtures with cereals, the best cover crops from this point of view are the mixture and SLL type of peas. For SLL peas and mix of faba bean + SLL peas as a cover crop was share of undersowing on total yield of dry matter up to 16%. The lowest yield of dry matter was recorded in cereals and some mix of cereals and pod-bearing plants used as cover crop. There was a share of undersowing on total yield of dry matter ranged from 1 to 5%. In yield comparison of undersowing in covering crops and without covering crops the yield of dry matter of red clover ranged at cereals + pod-bearing plants 3–15% and pod-bearing plants or their mixtures from 16 to 21%.

If the yields of dry matter given by the undersown crops into the highest yielding cover crops (cereals), were compared with the least yielding cover crop (leguminous), then the yield decrease was 0.46–0.48, i.e. by 85–89% less than in the cover leguminous crop. Statistical evaluation proved high significance levels among individual variants.

The yields of stubble cuts and the yields of dry matter in the second year of vegetation are the crucial indices of the cover crop suitability for undersowing crop establishments. The cover giving high yields in the year of their establishment have need not to be the most suitable for the qualitative and persistent stands of red clover crops.

Results of stubble cut and individual cuts from the second year of vegetation, included total yields, are presented in Table 2.

The results given in Table 2 indicate that the lowest yields, both the stubble cuts and in the second vegetation year, were obtained in the cover crops consisted of barley with pea. In these mixtures of cereals and pea occurs by more overshadowing and therefore suppressing of undersowing in the first year of vegetation, which had been also displayed in the second year of vegetation. There was observed up to 50% decrease of yield in stubble cut compared to the control without cover crop. In the second year of vegetation decrease of yield of cover crop wheat + SLL pea was caused mainly by the first cut, for about 18%. This yielding deficit was fully covered by the second and third cuts. In other cover crops yield in all cuts was comparable in the second year of vegetation. Statistical evaluation did not prove any statistical significant differences, how it can be seen in the results in Table 2.

Bounds of 12 $t.ha^{-1}$ of dry matter for evaluation of yields of plant stands at establishing into different cover crops were chosen with regard to average yield in practice (8 $t.ha^{-1}$ national average yield), in 67% potential yields ability of red clover in plot experiments.

Higher yielding ability of the cover crops – wheat + SLL pea; SLL pea; faba bean + SLL pea was caused by the

Table 1. Yield of dry matter of cover crop with undersown red clover crop in the year of establishing in $t.ha^{-1}$ (Červený Újezd 1992–1997)

Cover crop	Cover crop			Undersown crop	Weeds	Total yield
	cereals crop	leguminous crop	total			
SLL pea	0	5.63	5.63	0.62	0.19	6.44
Wheat + SLL pea	4.33	2.80	7.13	0.60	0.18	7.91
Barley + SLL pea	5.71	1.41	7.12	0.05	0.04	7.21
Faba bean + SLL pea		faba bean 1.65 pea 2.20	3.85	0.77	0.24	4.86
Without cover crop				2.25	1.98	4.23
LSD 0.05			5.933**	0.858**	0.526*	6.134**
D_{min}			0.032	0.001	0.031	0.001

* statistically significant
SLL = semileafless pea with reduced leaf area

Table 2. Yield of dry matter of red clover in separated cuts and total yield in t.ha^{-1} (Červený Újezd 1992 –1997)

Cover crop	Yield of stubble cut	Total yield in a year of establishment	Yield in a second year of vegetation				Total yield for 2 years of growing
			1st cut	2nd cut	3rd cut	total	
SLL pea	1.868	7.247	6.86	3.86	1.77	12.49	19.74
Wheat + SLL pea	2.023	9.937	5.65	4.67	2.72	13.04	22.98
Barley + SLL pea	1.952	9.162	6.66	3.54	1.75	11.95	21.11
Faba bean + SLL pea	2.423	7.286	6.71	3.84	1.77	12.32	19.61
Without cover crop	2.49	6.73	5.67	3.26	2.12	11.05	17.78
LSD 0.05	2.151**	8.071**	6.310	3.834	2.026	12.170	20.244*
D_{min}	0.016	0.028	0.181	0.252	0.396	1.014	0.858

yield increase of the first cut in the second vegetation year, i.e. more than 1 t.ha^{-1} in comparison with the traditional cover crops (oats or barley + leaf pea). SLL pea has, as a cover crop, some advantages comparing with previously used pea stands. In those cover crops in two other cuts occurred in composition of yields with stands established with other cover crops. Yield increasing in the first cut comparing with other cover crops can be explained by influence of favorably condition for development and therefore even better wintering of undersowing red clover. Influence of quality of established plant stands of undercrops could have lower degree of lodging of pea which was used in cover crops. Ordinary leaf peas with cereals, how it was observed in earlier experiments with cover crops, are in time of their complete ripeness lodged and in those plant stands inconsiderable suppressing of undercrops occurs. Higher yield of stubble cut of red clover in leaf type of peas, as reported by Vrzal and Fogl (1999), was caused by earlier harvested cover crops with regard to ripeness (ripeness of green pods). In case of later harvested this cover crop occurs to lodging of plant stands and therefore for influence fully stands of undercrop of red clover.

Comparing dry matter yields of red clover stands established into the cover crops with stands established without cover crops, we came to conclusion, that in the second year of vegetation the most of cover crops reached comparable yields. This result is in discrepancy with an opinion of Schmid (1969), that undercrop established without cover crop is essentially more yielding in the second year of vegetation than undersowing established into the cover crops.

Pea with reduced leaf areas (SLL) as a cover crop is examined in last years. SLL pea has, as a cover crop, some advantages compared with previously used pea stands. Primarily, considering the character of the plant, the pea stands are practically not lodging till the phase of green pods. The second priority is in the low ability of shading of the undersown crop during its harvest period. These pea crops implemented the prerequisites for the establishment of clover stands in the quality of cover crop mainly in the combination with spring wheat. The plant numbers and stand complexity in the second vegetation year are the favorable prerequisites for more persisting and higher yielding clover stands than established into unsuitable or less suitable cover crops. Among disadvantages SLL pea belongs to lower yielding ability of cover crop, higher number of annual weeds because lower overshadowing, which can be removed by cuts of cover crop. Other disadvantage of this type of pea consists in the character of plant, too. Leaf tendrils of each plant are mutually perfectly interlaced and there is a danger of pulling out of young plants of red clover crops.

Cereals as cover crops for legumes were used all the time of establishing red clover crops. From cereals cover crops as the best kind are those cereals, which have low tillering ability and therefore ability of regulating of plants number on area. This types of cover crop plants developed more suitable presumption for establishing of quality red clover crops stands. Spring wheat and spring barley were recommended as suitable cereals.

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ABSTRAKT

Zakládání porostů jetele lučního do různých krycích plodin

Biologická výnosová schopnost jetelovin je velmi vysoká. Pohybuje se podle podmínek v rozmezí 14 až 24 $t.ha^{-1}$ sušiny. Jedna z hlavních příčin, proč je v praxi využívána výnosová schopnost jetelovin pouze ze 40 až 50 %, je dána problémy při zakládání jetelovin do nesprávně volených krycích plodin.V letech 1992 až 1997 byly zakládány pokusy na Výzkumné stanici Agronomické fakulty ČZU v Červeném Újezdě. Byla sledována výnosová schopnost krycí plodiny, podíl jednotlivých složek na celkovém výnosu, výnosy strništní seče a jednotlivých sečí ve druhém roce vegetace. Velikost parcely činila 24 m^2, sklizňová plocha 10 m^2. Pokus byl zakládán metodou znáhodněných bloků ve čtyřech opakováních. Bylo sledováno pět variant krycích plodin: 1. kontrola – výsev jeteloviny bez krycí plodiny; 2. hrách s redukovanou listovou plochou (0,5 mil. klíčivých zrn [dále k. z.].ha^{-1}); 3. ječmen + hrách typu semileafless (SLL) s redukovanou listovou plochou (1 mil. + 0,5 mil. k. z. ha^{-1}); 4. pšenice + hrách SLL 250 mm (1 mil. + 0,5 mil. k. z. ha^{-1}); 5. bob + hrách SLL (0,3 mil. + 0,5 mil. k. z. ha^{-1}). Průměr srážek za 30 let činil 493 mm, za vegetační období 333 mm. Z dosažených výsledků vyplývá, že nejvyššího výnosu v roce založení z krycích plodin pro jetel luční dosahovaly obilniny s hrachem SLL. Výnosy obilnin bývají v průměru oproti luskovinám u ječmene vyšší o 33 % a u pšenice o 40 %. U směsí obilniny a luskoviny bylo zaznamenáno zvýšení výnosů oproti čistým porostům luskovin o 32 až 38 %. Na výnos krycí plodiny v jednotlivých letech má vliv řada ukazatelů – především průběh klimatických podmínek. Proto významným hodnocením vedle výnosu krycí plodiny je výnos podsevu a vztah těchto výnosů navzájem. Na základě výsledků uvedených v tab.1 je zřejmé, že nejvyšší výnosy podsevu byly dosaženy převážně u krycích plodin zakládaných do směsí obilniny s luskovinou. Při porovnávání výnosu sušiny podsevu do nejvýnosnější krycí plodiny (obilniny) a nejméně výnosné krycí plodiny (luskoviny) byl zaznamenán pokles výnosu oproti krycí plodině luskovině o 0,46 až 0,48 $t.ha^{-1}$, tj. o 85 až 89 %. Statistickým hodnocením byla prokázána vysoká hladina průkaznosti mezi jednotlivými variantami. Výnos strništní seče a výnosy sušiny v druhém roce vegetace jsou rozhodujícím ukazatelem vhodnosti krycí plodiny při zakládání podsevů. Krycí plodina, která poskytuje v roce založení vysoký výnos, nemusí být vždy vhodná pro kvalitní založení a vytrvalost porostu jetele lučního. Při hodnocení výsledků uvedených v tab. 2 se zakládáním jetele lučního do krycí plodiny luskoviny a obilniny vyplývá, že nejnižších výnosů jak strništní seče, tak ve druhém roce vegetace bylo dosaženo u krycích plodin ječmene s hrachem. Vyšší výnosová schopnost porostů v krycí plodině pšenice + hrách SLL; hrách SLL a bob + hrách SLL byla dána především nárůstem výnosu v první seči druhého roku vegetace, a to o více než 1 $t.ha^{-1}$ oproti klasickým krycím plodinám (oves nebo ječmen + hrách listový). Hrách SLL jako krycí plodina oproti hrachům využívaným dříve má několik předností. V prvé řadě, vzhledem k charakteru rostliny, je porost hrachu až do fáze zelených lusků prakticky nepoléhavý. Další předností této krycí plodiny je menší zastínění podsevu v období sklizně. Pro zakládání porostů jetelovin splňovaly tyto hrachy požadavky na kvalitu krycí plodiny především v kombinaci s jarní pšenicí. Šířka řádků je doporučována v rozmezí 250 až 375 mm. Porosty jeteloviny byly založeny s optimálními počty rostlin a především s vysokou výnosovou schopností v druhém roce vegetace. Počty rostlin a kompletnost porostu ve druhém roce vegetace dávají dobrý předpoklad vytrvalejších a výnosných porostů jetelovin.

Klíčová slova: jetel luční; krycí plodiny; výnos sušiny

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The content of plant phenolics in meadow grasses and its relation to nutritive value of fodder

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ABSTRACT

A total of 436 samples of 109 species of 15 families was collected from grasslands in the area of Jevíčko in the period of three years. Prediction of the index of potential negative activity of phenols (IANP) and of the parameters of fodder quality was carried out with a NIRSystems 6500 apparatus. The highest IANP value was determined for *Hypericum perforatum* at the stage of florescence (232), the lowest for *Vicia sepium* at the vegetative stage (14). The IANP of meadow species was significantly higher at the florescence stage ($P_{0.95}$) than at the vegetative stage, the influence of harvest year was significant in one case. Significant interspecific differences in IANP can be determined more often for families that exhibit higher average value of IANP (> 80), e.g. for *Rosaceae*, *Hypericaceae*, *Asteraceae*, *Plantaginaceae*. On the contrary, for families with an average value of IANP < 50, significant interspecific differences occur occasionally. A significant intraspecific variability was found out for *Taraxacum officinale*. In the model experiment a standard addition of the extracts of nine meadow plants to test samples of grasses and legumes decreased activity of proteases a cellulases in the digestive liquor. From the practical point of view these effects will have to be studied also in the mixture of botanical species in the rates that occur in mixed grasslands.

Keywords: meadow plants; plant phenols; IANP; antinutritive substances; digestibility; nutritive value

Following the decrease of intensity of grassland and meadows management (esp. the level of fertilizing and the number of harvests or grazing cycles, resp.) the number of plant species increases as well as the proportion of herbs. Some herbs contain secondary metabolites that can improve or worsen fodder quality (e.g. palatability, dry matter intake, nutritive value, use of nutrients in the animal body), and technological characteristics (e.g. ensilability, aerobic stability of silage). Many herbs had a very low content of fermentable carbohydrates, high buffering capacity or distinctive abiotic effects, whereas other herbs did not (Weißbach 1998). That makes herbs different from grasses because their silage characteristics among botanical species do not differ significantly. Meadow plants from families *Asteraceae*, *Rosaceae* and *Plantaginaceae* (Jeangros et al. 1999) exhibited a specific activity.

Phenolics make a very numerous group that is well-represented in plants (Klejdus and Kubáň 1999). They are usually changed by enzymatic degradation and chemical modification in animal rumen that they do not get into milk and cheese as such (Scehović et al. 1998). Just some terpenes and higher aldehydes get into cheese from plants in an almost identical form (Jeangros et al. 1999).

The proposed project links to previous works (Míka et al. 1998) and greatly extends the spectrum of meadow plants from the point of determination of the index of potential negative activity (IANP). The model experiment determined the influence of inhibitors from selected plant species on pepsin-cellulases solubility of grasses and legumes *in vitro*. These results were discussed so that links to silage characteristics could be found in order to assess the significance of IANP and its availability for establishing more precise fodder value of individual species of meadow plants.

MATERIAL AND METHODS

From the grasslands in the area of Jevíčko a total of 436 samples was selected from 109 species from 15 families, from each botanical species one average sample in one term. Sample collection was carried out at vegetative stage (in 1997) and at generative stage (florescence) from 1996 till 1998. The samples were dried at 55 °C and their IANP and parameters of nutritive value by NIRS technique (Míka et al. 1998) were determined. The results were statistically evaluated with a view to differences among families (Table 1), development stages (Table 2) and harvest years (Table 3). Next in 1997, for 24 plants of *Taraxacum officinale* was IANP determined at the stage of heading and its intraspecific variability was statistically evaluated. Then, the effect of plant extracts of 9 meadow plants was evaluated (with measured values of IANP) on the activity of proteases and cellulases in the digestive liquor in the form of determination of digestibility of organic matter *in vitro* (OMD) of three test samples (Figs. 1–3). Inhibitors were extracted from dry

Table 1. The IANP and nutritive value (in dry matter) of meadow plants according to families at the vegetative stage of growth

Family	Number of analyzed species	IANP	OMD	NEL ($MJ.kg^{-1}$)	N×6.25 ($g.kg^{-1}$)	PDIN ($g.kg^{-1}$)	WSC ($g.kg^{-1}$)
Asteraceae	26	132	75.9	6.85	133	93	84
Boraginaceae	3	84	77.0	6.23	131	73	108
Campanulaceae	2	29	74.1	6.20	91	55	55
Daucaceae	7	96	79.0	7.15	189	127	83
Geraniaceae	3	80	78.2	6.73	180	114	80
Hypericaceae	2	173	71.0	6.58	102	57	43
Lamiaceae	8	51	75.3	6.66	183	105	59
Plantaginaceae	3	138	73.6	6.51	144	81	72
Poaceae	17	41	72.4	6.75	121	75	80
Polygonaceae	5	95	74.9	6.54	113	71	36
Ranunculaceae	2	93	76.7	5.90	130	68	151
Rosaceae	6	176	75.9	5.52	146	73	84
Scrophulariaceae	7	91	72.5	5.90	115	68	58
Silenaceae	3	35	73.3	5.75	138	81	65
Viciaceae	15	53	77.0	6.57	192	118	62
LSD $P_{0.95}$	.	34	2.7	0.42	36	15	21
LSD $P_{0.99}$	.	45	3.6	0.55	48	20	28

IANP = index of negative potential activity of phenols (according to Scehović)
OMD = organic matter digestibility
NEL = netto energy of lactation
N×6.25 = protein
PDIN = PDIA + PDIMN (PDIA = the dietary protein undegraded in the rumen, but truly digestible in the small intestine; PDIMN microbial protein that could be synthesized in the rumen from the dietary N, when energy and other nutriens are not limiting), protein digestible in intestine
WSC = water soluble carbohydrates

Table 2. The IANP and nutritive value (in dry matter) of meadow plants at the vegetative and generative stage of growth

Stage of growth	Number of analyzed species	IANP	OMD	NEL ($MJ.kg^{-1}$)	N×6.25 ($g.kg^{-1}$)	PDIN ($g.kg^{-1}$)	WSC ($g.kg^{-1}$)
Vegetative	109	91	75.1	6.38	141	84	75
Generative	327	113	70.6	5.90	117	69	92
LSD $P_{0.95}$	.	16	2.2	0.29	22	13	17
LSD $P_{0.99}$	.	21	2.9	0.39	29	17	23

Table 3. The IANP and nutritive value (in dry matter) of meadow plants at the generative stage of growth in harvest years 1996–1998

Harvest year	Number of analyzed species	IANP	OMD	NEL ($MJ.kg^{-1}$)	N×6.25 ($g.kg^{-1}$)	PDIN ($g.kg^{-1}$)	WSC ($g.kg^{-1}$)
1996	109	106	70.4	5.91	118	70	96
1997	109	103	69.7	5.85	113	66	93
1998	109	129	71.8	5.93	120	71	88
LSD $P_{0.95}$	.	25	2.0	0.17	25	12	15
LSD $P_{0.99}$	.	33	2.6	0.22	33	16	20

samples of meadow plants using the method by Scehović (description in Míka et al. 1998) and these were added by the method of standard addition into digestive liquor with OMD determination *in vitro* (Jones and Hayward 1995) of red fescue cv. Táborská (from 1st cut), perennial ryegrass cv. Talon (from 2nd cut of five-harvest year) and red clover cv. Tábor (from 1st cut). The selection of species was done according to Weißbach (1998), who presents them in descending order with a respect to positive influence on silage fermentation. The model experiment was to verify if inhibitory effects of individual species of meadow plants on digestion of medium and high quality grass and clover are similar. The experiment was evaluated by Spearman's test according to the order of species. Value $r_S = 1$ represents a complete identity of the order, $r_S = -1$ indicates just the opposite order.

RESULTS

The highest IANP value was determined for *Hypericum perforatum* at the stage of florescence (232), the lowest for *Vicia sepium* at the vegetative stage (14). The average value of IANP from individual species for given family was between 176 (*Rosaceae*) and 29 (*Campanulaceae*). Differences between some families were significant (Table 1). In one family there occur species with a high value and also very low value of IANP. For example, in *Asteraceae* family, *Leonthodon hispidus* (185), *Bellis perenis* (172) and *Achillea milefolium* (165) belong to the former ones, while *Arctium minus* (19), *Tragopogon pratensis* (22), and *Cirsium canum* (24) to the latter ones. Great differences between species of the same family can be found in all families with average IANP value > 80 whereas families with low average value had relatively little differences between species. For example, for *Poaceae* family maximum difference was determined between 58 (*Dactylis glomerata*) and 17 (*Lolium perenne*).

IANP value of meadow plants at the stage of florescence was significantly higher ($P_{0.95}$) than in the vegetative stage (Table 2). The influence of harvest year was significant in one case (1998 compared to 1997; $P_{0.95}$) – Table 3. In 1997 IANP value was determined for 24 plants of *Taraxacum officinale* at the stage of florescence. The values were between 42 and 176 ($\bar{x} \pm s$ was 132 ± 56).

The IANP value relation to the parameters of nutritive value of meadow plants fodder was mostly insignificant ($r < 0.1$; $n = 436$), as well as for families ($r < 0.17$; $n = 60$) and so it is not mentioned.

After addition of the extract of 8 herbs or one clover (Fig. 1) into pepsin-cellulases liquor with a grass of medium quality (*Festuca rubra*) – Fig. 1, or high quality fodder grass (*Lolium perenne*), resp. – Fig. 2, or *Trifolium pratense* (Fig. 3), individual species of meadow plants decreased OMD of tested samples almost in identical order with that one by Weißbach (1998) during fermentation process of silage ($r_S = 0.767$, 0.800 and 0.917, resp.). The decrease of OMD of *Lolium perenne* was strongly in relation with IANP of corresponding herbal samples from which extracts were made ($r = -0.771^*$; $n = 9$) and for *Trifolium pratense* ($r = -0.699^*$; $n = 9$), however for *Festuca rubra* insignificantly ($r = -0.516$ N.S.; $n = 9$).

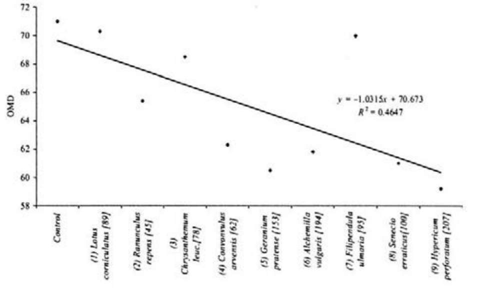

Figure 1. The influence of standard addition of inhibitors from 9 meadow plants on organic matter digestibility (OMD) *in vitro* *Festuca rubra*, cv. Táborská

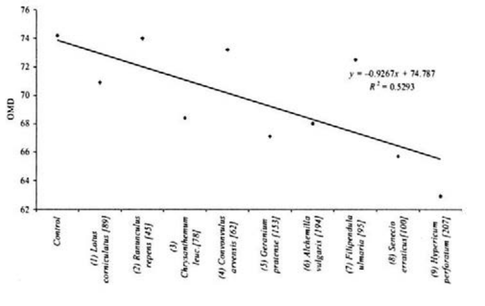

Figure 2. The influence of standard addition of inhibitors from 9 meadow plants on organic matter digestibility (OMD) *in vitro* *Lolium perenne*, cv. Talon

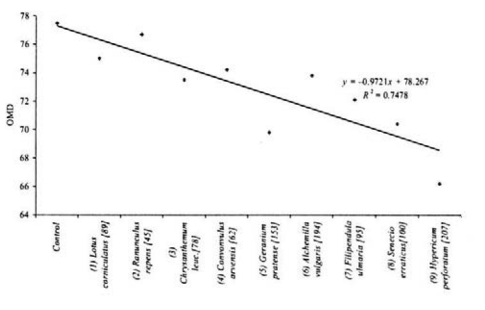

Figure 3. The influence of standard addition of inhibitors from 9 meadow plants on organic matter digestibility (OMD) *in vitro* *Trifolium pratense*, cv. Tábor

Used extracts from meadow plants: ( ) positive influence on silage fermentation in descending order according to Weißbach (1998); [ ] the IANP value of given sample from which the extract was made

DISCUSSION

Significant interspecific differences of IANP can be determined with higher frequency for families that show higher average value of IANP (> 80), for example, *Rosaceae*, *Hypericeae*, *Asteraceae*, and *Plantaginaceae*. On the contrary, if the family has average value of IANP < 50, significant interspecific differences occur rarely (similarly Scehović 1995; Míka et al. 1998; Jeangros et al. 1999). In relative comparison of IANP in collections of samples of fodder of different age, from different locations and different harvest years, botanical species with a high value of IANP regularly take top places (for example, *Hypericum perforatum*, *Alchemilla vulgaris*, and *Leonthodon hispidus*). Also Scehović, Jeangros (1994) rank *Alchemilla vulgaris* together with *Geranium silvaticum* and *Salvia pratensis* to species with relatively highest and stable value of IANP in different conditions. On the contrary, species with a lower average value of IANP (*Taraxacum officinale*, *Trifolium pratense*, and *Lotus corniculatus*) were found significant intraspecific differences (for the first species 51–150, for the second 54–120 and for the third 39–100), and these results correspond to our findings for *Taraxacum officinale* from around Jevíčko. Concerning this, Klimeš (oral message, 2000) suggests using IANP in the process of breeding wild plants for establishing flowery meadows as one criterion of selection so that the negative influence of phenolics on feeding value decreased. Before this can be done, natural variability of feature, inheritance and genetic correlation with rare plant qualities (e.g. yield, persistence, and disease resistance) have to be evaluated. Presently State Variety Trials test ecotypes and first varieties of *Plantago lanceolata* in replications in pure growth and in mixture (Mosimann and Lehmann 1999) in Switzerland. In New Zealand, two varieties, cvs. Tonic and Lancelot, of this species are grown for seeds, for their antibiological effects they are put into some mixtures for extensive pastures or into grass mixtures for silage.

The fact that no herb stopped lactic fermentation during silage preparation is significant (Weißbach 1998). The strongest positive influence on fermentation has *Lotus cornitulatus* (a species with a relatively low value of IANP), the weakest influence has *Hypericum perforatum* (a species with a very high value of IANP). Some species slow down degradation of lactic acid and creation of butteric acid. Positive influence on aerobic stability of ready silage showed leguminoses, next also *Ranunculus* sp., *Galium* sp., *Filipendula ulmaria* and *Centaurea jacea*. On the contrary, unstable silage occurred when herbs from the families of *Daucaceae* and *Polygonaceae* were included in silage. Proteolysis in silage is significantly decreased by *Alchemilla alpina*, *Filipendula ulmaria*, *Geranium pratense*, *Plantago lanceolata*, and *Sanguisorba officinalis*, that is by species known for their positive influence on milk production. Ammonia occurs in such silage in unusual low concentration. It is possible that secondary metabolites included in them react also in rumen as substances protecting proteins from dissemination (Weißbach 1999).

Although species with a high value of IANP (*Alchemilla vulgaris*, *Plantago lanceolata*, etc.) can exhibit high value *in vitro*, this digestibility is definitely overestimated because of low concentration of dry matter and its dilution in fermentation condition of the method *in vitro* (Jones and Hayward 1975) about 12–15× compared to the concentration in the rumen of ruminants and as a result of activity of potentially active principles (Míka et al. 1998; Scehović, written message). On the contrary, a standard addition of extracts of these herbs to test samples of grasses and legumes with a high digestibility (Figs. 2, 3) caused significant decrease of activity of proteases and cellulases in digestive liquor.

To quantify these effects, Scehović (1999) establishes Index of Potential Fermentation Activity (IAFP) determined on the basis of the amount of released gas during fermentation (extraction of the sample of tested species by artificial saliva and rumen fluid with added glucose) and pH decrease. Families rich in phenolics (phenolic acids, phenol polymers), e.g. *Rosaceae* and *Geraniaceae*, cause the most intensive inhibition. Presence of toxic organic acids in species from family *Polygonaceae* or alkaloids from family *Ranunculaceae* and *Chenopodiaceae*, resp., mucuses and terpenes from *Plantaginaceae*, *Asteraceae*, and *Daucaceae* partly explain their influence on fermentation, however the quantification is not simple. The negative influence of substances diluted in extract is probably direct, whereas phenolics bound to matrix influence the process and results of fermentation indirectly (Scehović 1999). Families *Poaceae* and *Viciaceae* represent a group with a greatest stimulative effect on fermentation. In practice these effects will have to be studied also in mixture of botanical species in the rates occurring in mixed grasslands.

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ABSTRAKT

Obsah fenolických látek v lučních rostlinách a jeho vztah k výživné hodnotě píce

Z travních porostů v okolí Jevíčka bylo během tří let vybráno 436 vzorků od 109 druhů z 15 čeledí. Predikce indexu potenciální negativní aktivity fenolů (IANP) a parametrů kvality píce byla provedena na přístroji NIRSystems 6500. Nejvyšší hodnota IANP byla stanovena u *Hypericum perforatum* ve fázi kvetení (232), nejnižší u *Vicia sepium* ve vegetativní fázi (14). IANP u lučních druhů ve fázi kvetení byl významně vyšší ($P_{0,95}$) než ve vegetativní fázi, vliv ročníku sklizně byl významný v jednom případě. Významné interspecifické rozdíly v IANP lze stanovit s větší četností v čeledích, které vykazují vyšší průměrnou hodnotu IANP (> 80), např. u *Rosaceae*, *Hypericaceae*, *Asteraceae*, *Plantaginaceae*. U čeledi s průměrnou hodnotou IANP < 50 se významné interspecifické rozdíly naopak vyskytují jen ojediněle. Z nich jsme jako příklad vybrali *Taraxacum officinale* a u ní jsme zjistili významnou intraspecifickou variabilitu. Pokud se přistoupí ke zušlechťování některých druhů plané flóry pro květnaté louky (*Achillea milefolium*, *Plantago lanceolata*, *Tragopogon pratensis* aj.), IANP by se měl použít jako jedno z kritérií selekce s cílem programově u nich snižovat negativní účinky fenolických látek na krmnou hodnotu. Ačkoliv druhy s vysokou hodnotou IANP (*Alchemilla vulgaris*, *Plantago lanceolata* aj.) mohou vykazovat vysokou hodnotu *in vitro*, tato stravitelnost je určitě nadhodnocována z důvodů nízké koncentrace vlákniny a jejího rozředění ve fermentačním prostředí metody *in vitro* přibližně 12- až 15krát v porovnání s koncentrací v trávicím traktu přežvýkavců, a tedy v důsledku působení potenciálně aktivních principů. Naopak, standardní přídavek výluhů z těchto bylin k testačním vzorkům trav a jetele s vysokou stravitelností vyvolal významné snížení aktivity proteáz a celuláz v trávicím médiu. Snížení OMD *Lolium perenne* souviselo významně s IANP příslušných vzorků bylin, z nichž byly výluhy připraveny ($r = -0{,}771^{*}$; $n = 9$) a u *Trifolium pratense* ($r = -0{,}699^{*}$; $n = 9$), avšak u *Festuca rubra* nevýznamně ($r = -0{,}516$ N.S.; $n = 9$). Negativní účinek látek rozpuštěných v extraktu je zřejmě přímý, zatímco fenolické látky vázané na matrici ovlivňují průběh a výsledek fermentace nepřímo. Čeledi *Poaceae* a *Viciaceae* představují skupinu s největším stimulačním účinkem na fermentaci. V praktickém pohledu však bude nutné tyto účinky studovat též ve směsi botanických druhů v poměrech vyskytujících se ve smíšených porostech.

Klíčová slova: luční rostliny; rostlinné fenoly; IANP; antinutriční látky; stravitelnost; výživná hodnota

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Lactuca serriola L. presence on the set-aside soil

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ABSTRACT

The possibility of *Lactuca serriola* L. infestation of sown stands of *Bromus catharticus* Vahl., *Arrhenatherum elatius* (L.) P. Beauv. ex J.S. et K.B. Presl, *Festuca pratensis* Huds., *Dactylis aschersoniana* Graebn., *Trifolium repens* L., *Medicago lupulina* L., *Lotus corniculatus* L., *Medicago media* Pers. and fallow under different management at setting the soil aside (3 times cut during vegetation with removing of the mass, 2 times mulching during vegetation, once mulching in the end of vegetation and once cut in the end of vegetation with removing of the mass). Height, number of dry mass yield of *Lactuca serriola* L. were measured. *Lactuca serriola* L. infested especially legumes stands harvested once in the end of vegetation period. Its share in the total dry mass yield of a year reached in average 16% in the stands of *Medicago lupulina* L. and 36% in *Trifolium repens* L. stands. Grass stands were relatively little infested or not at all. *Lactuca serriola* L. presence in the stands was influenced especially by the sown species. The infestation by *Lactuca serriola* L. was not significantly influenced by the stand management. Multiple harvest of the stands during the vegetation (3 times cut, 2 times mulching) prevented seeds creating and ripening of that species. The weed infestation in the year 1999 was significantly higher in comparison with the year 1997.

Keywords: arable land; setting aside; grasses; legumes; mulching; cutting; *Lactuca serriola* L.

The contemporary economic situation in the Czech Republic and the prepared entrance of the country to the EU are the reasons of restricting of agriculture production on the arable land. That is why the superfluous arable land is set aside. One of the aims of the setting the soil aside is to prevent weed infestation of the land and spreading of the weeds to the surrounding fields. One of the weeds of such managed land is also *Lactuca serriola* L.

It is an annual or biennial species that grows mostly on the edges of the fields, railway embankments, road margins and dumps (Hron and Kohout 1988; Hanf 1990). Hron and Zejbrlík (1979) mention, that it sometimes also occurs on drier insufficiently treated meadows. In the last time it also spreads to the set aside arable land. Its occurrence was noted so on more years wild fallow, especially in the first years after their establishment (Oesau 1992; Jüttersonke and Arlt 1994; Hintzsche 1994; Dittmann 1995), as on annual ones (Glemnitz and Augustin 1995). *Lactuca serriola* L. spreads from the set aside fields to cultivated ones especially in the direction of dominant winds. It is not generally among weeds of arable land, but its excessive occurrence in stands of winter rape (Zellner 1993) and its resistance to ALS-inhibitors in cereal crops were noted (Walter 1994).

MATERIAL AND METHODS

A field plot trial (area of a plot 30 m^2) was established in the experimental field of the University of Agriculture in Prague in the year 1996 (chernozem, 281 m above the sea level). *Bromus catharticus* Vahl. 50 $kg.ha^{-1}$, *Arrhenatherum elatius* (L.) P. Beauv. ex J.S. et K.B. Presl 50 $kg.ha^{-1}$, *Festuca pratensis* Huds. 30 $kg.ha^{-1}$, *Dactylis aschersoniana* Graebn. 18 $kg.ha^{-1}$, *Trifolium repens* L. 15 $kg.ha^{-1}$, *Medicago lupulina* L. 20 $kg.ha^{-1}$, *Lotus corniculatus* L. 15 $kg.ha^{-1}$and *Medicago media* Pers. 18 $kg.ha^{-1}$ were sown to the rows of 125 mm space in the spring. A part of the area was left as a wild fallow (self overgrown) and one plot represents a black fallow (chemically maintained without plant cover) The stands were chemically treated against weeds only in the year of establishment (1996). The stands are 3 times cut with the mass removal, two times mulched during a vegetation period, once mulched in the end of vegetation or once cut in the end of vegetation with the mass removal. The mulching was done by the mulcher AS 27/2 Enduro (Germany).

The plants of *Lactuca serriola* L. were cut before each stand harvest (the area 14 m^2) and then the number of plants per ha, average height of them and their share in the total dry mass yield were measured. The plants were cut in the height 40–50 mm (corresponding with the height of the stubble). The terms of the cuts in the years 1997–1999 are documented in Table 1.

The statistical evaluation was done by analysis of variance in the Statgraphics programme, version 4.2, Tukey's method.

RESULTS AND DISCUSSION

In the year 1997 the three times cut plots were infested with *Lactuca serriola* only in the 3rd cut (sown by *Trifolium repens* and *Lotus corniculatus*) (Table 4). In the

Table 1. Terms of harvests in the years 1997–1999

Year	Way of treatment						
	3× cuts			2× mulching		1× mulching	1× cut
	1.	2.	3.	1.	2.		
1997	11. 6.	15. 7.	20. 10.	11. 6.	20. 10.	20. 10.	20. 10.
1998	19. 5.	29. 7.	1. 10.	30. 6.	1. 10.	1. 10.	1. 10.
1999	24. 5.	2. 8.	10. 10.	20. 6.	10. 10.	10. 10.	10. 10.

Table 2. Yield of sown crops ($t.ha^{-1}$), all weeds and *Lactuca serriola* L. under different stand management in the years 1997–1999

Harvest	Crop	1997			1998			1999		
		crop	weed	*Lactuca serriola*	crop	weed	*Lactuca serriola*	crop	weed	*Lactuca serriola*
3× cuts	*Bromus catharticus*	9.810	0	0	3.921	0.089	0.002	1.767	0.253	0.017
	Arrhenatherum elatius	8.920	0.005	0	4.220	0.231	0	4.361	0.787	0.001
	Festuca pratensis	4.630	0.016	0	1.835	0.322	0	1.788	0.637	0.002
	Dactylis aschersoniana	3.100	0.011	0	2.146	0.270	0	2.767	0.898	0.009
	Trifolium repens	7.630	0.003	0.003	2.526	0.750	0.001	2.577	4.390	1.149
	Medicago lupulina	9.190	0.046	0	0.172	3.579	0.159	1.970	3.429	0.040
	Lotus corniculatus	9.100	0.024	0.009	6.887	1.095	0	11.376	1.369	0.056
	Medicago media	12.500	0	0	6.495	0.004	0	13.006	0.107	0.003
2× mulching	fallow	0	6.870	0.059	0	3.920	0.073	0	6.040	0.015
	Bromus catharticus	7.850	0	0	4.026	0.049	0	4.417	0.292	0.001
	Arrhenatherum elatius	9.540	0	0	5.631	0.374	0	6.293	0.604	0
	Festuca pratensis	4.880	0.011	0	2.487	0.491	0	3.678	1.691	0
	Dactylis aschersoniana	3.460	0.022	0	3.044	1.085	0	3.955	2.859	0
	Trifolium repens	4.610	0.097	0	1.148	3.585	0.010	0.188	6.380	0.555
	Medicago lupulina	6.890	3.330	0	0.223	6.241	0.609	0.697	8.661	0.114
	Lotus corniculatus	6.300	0.220	0.043	5.112	2.176	0.035	4.183	3.687	0.060
	Medicago media	8.400	0.004	0	7.929	0.119	0.002	7.796	0.070	0.046
1× mulching	fallow	0	5.400	0.028		3.966	0.008	0	4.601	0.019
	Bromus catharticus	5.460	0	0	3.781	0.286	0	2.355	0.573	0.183
	Arrhenatherum elatius	5.980	0.051	0	5.375	0.361	0	4.283	0.470	0
	Festuca pratensis	4.000	0.102	0	1.875	0.633	0	3.009	1.303	0
	Dactylis aschersoniana	3.540	0.32	0	1.998	0.339	0	2.942	1.420	0
	Trifolium repens	0.730	0.150	0.031	0.032	2.281	0.894	0	3.197	2.338
	Medicago lupulina	0.170	1.540	0.089	0.045	3.150	1.049	0	4.549	0.304
	Lotus corniculatus	2.070	0.921	0.054	1.637	1.169	0.026	1.6991	2.399	0.017
	Medicago media	4.440	0.059	0.059	2.685	1.245	0	3.0356	1.252	0.004
1× cut	fallow	0	3.350	0.032	0	3.157	0.009	0	3.068	0.0003
	Bromus catharticus	7.280	0	0	2.543	0.157	0.004	2.6677	0.434	0.001
	Arrhenatherum elatius	7.130	0	0	4.811	0.048	0	4.2100	0.046	0
	Festuca pratensis	4.780	0.082	0	2.024	0.527	0.020	3.0101	0.677	0.005
	Dactylis aschersoniana	4.180	0.043	0	2.531	0.811	0	3.6326	0.802	0.001
	Trifolium repens	0.490	0.01	0	1.532	2.842	2.828	0	5.160	1.860
	Medicago lupulina	0.120	2.990	0.403	0	3.807	1.406	0	3.967	0.074
	Lotus corniculatus	2.320	0.700	0.303	1.449	1.265	0.100	1.4651	3.068	0.025
	Medicago media	3.560	0.157	0.123	2.177	1.611	0.004	1.4542	3.247	0

year 1998 its occurrence was noted in the stand of *Medicago lupulina* already in the first cut. *Lactuca serriola* infested *Bromus catharticus* and *Trifolium repens* plots in the 2nd cut and *Bromus catharticus* and *Medicago lupulina* in the 3rd cut. In the 4th year of vegetation (1999) plants of *Lactuca serriola* were found in all legume stands in the 1st cut. The grass stands were infested especially in the 2nd cut. *Lactuca serriola* was mostly present in the stands of *Trifolium repens.* Its share in the total dry mass yield was 17% in the 4th year of vegetation (1999), while it was only 0.03% in the previous years. Dry mass yields and number of *Lactuca serriola* plants in particular cuts and years are together with their average height in Table 4. The plants of *Lactuca serriola* did not manage to create seeds under this way of stand treatment. The above-ground dry mass production of sown stands is documented in Table 2 and the total numbers of *Lactuca serriola* plants per m^2 in Table 3.

The stands mulched 2 times during a vegetation also showed an increasing weed infestation in dependence on the time after setting the soil aside. In the year 1997 the weed infestation appeared as late as in the 2nd cut (*Lotus corniculatus* and the fallow) (Table 5). In the 3rd year of vegetation (1998) *Lactuca serriola* appeared in the 1st and 2nd harvest (mulching) of the fallow and in the stands of *Medicago lupulina* and *Trifolium repens. Lotus cornitulatus* and *Medicago media* stands were infested only in the 1st mulching (Table 5). In the 1st harvest of the year 1999 *Lactuca serriola* grown in the fallow and in all the legumes; in the 2nd mulching in the stands of *Bromus catharticus*, *Trifolium repens* and in the fallow. During the years 1997–1999 *Lactuca serrio-*

Table 3. Number of *Lactuca serriola* L. plants (.ha^{-1}) in the stands of sown crops in the years 1997–1999

Harvest	Crop	1997	1998	1999
3× cuts	*Bromus catharticus*	0	4 286	100 714
	Arrhenatherum elatius	0	0	5 000
	Festuca pratensis	0	0	2 857
	Dactylis aschersoniana	0	0	22 857
	Trifolium repens	714	714	412 857
	Medicago lupulina	0	82 143	77 857
	Lotus corniculatus	2 857	0	70 000
	Medicago media	0	0	7 857
2× mulching	fallow	15 714	14 285	23 571
	Bromus catharticus	0	0	1 429
	Arrhenatherum elatius	0	0	0
	Festuca pratensis	0	0	0
	Dactylis aschersoniana	0	0	0
	Trifolium repens	0	5 714	307 143
	Medicago lupulina	0	292 142	460 000
	Lotus corniculatus	5 000	25 000	86 429
	Medicago media	0	714	35 000
1× mulching	fallow	1 429	9 285	27 143
	Bromus catharticus	0	0	28 571
	Arrhenatherum elatius	0	0	0
	Festuca pratensis	0	0	0
	Dactylis aschersoniana	0	0	0
	Trifolium repens	1 429	162 143	390 714
	Medicago lupulina	1 429	338 571	229 286
	Lotus corniculatus	2 857	24 286	14 286
	Medicago media	4 286	0	3 571
1× cut	fallow	714	12 143	714
	Bromus catharticus	0	2 143	2 143
	Arrhenatherum elatius	0	0	0
	Festuca pratensis	0	3 571	2 857
	Dactylis aschersoniana	0	0	714
	Trifolium repens	0	408 571	259 286
	Medicago lupulina	7 857	665 000	40 000
	Lotus corniculatus	12 143	58 571	12 857
	Medicago media	5 714	714	0

Table 4. Yield, number and average height of *Lactuca serriola* L. plants (3 times cut stands)

Crop	Year	Yield ($t.ha^{-1}$)			Number of plants ($.ha^{-1}$)			Height of plants (m)		
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Bromus catharticus	1998		0.0008	0.001		4 286	714		0.049	0.210
	1999		0.015	0.002		88 571	12 143		0.147	0.167
Arrhenatherum elatius	1999		0.0006			5 000			0.155	
Festuca pratensis	1999		0.002			2 857			0.248	
Dactylis aschersoniana	1999		0.008	0.001		21 429	1 429		0.193	0.610
Trifolium repens	1997			0.003			714			0.625
	1998		0.0007			714			0.176	
	1999	0.319	0.806	0.025	158 571	195 714	58 571	0.232	0.650	0.139
Medicago lupulina	1997			0.009			2 857			0.704
	1998	0.156		0.004	75 714		6 429	0.312		0.164
	1999	0.004	0.032	0.003	14 256	47 143	16 429	0.137	0.265	0.134
Lotus corniculatus	1999	0.028	0.028		60 000	10 000		0.213	0.607	
Medicago media	1999	0.003			7 857			0.235		

Table 5. Yield, number and average height of plants of *Lactuca serriola* L. (2 times mulched stands)

Crop	Year	Yield ($t.ha^{-1}$)		Number of plants ($.ha^{-1}$)		Height of plants (m)	
		1st mulching	2nd mulching	1st mulching	2nd mulching	1st mulching	2nd mulching
Fallow	1997		0.059		15 714		0.618
	1998	0.044	0.028	3 517	10 714	0.390	0.364
	1999	0.004	0.011	19 286	4 286	0.088	0.470
Bromus catharticus	1999		0.001		1 429		0.298
Trifolium repens	1998	0.004	0.006	2 857	2 857	0.295	0.388
	1999	0.547	0.008	298 571	8 571	0.568	0.341
Medicago lupulina	1998	0.457	0.152	198 571	93 571	0.439	0.395
	1999	0.114		460 000		0.306	
Lotus corniculatus	1997		0.043		5 000		0.880
	1998	0.035		25 000		0.315	
	1999	0.060		86 429		0.314	
Medicago media	1998	0.002		714		0.642	
	1999	0.046		35 000		0.298	

la mostly spread in the stands of *Medicago lupulina* and *Trifolium repens*. *Lactuca serriola* share in the dry mass yield was 9% (1998) and 1% (1999) in the stands of *Medicago lupulina* and 0.2% (1998) and 9% (1999) in *Trifolium repens* (Table 2). Neither under this way of treatment *Lactuca serriola* managed to create seeds, but some of the plants flowered. There were 5–16 thousand plants in individual terms of cuts in the 2nd year of vegetation, 700–199 thousand plants per ha in the 3rd year of vegetation and 1–460 thousand plants per ha in the 4th year of vegetation (Table 5). The grass stands were less weed infested (Table 3). There were no plants of *Lactuca serriola* in 2 times mulched stands of *Arrhenatherum elatius, Festuca pratensis* and *Dactylis aschersoniana*.

The strongest infestation was on the plots harvested once per year (cut or mulched). In the variant once mulched the stand of *Medicago lupulina* was the most infested one with *Lactuca serriola* in the 2nd year of vegetation (1997), *Medicago lupulina* and *Trifolium repens* in the years 1998 and 1999 (Table 2). The share of *Lactuca serriola* in the total dry mass yield of *Medicago lupulina* was 32% (1998) and 7% (1999) and 39% (1998) or 73% (1999) of *Trifolium repens* yield, resp. The grass stands were not infested with this weed, only some plants of *Lactuca serriola* appeared in the stand of *Bromus catharticus* (Tables 2 and 3) in the 4th year of vegetation (1999). The average height of the plants was 0.5–1.2 m (Table 6) during the years 1997–1999. A similar trend of weed infestation was also in the plots cut once per year

Table 6. Average height of *Lactuca serriola* L. plants (once cut or mulched stands)

Crop	Year	Height of plants (m)	
		1st mulching	1st cut
Fallow	1997	1.235	1.773
	1998	0.495	0.326
	1999	0.550	0.483
Bromus catharticus	1998		0.669
	1999	0.816	0.543
Festuca pratensis	1998		0.771
	1999		0.690
Dactylis aschersoniana	1999		0.614
Trifolium repens	1997	1.062	
	1998	0.801	0.986
	1999	1.154	1.309
Medicago lupulina	1997	1.180	1.404
	1998	0.790	0.742
	1999	0.667	0.783
Lotus corniculatus	1997	1.017	1.240
	1998	0.558	0.552
	1999	0.615	0.723
Medicago media	1997	0.978	1.143
	1998		0.954
	1999	0.669	

with the mass removing. In the year 1997 the trial plots with legumes (except *Trifolium repens*) and fallow were in average 3 times more infested than once mulched ones (Table 2). In the year 1998 the stands of *Medicago lupulina* and *Trifolium repens* were strongly infested with *Lactuca serriola.* Its share in the total yield of dry mass was 37% in *Medicago lupulina* and 65% in *Trifolium repens*. But these stands were less infested with *Lactuca serriola* in the following year (1999), because of the grass weeds spreading especially *Agropyron repens* (Tables 2 and 3). The average height of *Lactuca serriola* plants was 0.33 to 1.77 (Table 4) in the years 1997–1999, the plants were

Table 7. Average yield of *Lactuca serriola* L. ($t.ha^{-1}$) in sown crops stands

Crop	Year			Average
	1997	1998	1999	
Fallow	0.030	0.023	0.009	0.020
Bromus catharticus	0	0.002	0.051	0.017
Arrhenatherum elatius	0	0	0.0003	0.0001
Festuca pratensis	0	0.005	0.002	0.002
Dactylis aschersoniana	0	0	0.003	0.001
Trifolium repens	0.009	0.933	1.476	0.806
Medicago lupulina	0.123	0.806	0.133	0.354
Lotus corniculatus	0.102	0.040	0.040	0.061
Medicago media	0.046	0.002	0.013	0.020
D_{min} ($\alpha = 0.05$)	0.166	0.983	0.546	0.423

brown and mostly dry in all the plots cut or mulched once per year. The seeds were not present on the plants yet.

The general evaluation of the sown species ability to reduce *Lactuca serriola* spreading showed that especially *Medicago lupulina* and *Trifolium repens* are not able to compete with this weed. Just in the year 1998 the number of *Lactuca serriola* plants was significantly higher in the stands of *Medicago lupulina* in comparison with the stands of the other species (except *Trifolium repens*) and the fallow. There was no significant difference among the dry mass yields of the sown species. In the year 1999 the number of weed plants in the stands of *Medicago lupulina* and *Trifolium repens* was significantly higher than in the other stands (Table 8). In the 3rd year of setting the soil aside (1999) the yield of *Lactuca serriola* in the stand of *Trifolium repens* was significantly higher in comparison with the other stands (Table 7). *Medicago media* was the less infested legume. These results have not sustained a significantly different influence of different ways of stands treating on *Lactuca serriola* appearance in the stands of the searched sown crops and fallow until now.

Table 8. Average number of *Lactuca serriola* L. plants ($.ha^{-1}$) in sown crops stands

Crop	Year			Average
	1997	1998	1999	
Fallow	4 464	8 928	12 857	8 750
Bromus catharticus	0	1 786	33 214	11 667
Arrhenatherum elatius	0	0	1 250	417
Festuca pratensis	0	893	1 429	774
Dactylis aschersoniana	0	0	5 893	1 964
Trifolium repens	536	144 286	342 500	162 440
Medicago lupulina	2 322	344 464	201 786	182 857
Lotus corniculatus	5 714	26 964	45 893	26 191
Medicago media	2 500	357	11 607	4 821
$D_{min.}$ ($\alpha = 0.05$)	6 813.4	211 294.1	146 236.7	121 382.4

Increasing of legume stands weed infestation, especially under once harvesting per year (once cut or mulch) is probably caused by their leaves drying, falling and as a result of it uncovering of the soil surface. Such stands do not allow to germinate to the seeds of *Lactuca serriola* spread by wind (Jütersonke and Arlt 1994), but they also give suitable conditions for the growth of the young weed plants. Only 2 or 3 harvests per year prevented seeds creating and ripening of *Lactuca serriola* in our trial.

In spite of no significant difference in weed infestation in dependence on different ways of stands treatment it is possible, on the basis of lower average yield and plant number of *Lactuca serriola* on the plots once mulched in comparison with the once cut ones, to see a reduction of number of *Lactuca serriola* young plants by mulching.

Lactuca serriola spreading on the land treated by these ways occurs in the first years after setting the land aside. Oesau (1992), Dittmann (1995), Hintzsche (1994) and Jütersonke and Arlt (1995) present a similar knowledge.

Increasing of *Lactuca serriola* plants number from the year 1997 to the year 1999 (the 4th year of vegetation) was statistically significant ($\alpha = 0.05$). A contingent decreasing of *Lactuca serriola* infestation of sown crop stands (especially legumes) in later years after setting the soil aside is probably caused by the other species spreading (particularly grasses) that cover the soil surface and do not permit germinating of the weed seeds that are spread by wind.

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ABSTRAKT

Výskyt druhu *Lactuca serriola* L. na půdě uváděné do klidu

Lactuca serriola L. je jedním z důležitých plevelů vyskytujících se na půdách uvedených do klidu. Proto byla v letech 1997 až 1999 sledována možnost zaplevelení těchto ploch osetých kulturními druhy *Bromus catharticus* Vahl., *Arrhenatherum elatius* (L.) P. Beauv. ex J.S. et K.B. Presl, *Festuca pratensis* Huds., *Dactylis aschersoniana* Graebn., *Trifolium repens* L., *Medicago lupulina* L., *Lotus corniculatus* L., *Medicago media* Pers. a divokých úhorů druhem *Lactuca serriola* L. při různých způsobech jejich obhospodařování (3× seč v průběhu vegetace s následným odvozem hmoty, 2× mulč v průběhu vegetace, 1× mulč na konci vegetace a 1× seč na konci vegetace s následným odvozem hmoty). U druhu *Lactuca serriola* L. byla sledována výška, počet a výnos sušiny rostlin. Výskyt druhu *Lactuca serriola* L. v porostech byl ovlivněn zejména druhem kulturní rostliny. Způsob ošetření neovlivnil průkazně zaplevelení druhem *Lactuca serriola* L. Z 3× sečených porostů byly nesilněji zapleveleny porosty *Trifolium repens* L., kde se *Lactuca serriola* L. v roce 1999 podílela na celkovém výnosu sušiny 17% a počet rostlin činil 412 tisíc.ha^{-1} (tab. 3). Ze sledovaných jetelovin potlačoval nejlépe druh *Lactuca serriola* L. porost *Medicago media* Pers. (tab. 2). Ve čtvrtém roce vegetace (1999) došlo k zaplevelení i všech porostů trav. V porostech 2× mulčovaných se za sledované období (1997 až 1999) *Lactuca serriola* L. nejvíce rozšířila v porostech *Medicago lupulina* L.a *Trifolium repens* L. Na parcelách s porostem *Medicago lupulina* L. se tento druh podílel 9 % (1998) a 1 % (1999) na výnosu celkové sušiny biomasy a v porostu *Trifolium repens* L. 0,2 % v roce 1998 a 9 % v dalším roce vegetace. V porostech *Arrhenatherum elatius* (L.) P. Beauv. ex J.S. et K.B. Presl se nevyskytly žádné rostliny *Lactuca serriola* L. Počet rostlin byl v letech 1997 až 1999 na 2× mulčovaných porostech v rozmezí 3 až 460 tisíc.ha^{-1} (tab. 3). Vícenásobné ošetření porostů v průběhu vegetace (3× seč, 2× mulč) zamezilo tvorbě a dozrávání nažek tohoto plevelného druhu. Na 1× sklízených parcelách (1× seč a 1× mulč) zaplevelila *Lactuca serriola* L. zejména porosty jetelovin, kde její podíl na celkovém výnosu sušiny dosahoval v průměru 16 % v porostech *Medicago lupulina* L. a 36 % v *Trifolium repens* L.

Výnos sušiny biomasy druhu *Lactuca serriola* L. byl v porostech *Trifolium repens* L. ve čtvrtém roce vegetace 2,34 t.ha^{-1} (1× mulč) a 1,86 t.ha^{-1} (1× seč). Nejméně byla z jetelovin zaplevelená *Medicago media* Pers. Porosty *Arrhenatherum elatius* (L.) P. Beauv. ex J.S. et K.B. Presl, *Festuca pratensis* Huds. a *Dactylis aschersoniana* Graebn. 1× mulčované nebyly druhem *Lactuca serriola* L. zapleveleny. Porosty *Arrhenatherum elatius* (L.) P. Beauv. ex J.S. et K.B. Presl sečené 1× ročně zamezily rozvoji druhu *Lactuca serriola* L. Celkový počet rostlin *Lactuca serriola* L. činil v 1× sklízených porostech trav a jetelovin 714 až 665 tisíc. ha^{-1} a průměrná výška rostlin byla v rozmezí od 0,33 do 1,77 m (tab. 6). Při jednom ošetření porostů na konci vegetace byly rostliny *Lactuca serriola* L. v době sklizně na všech parcelách (1× mulč a 1× seč) hnědé, většinou suché. Nažky již nebyly na rostlinách přítomny. Pokusy potvrdily možnost zaplevelení porostů pícnin na orné půdě uvedené do klidu a na úhorech druhem *Lactuca serriola* L. Celkové zaplevelení v roce 1999 bylo ve srovnání s rokem 1997 průkazně vyšší. Průměrný výnos a počet rostlin *Lactuca serriola* L. v porostech kulturních rostlin v letech 1997 až 1999 dokumentují tab. 7 a 8. Statistické hodnocení bylo provedeno analýzou rozptylu v programu Statgraphics, verze 4.2, metoda podle Tukeye.

Klíčová slova: orná půda; ukládání do klidu; trávy; jeteloviny; mulč; seč; *Lactuca serriola* L.

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Evaluation of yield structure and quality in durum wheat cultivars

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ABSTRACT

Spring durum wheat cultivars were evaluated under conditions of Prague locality for two years (1997 and 1998) and compared with control bread wheat cultivars Sandra and Munk. Traits like phenological characteristics, response to diseases, spike traits and yield components were evaluated. Average period of tested durum wheat accessions from plant emergence to heading was 50.5 days. Flowering started 7 days after heading and wax ripeness stage was reached 40.5 days after flowering. Hungarian cultivar Belladur was the latest in recorded developmental stages. Plant height varied from 50 to 79 cm. Consequently, their resistance to lodging was high. Durum wheat cultivars were susceptible to powdery mildew. Significantly high level of resistance of all durum wheat accessions was found for stem rust. Incomplete resistance to yellow rust was recorded only in Semperdur, Fenice and Belladur. In spike length, number of spikelets per spike and number of kernels per spike, the durum wheat accessions did not reach the level of control cultivars. Important yield component 1000 kernels weight of most tested durum cultivars was higher than 50.0 grams. The highest harvest index and grain yield were recorded in control bread wheat accessions. Austrian cultivar Semperdur and Hungarian Belladur were the highest productive durum wheat accessions. Relatively stable manifestation in the both years of evaluation was found in the following traits: lodging, stem and yellow rusts resistance, spike length, number of kernels per spikelet, 1000 kernels weight, and harvest index.

Keywords: durum wheat; phenological characteristics; diseases; spike characteristics; yield components

Gathering, evaluation and utilisation of wheat genetic resources at the territory of the Czech Republic have had long-term tradition. In former Czechoslovakia there were gathered first wheat genetic resources and used in breeding immediately after the World War I. Since that time the collection has been increased gradually till now, when it contains 9804 accessions. The collection is evaluated, documented and maintained in the Czech gene bank at the Research Institute of Crop Production, Prague-Ruzyně.

Durum wheat sub-collection consists of 832 accessions. Among them botanical varieties *leucurum* (238), *hordeiforme* (143) and *leucomelan* (107) are the prevailing ones. The most of them are spring cultivars.

Because of climate conditions, which are not much suitable for durum wheat growing, this crop is grown in the warmest regions of the Czech Republic. Only two cultivars of the crop are registered in the country – Soldur – winter durum and Grandur – spring cultivar of durum wheat.

The evaluation of our collection is aimed at description of durum wheat accessions under conditions of marginal region of growing and selection of the most suitable genotypes for such conditions. These materials could be used in breeding of new cultivars for similar conditions.

MATERIAL AND METHODS

Durum accessions were evaluated within the system of wheat genetic resources evaluation, likewise all new accessions which enter the collection. Each accession is evaluated for two years.

Locality description. Experimental field is located at the periphery of Prague (latitude N 50°05', longitude E 14°18', altitude 355 m) that is characterised by long-term average amount of precipitation per year 472 mm and average annual temperature 7.9°C. The warmest month is July (17.6°C).

Seed material used in experiment. In the period 1997 and 1998, 11 spring durum accessions were evaluated (Table 1), plus two bread spring wheat control cultivars registered in the country.

Cultural practice and evaluation during vegetation. The experiments were sown on April 2, 1997 and March 27, 1998, resp., that is normal time for spring wheat sowing in Prague locality. Sowing rate was 4.5 mil. germinant grains per hectare. Treated seeds were sown on the plots 4 m^2 in one replication. Weed was chemically controlled at the beginning of vegetation period (Granstar 75 WG in combination with Starane 250 EC). Phenological characters and responses to diseases were scored according to descriptor list (Bares et al. 1985)

Post-harvest analyses. Yield components and spike traits were analysed in 30 spikes per accession. Seed samples were analysed from the quality viewpoint; crude protein content by Kjeldahl method (Kjeltec Auto 1030 Analyzer), wet gluten content and gluten index on Glutomatic 2200.

Statistical analyses. Data for each trait from two years of evaluation were statistically analysed by multiple

Table 1. Durum wheat accessions evaluated in the period 1997–1998

Accession name	Botanical variety	State of origin
Alcatraz	*T. durum* var. *italicum*	MEX
Anade	*T. durum* var. *leucurum*	MEX
Aramides	*T. durum* var. *erythromelan*	MEX
Avetrilla	*T. durum* var. *melanopus*	MEX
Bejah	*T. durum* var. *valenciae*	MEX
Belladur	*T. durum* var. *leucurum*	HUN
Brzina	*T. durum* var. *melanopus*	MEX
Buttah	*T. durum* var. *leucomelan*	MEX
Fenice	*T. durum* var. *leucurum*	ITA
Semperdur	*T. durum* var. *leucurum*	AUT
Yazi	*T. durum* var. *leucomelan*	MEX
Munk – control	*T. aestivum* var. *lutescens*	DEU
Sandra – control	*T. aestivum* var. *aestivum*	CZE

analysis of variance and contrasts were tested by Tukey *HSD* test on 95 percent significance level.

RESULTS AND DISCUSSION

Phenological characteristics

Phenological traits were recorded to describe growth and development stages during vegetation period (Table 2). The periods were counted from the date of plant emergence.

Period from emergency to heading ranged from 48.5 (Alcatraz, Anade, Bejah and Brzina) to 56 (Belladur) days. Hungarian cultivar Belladur was extremely late and differed significantly from other cultivars except bread wheat Munk.

Flowering stage started 7 days after heading. Cultivar Brzina was the earliest (54.5 days) and Belladur the latest (61.5 days) in this trait. That was very different from data describing period from sowing to flowering in a Mediterranean environment which ranged from 132 to 148 days (Motzo et al.1996).

Wax ripeness stage was reached by tested cultivars 40.5 days after flowering that means that grain-filling period was relatively long. This period has a strong influence on grain yield via grain weight (Siname et al. 1993). The early cultivars Anade (95.0 days), Alcatraz (95.5 days) and Yazi (96.0 days) differed significantly from the latest ones – Fenice and Belladur (100.5 days).

Hungarian cultivar Belladur was the latest within the tested set in all three evaluated stages.

Plant height and lodging

Plant height of all tested cultivars was relatively short (62.3 cm in average) and did not exceed 80 cm. Italian cultivar Fenice was the shortest (50 cm) and on the other hand the Mexican Buttah was the tallest one (79 cm).

Higher susceptibility to lodging was recorded in the taller cultivars (Table 3). Anade, Buttah and Yazi, the most lodging susceptible cultivars belonged to the group with longer straw – they were taller than average plant height of the set.

Examined durum wheat accessions, in general, were short and consequently highly resistant to lodging.

Table 2. Phenological traits of tested durum wheat cultivars (1997–1998)

Emergence – heading			Emergence – flowering			Emergence – wax ripeness		
cultivar	days		cultivar	days		cultivar	days	
Alcatraz	48.5	a	Brzina	54.5	a	Anade	95.0	a
Anade	48.5	a	Sandra	55.5	ab	Alcatraz	95.5	ab
Bejah	48.5	a	Semperdur	56.0	ab	Yazi	96.0	ab
Brzina	48.5	a	Fenice	56.5	ab	Brzina	96.5	abc
Buttah	49.0	a	Munk	56.5	abc	Buttah	97.0	abc
Yazi	49.0	a	Alcatraz	57.0	abc	Aramides	98.0	abc
Aramides	50.0	ab	Buttah	57.0	abc	Avetrilla	98.0	abc
Avetrilla	50.0	ab	Yazi	57.5	abc	Bejah	98.0	abc
Semperdur	50.0	ab	Anade	58.0	abc	Sandra	99.0	abc
Sandra	52.5	bc	Bejah	58.0	abc	Munk	99.0	abc
Fenice	52.5	bc	Aramides	59.0	bc	Semperdur	99.5	bc
Munk	53.5	cd	Avetrilla	59.5	bc	Fenice	100.5	c
Belladur	56.0	d	Belladur	61.5	c	Belladur	100.5	c
Average	50.5			57.4			97.9	
Year differences	**			**			**	

** significant differences between years of testing *P* (0.05)

Table 3. Plant height and lodging resistance of tested durum wheat cultivars (1997–1998)

Plant height			Lodging		
cultivar	(cm)		cultivar	score	
Fenice	50.0	a	Anade	7.0	a
Aramides	52.5	ab	Buttah	7.5	ab
Alcatraz	56.0	ab	Yazi	8.0	abc
Bejah	57.5	ab	Alcatraz	8.5	bc
Belladur	57.5	ab	Aramides	8.5	bc
Avetrilla	60.5	ab	Munk	8.5	bc
Sandra	65.0	abc	Sandra	9.0	c
Anade	65.0	abc	Avetrilla	9.0	c
Brzina	65.0	abc	Bejah	9.0	c
Yazi	66.5	bc	Brzina	9.0	c
Semperdur	67.5	bc	Fenice	9.0	c
Munk	68.0	bc	Semperdur	9.0	c
Buttah	79.0	c	Belladur	9.0	c
Average	62.3			8.5	
Year differences	**				

** significant differences between years of testing *P* (0.05)

Disease resistance

Resistance to powdery mildew was evaluated under natural infection, but responses of wheat cultivars to stem and yellow rusts were tested under infection pressure in infection nursery (Table 4).

All durum wheat accessions were relatively susceptible to powdery mildew. Only cultivars Anade, Belladur and Yazi had the same or higher resistance level as control bread wheat Sandra. Susceptibility of another durum wheat set to powdery mildew and other fungal diseases described also Casulli (1990). The most resistant was control cultivar Munk.

Significantly higher level of resistance of all durum cultivars was found when their response to stem rust was compared with control bread wheat cultivars. Control cultivars were scored 3.0, resp. 3.5 only. On the other hand, durum cultivars showed the level of resistance between 6.0 (Aramides and Semperdur) and 9.0 (Belladur). The Austrian durum wheat Belladur was the most resistant to the stem rust.

Another Austrian durum wheat Semperdur showed, on the other hand, the lowest resistance to yellow rust (5.0). Also Italian Fenice and Hungarian Belladur were less resistant to this disease. Bread wheat control cultivars and all Mexican durum wheat accessions were fully resistant to that rust species.

Spike traits

Samples of 30 spikes per accession were analysed after harvest to describe their structure and identify important yield elements (Table 5).

Spike length of durum wheat accessions ranged from 4.7 cm (Buttah, Yazi, Alcatraz) to 5.8 cm (Fenice). But all of them had significantly shorter spikes than bread wheat cultivars Sandra (7.6 cm) and Munk (7.7 cm).

Similarly the control cultivars differed significantly from all durum accessions in number of spikelets per spike. Within the set of durum cultivars that had number of spikelets per spike in the range from 11.5 (Alcatraz) to 13.2 (Yazi) there were no significant differences.

Table 4. Disease resistance of tested durum wheat cultivars (1997–1998)

Powdery mildew			Stem rust			Yellow rust		
cultivar	score		cultivar	score		cultivar	score	
Bejah	3.0	a	Munk	3.0	a	Semperdur	5.0	a
Alcatraz	3.5	a	Sandra	3.5	a	Fenice	7.5	ab
Avetrilla	3.5	a	Aramides	6.0	b	Belladur	7.5	ab
Brzina	3.5	a	Semperdur	6.0	b	Sandra	9.0	b
Semperdur	3.5	a	Bejah	6.5	b	Munk	9.0	b
Buttah	4.0	a	Anade	7.0	bc	Alcatraz	9.0	b
Fenice	4.0	a	Avetrilla	7.0	bc	Anade	9.0	b
Aramides	5.5	ab	Brzina	7.0	bc	Aramides	9.0	b
Sandra	6.0	ab	Buttah	7.0	bc	Avetrilla	9.0	b
Anade	6.0	ab	Yazi	7.0	bc	Bejah	9.0	b
Belladur	6.0	ab	Fenice	7.0	bc	Brzina	9.0	b
Yazi	6.5	ab	Alcatraz	7.5	bc	Buttah	9.0	b
Munk	8.0	b	Belladur	9.0	c	Yazi	9.0	b
Average	4.8			6.4			8.5	
Year differences	**							

** significant differences between years of testing *P* (0.05)

Table 5. Spike traits of tested durum wheat cultivars (1997–1998)

Spike length			Number of spikelets per spike			Number of kernels per spike			Number of kernels per spikelet			Grain weight per spike			1000 kernels weight		
cultivar	(cm)		cultivar			cultivar			cultivar			cultivar	(g)		cultivar	(g)	
Buttah	4.7	a	Alcatraz	11.5	a	Bejah	14.2	a	Bejah	1.2	a	Bejah	0.7	a	Sandra	43.9	a
Yazi	4.7	a	Anade	11.8	a	Avetrilla	17.3	ab	Avetrilla	1.4	ab	Alcatraz	0.8	ab	Alcatraz	45.2	a
Alcatraz	4.7	a	Semperdur	11.9	a	Brzina	18.2	abc	Fenice	1.4	ab	Brzina	0.8	ab	Anade	45.5	a
Bejah	4.9	ab	Buttah	12.0	a	Fenice	18.4	abc	Brzina	1.4	ab	Avetrilla	0.9	abc	Bejah	47.9	ab
Brzina	5.1	abc	Bejah	12.1	a	Alcatraz	19.1	abc	Alcatraz	1.6	abc	Fenice	1.0	abcd	Munk	48.0	ab
Aramides	5.2	abc	Brzina	12.5	a	Semperdur	20.9	abcd	Semperdur	1.8	abc	Semperdur	1.0	abcd	Yazi	48.9	ab
Anade	5.2	abc	Avetrilla	12.7	a	Aramides	24.3	abcde	Aramides	1.9	abc	Aramides	1.1	bcde	Belladur	49.4	ab
Semperdur	5.5	abc	Aramides	12.9	a	Buttah	26.9	bcde	Sandra	2.0	abc	Anade	1.2	cde	Buttah	50.3	ab
Belladur	5.5	abc	Fenice	13.0	a	Anade	28.7	cde	Munk	2.2	bc	Belladur	1.3	cde	Brzina	50.4	ab
Avetrilla	5.6	bc	Belladur	13.0	a	Belladur	28.7	cde	Belladur	2.2	bc	Buttah	1.3	cde	Semperdur	52.9	ab
Fenice	5.8	c	Yazi	13.2	a	Yazi	30.6	de	Buttah	2.2	bc	Sandra	1.3	de	Aramides	53.9	ab
Sandra	7.6	d	Munk	15.7	b	Sandra	33.3	e	Yazi	2.3	bc	Yazi	1.4	de	Avetrilla	56.1	b
Munk	7.7	d	Sandra	16.8	b	Munk	34.0	e	Anade	2.4	c	Munk	1.5	e	Fenice	56.7	b
Average	5.5			13.0			24.2			1.8			1.1			49.9	
Year differences					**			**						**			

** significant differences between years of testing *P* (0.05)

Table 6. Harvest index and grain yield of tested durum wheat cultivars (1997–1998)

Harvest index			Grain yield		
cultivar			cultivar	(t.ha^{-1})	
Bejah	0.38	a	Bejah	2.1	a
Avetrilla	0.43	ab	Avetrilla	2.6	ab
Fenice	0.44	ab	Fenice	2.9	ab
Alcatraz	0.45	ab	Alcatraz	3.1	abc
Brzina	0.45	ab	Brzina	3.2	abcd
Buttah	0.48	ab	Anade	3.8	bcd
Aramides	0.50	ab	Aramides	3.9	bcd
Semperdur	0.50	ab	Buttah	3.9	bcd
Yazi	0.52	b	Yazi	3.9	bcd
Belladur	0.53	b	Semperdur	4.4	cde
Anade	0.53	b	Belladur	4.5	de
Sandra	0.55	b	Sandra	5.4	e
Munk	0.57	b	Munk	5.7	e
Average	0.49			3.8	
Year differences				**	

** significant differences between years of testing P (0.05)

Very different numbers of kernels in spike were recorded in the set of durum accessions (Bejah 14.2, Yazi 30.6). Similar differences among another durum wheat set but on higher level (37–61 grains) were described by (Motzo et al. 1996). The cultivars with the highest number of spikelets per spike (Belladur 13.0 and Yazi 13.2) had also the highest number of kernels per spike (28.7 and 30.6, resp.).

Number of kernels per spikelet within the group of durum accessions was very different; in Anade cultivar (2.4) two times higher than in Bejah (1.2). Control bread wheat cultivars Sandra and Munk had medium number of kernels in spikelet (2.0 and 2.2, resp.) that was slightly over the group average.

Also grain weight per spike – the trait with low heritability (Szwed-Urbas 1992) – of tested durum wheat cultivars was two times higher in Yazi (1.4 g) than in Bejah (0.7 g). Higher grain weight per spike was typical for bread wheat cultivars 1.3 g and 1.5 g, resp.

Large, heavy kernels were characteristic for many durum wheat cultivars. Their 1000 kernels weight ranged from 45.2 to 56.7 g and most of them had weight of 1000 kernels higher than 50.0 grams. It corresponds with the results from Mediterranean region (Motzo et al. 1996) where this parameter for largest seeds ranged from 51.8 to 59.5 grams. Control cultivars of bread wheat had smaller kernels (Sandra 43.9 g and Munk 48.0 g).

Average (1997–1998) harvest index (HI) of durum wheat accessions, as a ratio between grain weight and overground biomass weight, was very different (Bejah 0.38; Anade 0.53) (Table 6). Harvest index of Bejah cultivar was significantly different from the cultivars with HI higher than 0.5 (Yazi, Belladur, Anade). Bread wheat cultivars Sandra and Munk had highest HI (0.55 and 0.57, resp.). Harvest index is closely related to grain production but inversely correlated with grain protein content (Alessandroni et al. 1990)

Yield per hectare might be estimated very roughly on the basis of one plot – two years' average. Nevertheless, we can describe cultivar Bejah (2.1 t.ha^{-1}) as low productive, the group of cultivars Avetrilla, Fenice, Alcatraz and Brzina (2.6–3.2 t.ha^{-1}) as relatively low productive. On the

Table 7. Main quality parameters of tested durum wheat cultivars (1997–1998)

Crude protein content			Wet gluten content			Gluten index		
cultivar	(%)		cultivar	(%)		cultivar		
Munk	12.4	a	Sandra	25.1	a	Aramides	36.6	a
Yazi	12.8	a	Munk	26.6	a	Alcatraz	41.3	a
Sandra	13.0	a	Fenice	27.1	a	Brzina	49.2	a
Belladur	13.8	ab	Yazi	27.4	a	Belladur	49.8	a
Alcatraz	14.0	ab	Alcatraz	31.4	a	Bejah	51.5	a
Buttah	14.0	ab	Anade	31.4	a	Anade	57.4	a
Anade	14.0	ab	Semperdur	31.7	a	Buttah	66.3	a
Semperdur	14.1	ab	Belladur	31.9	a	Fenice	68.1	a
Brzina	14.7	ab	Buttah	32.5	a	Yazi	68.8	a
Avetrilla	15.2	ab	Brzina	32.9	a	Avetrilla	75.5	a
Aramides	15.3	ab	Avetrilla	36.2	a	Munk	75.6	a
Fenice	17.0	b	Aramides	38.1	a	Semperdur	81.5	a
Bejah	17.0	b	Bejah	41.3	a	Sandra	88.3	a
Average	14.4			31.8			62.3	
Year differences	**			**			**	

** significant differences between years of testing P (0.05) other hand, the Austrian durum wheat cultivar Semperdur and Hungarian Belladur were, under relatively similar conditions for which they have been bred, the most productive (4.4 and 4.5 $t.ha^{-1}$, resp.). None of durum accessions overcame grain yield of control bread wheat cultivars Sandra (5.4 $t.ha^{-1}$) and Munk (5.7 $t.ha^{-1}$).

Grain quality parameters

High crude protein content was identified in grain of most durum wheat cultivars and varied from 13.8 (Belladur) to 17.0% (Fenice and Bejah) (Table 7). Only Yazi had crude protein content (12.8%) on the level between the control bread wheat cultivars Munk (12.4%) and Sandra (13.0%). In this character Fenice and Bejah (17.0%) were significantly different from Yazi and the control bread wheat cultivars that had crude protein content 13.0% or lower. Average crude protein content (14.4%) corresponds with data from Western Canada (14.8%) from the period 1988–1996 (Anonym 1997).

Bread wheat cultivars with low protein content had also the lowest wet gluten content. Durum wheat accessions contained wet gluten in the range from 27.1 (Fenice) to 41.3% (Bejah). Unfortunately, owing to strong influence of growing conditions in the years of evaluation the differences among accessions were not significant. Also the average level of this trait (31.8%) is very similar to Western Canadian durum wheat (31.9%) (Anonym 1997).

Gluten index of tested wheat accessions was very variable; from 36.6 (Aramides) to 88.3 (control cultivar Sandra). For the same reason like in the case of gluten content also in gluten index the deep differences were not significant. But gluten quality (GI) of the Semperdur cultivar was very high – on the level of *T. aestivum* control cultivars.

Influence of growing (climatic) conditions – differences between years

Methodology, design of experiment and cultural practice during vegetation were the same in both years of the evaluation. That means that we can consider climate differences to be main source of differences between years of evaluation. The differences were tested by multiple analysis of variance and by Tukey *HSD*-test (Tables 2–7).

Significant differences between years of testing were found in all phenological phases (periods from plant emergence to heading, flowering and wax ripeness). It can be concluded that climatic conditions of the years when the set of wheat was evaluated had significant influence on stages of development. Significant differences between years were also found for plant height and powdery mildew.

The most of spike characteristics, except spike length, number of kernels per spikelet, 1000 kernels weight and HI were significantly different in the years of evaluation.

All quality parameters were influenced strongly by conditions during vegetation period.

Relatively stable manifestation was found in the traits like lodging, stem and yellow rust resistance, spike length, number of kernels per spikelet, 1000 kernels weight and harvest index.

CONCLUSIONS

Average period of tested durum wheat accessions including control bread wheat cultivars from plant emergence to heading was 50.5 days. Flowering started 7 days after heading and wax ripeness stage was reached 40.5 days after beginning of flowering. Hungarian cultivar Belladur was the latest in all recorded developmental stages.

All tested accessions had relatively short straw. Total plant height varied from 50 to 79 cm. Consequently their resistance to lodging was high, scored in the range 7–9, while the most of durum wheat accessions were fully lodging resistant (scored 9).

Control cultivar Sandra and durum wheat cultivars were relatively susceptible to powdery mildew. Significantly higher level of resistance of all durum wheat accessions was found when their response to stem rust was compared with control bread wheat cultivars. Not full resistance to yellow rust was recorded only in three cultivars Semperdur, Fenice and Belladur.

In the spike characteristics (spike length, number of spikelets per spike and number of kernels per spike), durum wheat accessions did not reach the level of the traits in control bread wheat cultivars. Number of kernels per spikelet within the group of durum accessions was very different; between 1.2 and 2.4 kernels. Important yield component 1000 kernels weight of most tested durum cultivars was higher than 50.0 grams. Control bread wheat cultivars had smaller seeds with lower 1000 kernels weight.

The highest harvest index and grain yield were recorded in control bread wheat accessions. Austrian cultivar Semperdur and Hungarian Belladur were the most productive durum wheat cultivars.

Relatively stable manifestation (non-significant differences between years of evaluation) was found in the following traits: lodging, stem and yellow rust resistance, spike length, number of kernels per spikelet, 1000 kernels weight and harvest index.

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ABSTRAKT

Zhodnocení struktury a kvality výnosu odrůd tvrdé pšenice

Odrůdy jarní tvrdé pšenice byly hodnoceny po dobu dvou let (1997 a 1998) v lokalitě Praha-Ruzyně a porovnávány s kontrolními odrůdami pšenice seté Sandra a Munk. Byly u nich ověřovány skupiny znaků jako fenologické charakteristiky, reakce na choroby, charakteristiky klasu a výnosové komponenty. U sledovaných vzorků tvrdé pšenice byla průměrná doba od vzejití do metání 50,5 dnů. Fáze kvetení začala 7 dní po metání a stadia voskové zralosti dosáhly vzorky v průměru 40,5 dne po kvetení. Maďarská odrůda Belladur byla v jednotlivých vývojových stadiích nejpozdnější. Výška rostlin se pohybovala mezi 50 a 79 cm a v důsledku toho byla vysoká jejich odolnost k poléhání. Odrůdy tvrdé pšenice byly citlivé k padlí travnímu. Průkazně vysoká hladina rezistence všech vzorků tvrdé pšenice byla naproti tomu zaznamenána u rzi travní. Neúplná rezistence ke rzi plevové byla zjištěna pouze u odrůd Semperdur, Fenice a Belladur. V délce klasu, počtu klásků v klasu a počtu zrn v klasu se vzorky tvrdé pšenice nevyrovnaly kontrolním odrůdám. V hmotnosti 1000 semen přesáhla většina hodnocených odrůd pšenice tvrdé hranici 50,0 g. Nejvyšší sklizňový index byl zjištěn u kontrolních odrůd pšenice seté. K nejvýnosnějším odrůdám tvrdé pšenice patřily maďarské odrůdy Semperdur a Belladur: Relativně stabilní projev byl v obou pokusných letech zjištěn u znaků: poléhání, odolnost ke rzi travní a plevové, délka klasu, počet zrn v klásku, HTS a u sklizňového indexu.

Klíčová slova: tvrdá pšenice; fenologické charakteristiky; choroby; charakteristiky klasu; výnosové komponenty

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Polymorphism of biochemical markers in selected varieties of spelt wheat (*Triticum spelta* L.)

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ABSTRACT

Esterase isozymes, proteins extracted in 2% glutathione buffer (separated in non-denaturing PAGE system) and seed proteins separated in SDS-PAGE were used as biochemical markers for evaluation of polymorphism level in three spelt wheat varieties, three breeders' spelt lines (originated from hybridisation between spelt and common wheat) and reference common wheat variety. Electrophoretic phenotypes and zymograms were evaluated by means of digital image analysis and Nei & Li coefficient of similarity was used to evaluate the relation of analysed genotypes. Entire evaluation of all three marker systems showed differences between common wheat variety Brea and spelt varieties and spelt breeders' lines. Also significant differences between old spelt varieties (Hercule, Altgolg and Rouquin) and new spelt breeders' lines were found.

Keywords: spelt wheat; biochemical markers; seed proteins; isozymes; electrophoresis

Spelt wheat (*Triticum spelta* L.) represents phylogenetically earlier form of bearded hexaploid wheats with genome constitution AA BB DD (Kling 1988). Winzeler and Rügger (1990) published that not hexaploid bearded form of *Triticum aestivum*, but spelt wheat is probably the initial independent form of culture wheat. Traditionally the highest spreading of spelt wheat as agricultural crop is in south-western Germany and in Switzerland.

The interest in cultivation of spelt wheat is increasing also in the Czech Republic at present. Because of spelt wheat exhibits a range of unfavourable growing and technological features, in recent years spelt is hybridised with common winter wheat (mainly English provenience). In European market there are available old traditional spelt varieties from Germany, Switzerland and Belgium and also new breeding materials with high rate of common wheat genome. Genetic differentiation between original common wheat and these hybrids of spelt and common wheat is very difficult as a result of high proportion of common wheat genome in these hybrids. These reasons led into utilisation of biochemical and molecular markers in identification of spelt wheat varieties (Harsch et al. 1997).

Proteins should be used as genetic markers: these biochemical systems performed a relatively high level of genetic polymorphism independent on environment conditions, the alleles at most isozyme loci are co-dominant, and cause no deleterious changes in plant phenotype, this co-dominance also allows homozygotes to be distinguished from heterozygotes. Isozymes (molecular forms of enzymes) or proteins without detectable enzymatic activity as seed storage proteins should be used as these biochemical markers (Sýkorová and Hadačová 1992).

Two methodological approaches are mostly used in spelt proteins studies. The most employed method is analysis of gliadins (identification of spelt varieties and lines and prediction of technological quality). The second method is based on analysis of total protein in conditions of SDS-PAGE (Radic-Miehle et al. 1997; Šašek and Černý 1997). Radic-Miehle et al. (1997) published standard electrophoretic patterns for both protein systems (gliadins and total SDS protein) typical for spelt genome and common wheat genome. Electrophoretic pattern of total SDS-protein separated in conditions of SDS-PAGE exhibited two variable zones characterised by molecular weight 40–50 kDa and 55–61 kDa. Unfortunately, as a result of high genetic affinity between spelt varieties it is sometimes almost impossible to distinguish different varieties. Harsch et al. (1997) reported that two German traditional spelt varieties Ostro and Oberkulmer exhibited identical gliadin patterns.

The objectives of this paper were to identify traditional varieties of spelt wheat (Hercule, Altgold, Rouquin) and Swiss new breeding lines (H 92.20, H 92.27, H 92.28) using electrophoretic analysis of biochemical markers. Three different biochemical systems were used for analyses: SDS – total protein, isozymes of esterases and proteins soluble in 2% glutathione. These marker systems were chosen according to our preliminary results (Dvořáček et al. 2000) and also poor availability of 2-chloroethanol (extraction reagent in standard gliadin analysis procedure) influenced the choice of these three systems.

MATERIAL AND METHODS

In this study 6 varieties of spelt wheat were analysed:
- two traditional Belgian varieties Hercule (in Tables and Figs. marked No. 1) and Rouquin (No. 3)
- one Swiss variety Altgold (No. 2)

– three Swiss new breeding varieties from breeding station Triemenhof-Girenbad: M 92.20 (No. 5), H 92.27 (No. 6), H 92.28 (No. 4)

Also one reference variety of common winter wheat (Brea) was analysed.

The whole grains were used for electrophoretic analyses and 20 grains were used for each marker system. Three marker systems were used: (1) esterase isozymes detected after PAGE, (2) proteins soluble in 2% glutathione buffer and separated in PAGE and (3) proteins pre-extracted in Na-phosphate buffer and separated in SDS–PAGE. Methods of extraction and electrophoretic separation are described in Dvořáček et al. (2000).

Dual slab electrophoretic system HOEFER SE600 was used for analysis. Electrophoretic phenotypes were digitised and evaluated using specialised software BioProfil 1D++ (Vilber Lourmart). Similarity matrixes were calculated using Nei & Li coefficient of genetic similarity and dendrograms were calculated using UPGA method and 95% confidence.

RESULTS AND DISCUSSION

Complex of esterase isozymes

Seed esterases exhibited relatively high number of bands detected on gels but level of polymorphism in this system was very low. Two zones of enzyme activity were detected: in high molecular weight part of the pattern ($Rf = 0.125$) two or three bands were detected (substrate specificity to α-naphthyl acetate and in lower molecular weight part of the pattern ($Rf = 0.438$) three bands were detected (substrate specificity to ß- naphthyl acetate – Fig. 1).

On the basis of esterase pattern evaluation the analysed genotypes were divided into three different groups. The first group was represented by a traditional spelt variety Altgold; the second group by a new breeding material M 92.20; and in the third group both traditional varieties (Hercule and Rouquin), new breeding materials (H 92.28, H 92.27) and common wheat variety (Brea) were concentrated (dendrogram in Fig. 2).

Complex of proteins soluble in 2% glutathione buffer

This marker system (native proteins – Čurn and Sáková 1999) exhibited relatively poor pattern consisting of 1–3 bands (MW = 30–33 kDa). Results of electrophoretic separation are demonstrated in Fig. 3. Again three groups of varieties were distinguished using this marker system. Spelt varieties Hercule, Rouquin and new breeding material H 92.27 were concentrated into first homology group. Spelt variety Altgold and new breeding lines M 92.20 and H 92.28 formed the second group. Third group was represented by common wheat variety Brea, which was considerably different from spelt varieties and hybrid genotypes (dendrogram in Fig. 4 and Table 1).

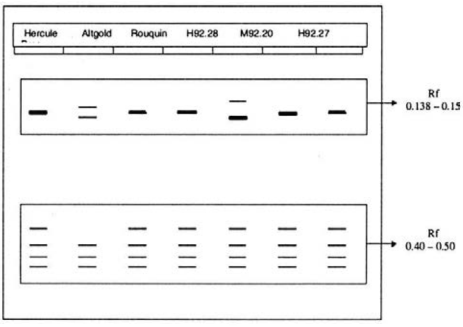

Figure 1. Pattern of leaf esterases

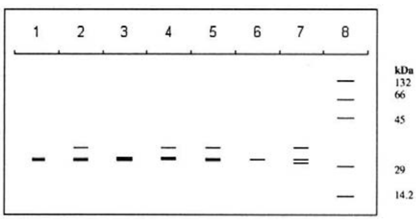

Figure 3. Pattern of seed proteins extracted using 2% glutathione buffer

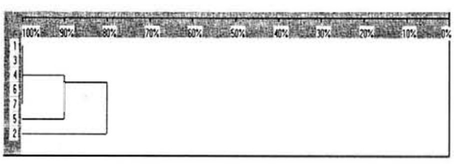

Figure 2. Dendrogram of leaf esterase isozymes, Nei-Li coefficient

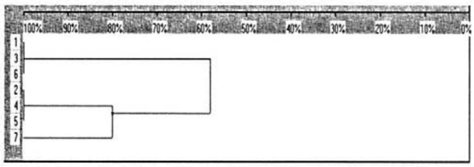

Figure 4. Dendrogram of seed proteins (extraction in 2% glutathione buffer), Nei-Li coefficient

Table 1. Molecular weight (in kDa) for seed proteins extracted using 2% glutathione buffer

	MW-RF							
	L1	L2	L3	L4	L5	L6	L7	L8
1	30.872	32.885	30.872	32.885	33.000	30.730	33.000	132.000
2		30.730		31.009	30.730		30.872	66.000
3							30.114	45.000
4								29.000
5								14.200

L1–L7 = analysed wheat genotypes L8 = molecular weight marker

Total protein separated in conditions SDS-PAGE

This marker system exhibited very abundant pattern with three variable zones of bands localised in regions of molecular weights 116–80 kDa, 55–45 kDa and 36 kDa. Results of these analyses are shown in Fig. 5 and Table 2. Also in this case relatively high uniformity in analysed set of spelt varieties and spelt/common wheat hybrids (dendrogram in Fig. 6) is evident. Although it was possible to distinguish all analysed varieties, the similarity higher than 90% did not allow recommending this marker system for precise identification of spelt wheat varieties.

Final evaluation

Using BioProfil 1D++ software we evaluated all three marker systems. Output of this analysis is summarised in Table 3 and expressed in graphic form in dendrogram in Fig. 7. There is obvious high similarity between dendrograms in Figs. 6 and 7 and total protein separated in conditions of SDS-PAGE had decisive contribution in final expression of these three biochemical markers.

Using complete evaluation of all three marker systems considerable difference between referent winter wheat variety Brea and spelt genome is evident. Also significant difference between group of two new breeding materials (M 92.20 and H 92.27) and group of traditional spelt varieties (Hercule, Altgold, Rouquin) was recorded.

The analysis of total protein in conditions of SDS-PAGE was the crucial factor for final identification of spelt varieties. This confirmed results of other authors. Westermeier (1990) published that methods based on presence of detergent like SDS catch broader range of variation as a result of analysis of total protein pattern. Also Šašek and Černý (1997) utilised the method of electrophoretic separation in presence SDS for analysis of glutenins with HMW.

It is also in agreement with Radic-Miehle et al. (1998) and Harsch et al. (1997) who outline the problem of very difficult determination of spelt hybrids (hybrids between spelt and common wheat). This situation is confirmed by new breeding material H 92.28, which electrophoretic phenotypes are almost identical with spelt patterns. How-

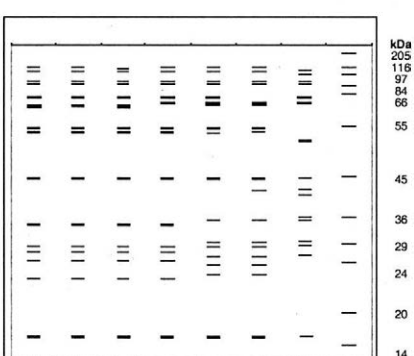

Figure 5. Pattern of seed proteins separated in SDS-PAGE

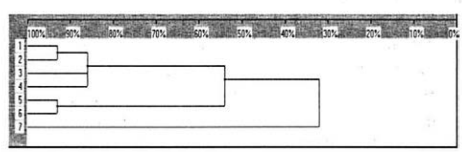

Figure 6. Dendrogram of seed proteins separated in SDS-PAGE, Nei-Li coefficient

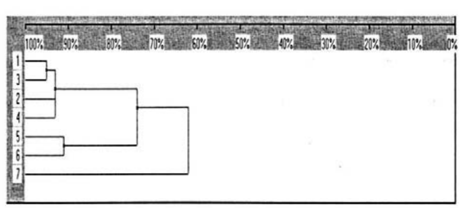

Figure 7. Dendrogram – entire evaluation of all three marker systems, Nei-Li coefficient

Table 2. Molecular weight (in kDa) for seed proteins separated in SDS-PAGE

	MW-RF							
	L1	L2	L3	L4	L5	L6	L7	L8
1	116.000	116.000	111.202	116.000	116.000	116.000	106.584	205.000
2	104.398	102.324	102.324	102.324	102.324	102.324	97.000	116.000
3	90.855	90.855	90.855	90.855	90.855	90.855	90.855	97.000
4	87.044	87.044	87.044	87.044	87.044	87.044	87.044	84.000
5	64.551	64.551	64.551	64.551	64.551	62.466	64.551	66.000
6	61.569	61.952	61.569	62.724	62.466	54.644	62.595	55.000
7	54.644	54.644	54.644	54.644	54.644	53.931	52.147	45.000
8	53.859	53.859	53.859	53.859	53.646	44.554	44.703	36.000
9	44.554	44.554	44.554	44.554	44.554	42.007	42.311	29.000
10	34.088	34.088	34.088	34.088	35.183	35.183	41.243	24.000
11	28.121	28.121	28.121	28.121	29.407	29.407	35.909	20.000
12	26.523	26.523	26.523	26.523	28.121	28.121	35.183	14.200
13	24.366	24.366	24.366	24.366	25.438	25.438	29.809	
14	21.793	21.793	21.793	21.793	23.517	23.517	28.560	
15	15.838	15.838	15.838	15.838	22.171	22.171	25.821	
16					15.762	15.762	15.991	

ever, no differences between traditional spelt varieties are high. Relatively higher differences are only between both Belgian varieties Hercule (No. 1) and Rouquin (No. 3), as shown on dendrogram in Fig.6.

Significant differences were found between new breeding material H 92.28 and two lines M 92.20 and H 92.28. Line H 92.28 was linked to spelt genome cluster. On the other side lines M 92.20 and H 92.28 were joined to corporate cluster with common wheat and different level of introgression of wheat genome is obvious in analysed new breeding materials.

Spelt wheat represents earlier form of bearded hexaploid wheats with relatively narrow genetic background and low variation in system of biochemical markers. Only one marker system is insufficient for unambiguous determination or identification of particular spelt variety. Further study in the sphere of identification spelt varieties should be focused on utilisation of more sensitive molecular markers, markers on the level of DNA.

Table 3. Similarity matrix (Nei-Li's coefficient) resulting from entire evaluation of all three marker systems

	L1	L2	L3	L4	L5	L6	L7
L1	1.00						
L2	0.93	1.00					
L3	0.95	0.88	1.00				
L4	0.93	0.91	0.88	1.00			
L5	0.76	0.78	0.71	0.83	1.00		
L6	0.74	0.68	0.70	0.77	0.91	1.00	
L7	0.62	0.61	0.62	0.70	0.71	0.65	1.00

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ABSTRAKT

Hodnocení polymorfismu biochemických markerů u vybraných odrůd pšenice špaldy (*Triticum spelta* L.)

Izoenzymy esteráz, proteiny extrahované v 2% glutationu a separované v nedenaturačních podmínkách PAGE a celkový protein zrna separovaný v podmínkách SDS-PAGE byly použity jako biochemické markery pro hodnocení polymorfismu tří odrůd špaldy, tří novošlechtění (kříženců špaldy a pšenice seté) a jedné referenční odrůdy pšenice seté. Získané elektroforeogramy byly hodnoceny metodickým postupem digitální obrazové analýzy a pomocí koeficientu podobnosti (Nei&Li) byla hodnocena příbuznost analyzovaných genotypů. Při celkovém hodnocení všech tří sledovaných markerovacích systémů je možné shledat diferenci mezi referenční odrůdou pšenice seté Brea a genomem pšenice špaldy. Dále byla nalezena výrazná diference mezi skupinou dvou novošlechtění M 92.20 a H 92.27 a skupinou tradičních odrůd Hercule, Altgold a Rouquin, k nimž bylo přiřazeno novošlechtění H 92.28.

Klíčová slova: pšenice špalda; biochemické markery; zásobní proteiny; isoenzymy; elektroforéza

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Variability of storage proteins in some Czech landraces, modern and obsolete cultivars of wheat (*Triticum aestivum* L.)

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ABSTRACT

A set of Czech landraces and obsolete cultivars was evaluated with respect to the allelic variations at *Gli-1*, *Glu-1* and *Glu-3* loci. PCR based technique was used for LMW-GSs evaluation. The method was proved to be suitable for study of wheat germplasm with respect to LMW-GSs allelomorphism. A higher diversity was found in the landraces and obsolete cultivars as compared with modern ones. Especially short amplification products corresponding to LMW-GSs with shorter central repetitive domains are missing in modern cultivars. Landraces and obsolete cultivars originating in the Czech Republic were proved to be a source of important variability with respect to LMW-GSs and could be used as a source of alternative alleles. Gliadin and HMW-GS allelomorphism evaluated on the protein level were similar to those found in comparable sets of wheat genotypes. No significant relationships were discovered between gliadins and LMW-GS patterns based on clustering, even when *Gli-1* and *Glu-3* loci are localised close to each other on the same chromosome arms.

Keywords: low-molecular-weight (LMW) glutenin; high-molecular-weight (HMW) glutenin; gliadin; diversity; wheat; landraces; genetic resources

Modern plant breeding and agronomic practises have regrettably eroded the genetic diversity of original local cultivars and landraces and resulted in narrowing of the genetic base of present cultivars in many crops. Landraces and obsolete cultivars (for definition see Gepts 1993) have been studied and exploited along with modern genotypes in programs for genetic improvement of major crops such as wheat, barley or rice. Landraces and obsolete cultivars are considered to be an important source of valuable traits due to slightly different physiological characteristic (Plucknett et al. 1987; Kato and Yokoyama 1992; Zhang 1995). They could serve also as potential donors of genes for improvement of protein content (Blum et al. 1987; Ciaffi et al. 1991). Unusual alleles of storage proteins, especially of HMW glutenins, have been found in some landraces and obsolete cultivars as well (Ciaffi et al. 1993; Gregová et al. 1999).

Wheat storage proteins play an important role in determining dough technological quality. Wheat storage proteins are represented by two main classes of proteins: polymeric glutenins and monomeric gliadins. Glutenins comprise several types of subunits, which are bound by disulphide bridges and form extra large macromolecules in wheat endosperm (Wrigley 1996). Glutenins are usually divided into two groups according to their molecular weights – HMW (High Molecular Weight) and LMW (Low Molecular Weight) glutenin subunits (= GS). The structures of HMW-GSs and their encoding genes have been well characterised. HMW-GS genes have been localised at the *Glu-1* loci at the long arm of group 1 chromosomes. Up to now complete DNA coding sequences of subunits presented in cv. Cheyenne have been published (rev. Shewry et al. 1992). It was also proved, that some allelic variants are associated with good breadmaking quality (Payne 1987). LMW-GS genes have been localised at *Glu-3* loci at the short arm of group 1 chromosomes (Singh and Shepherd 1988) LMW-GSs genes have been studied mainly in durum wheat (*Triticum durum* L.) because of a high correlation between LMW-GS alleles and pasta-making quality (D'Ovidio et al. 1999). Several LMW-GS genes have been isolated from hexaploid wheat (Cassidy et al. 1998), as well. LMW-GSs are characterised less in comparison with HMW-GSs due to some difficulties with their resolution using one-dimensional (1D) protein polyacrylamide gel electrophoresis.

The characterisation of the genetic variability of wheat storage proteins in Czech landraces and obsolete cultivars of hexaploid wheat was the main aim of the present work. Up to now the allelomorphism of storage protein genes in wheats of Czech origin have been mostly studied by starch electrophoresis of gliadins and SDS-PAGE of HMW-GSs (Černý and Šašek 1996a, b). Special attention was devoted in our investigation to LMW-GS alleles, because LMW-GSs allelomorphism have not been studied in a similar set of wheat material up to now, even when their effect upon final bread making is apparent (Liu et al. 1996; Nieto-Taladriz et al. 1998; Lee et al. 1999).

MATERIAL AND METHODS

Plant material. Altogether 15 landraces and obsolete cultivars of wheat (*Triticum aestivum L.*) grown in 30's and 40's were evaluated in our study. Seven modern cultivars of Czech origin (Alka, Hana, Šárka, Sparta, Vala, Vlada, Vlasta) and Slovak cultivar Astella were used as a control material for LMW-GSs analysis. For more detail characterisation of plant material see Table 1a, b. Cv. Chinese Spring nulli/tetrasomic lines were used to confirm chromosome specificity of the PCR primers.

Gliadins characterization. Approximately 100 grains per each accession were ground into fine powder to get a complex sample containing all alleles included in the cultivar. Gliadins were extracted and subjected to polyacrylamide gel electrophoresis (PAGE) with Al-lactate buffer (pH = 3.1) as described by Metakovsky and Novoselskaya (1991). The gliadin patterns were evaluated by Gel Manager v1.5. Gel Manager software was used to obtain Pearson product moment coefficients for the comparison of gliadin patterns.

HMW glutenin subunits characterization. Approximately 100 grains per each accession was ground into a fine powder to get a complex sample containing all alleles included in the cultivar. Glutenins were extracted, separated and visualised according to the standard SDS-PAGE technique (Wrigley et al. 1992) and scored according to Payne (1987) nomenclature. The gels were photodocumented using Lucia D Image analysis.

Characterisation of LMW GS coding sequences by the use of PCR. DNA was extracted from bulked leaves of minimally 40 ten-day-old greenhouse cultivated plants following the Saghai-Maroof et al. 1984 protocol. DNA was dissolved in TE (10 mM TRIS-Cl, 1 mM EDTA, pH = 7.5) to the final concentration 50 ng.μl^{-1}. Following primer pairs proposed by Ciaffi et al. (1999) were used for the PCR amplification of LMW-GSs alleles:

Primer pair I:
5'-CGACAAGTGCAA TTGCGCAGATGGA-3' (forward)
5'-ACCTAGCAAGACGTTGTGGCATTGC-3' (revers)

Primer pair II:
5'-AGATGCATCCCTGGTTTGGAG-3' (forward)
5'-GAGGAATACCTTGCATGGGTT-3' (revers)

Primer pair III:
5'-AACCCATGCAAGGTATTCCTC-3' (forward)
5'-AATGGAAGTCATCACCTCAAG-3' (revers)

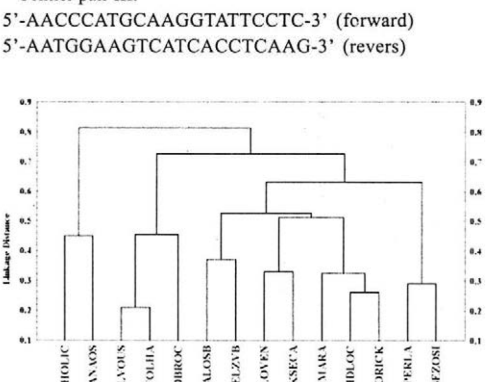

Figure 1. Association among landraces and old cultivars obtained on the basis of genetic similarity estimates (Gel Manager software) calculated from patterns of A-PAGE analysis of gliadins

PCR reaction was carried out in a reaction volume of 20 µl containing 1U *Taq* polymerase (Promega) and 1× corresponding *Taq* PCR buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 1 µM of each primer and 50 ng of template DNA. DNA was denatured 2 min. at 95°C, amplification conditions were 35 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 1 min followed by final extension 7 min at 72°C. PCR products were separated on capillary electrophoresis using ABI PRISM 310 Genetic Analyser (Perkin Elmer). Data were analysed in conjunction with GeneScan and Genotyper software.

Statistical analysis. A binary matrix reflecting specific LMW-GS PCR product presence (1) or absence (0) was generated for each accession. Pairwise distances between the accessions based on Dice's similarity metrics (Nei and Li 1979) were calculated with the use of RAPDALG program (The RAPDistance Package, Armstrong et al. 1994), which is suitable for binary data analysis. UPGMA-clustering, principal component and regression analysis were conducted using the statistical software package STATISTICA (StatSoft, Inc.). Diversity index for each PCR product was calculated according to Dahleen (1997).

RESULTS

Each of the evaluated accessions exhibited specific gliadins patterns. Only alleles, which have been described up to now in the world collection, were found within studied material. The minimal variations (only between α-gliadins) were found between cv. Valtická osinatá and Selecty Chrudimka. Cluster analysis based on gliadins variability divided the cultivars into the four main groups (Fig. 1). Kelčanská Holice and Hanácká osinatá formed one group, even when their quality parameters (Table 1a) are different. The second cluster consists of three accessions – Slapská vouska, Novodvorská Volha and Dobrovická červená, with different quality parameters. Accessions from the third group, which was the largest one, were also differing in the sedimentation test value and protein content. Two cultivars and landraces were grouped in the fourth cluster.

Variability was also found among cultivars in HMW-GS composition. The single signal per each locus was found in all accessions. It indicates, that there is only one major HMW line in each of evaluated accessions. All of the most common glutenin subunits were identified at *Glu-1A* loci (GS-null, 1, 2*), the GS 1 was the most frequent. Five GSs allelic combinations were found at *Glu-1B* loci and only two GSs allelic combinations were identified at *Glu-1D* loci. The 5 + 10 combination, which is important for good bread-making quality was found only at three cultivars. The gluten score varied between 5 and 10 (Table 2a). Only one allele per locus was found also in evaluated modern cultivars (Table 2b) using bulk analysis.

The PCR analysis of LWM-GS loci of the 15 obsolete cultivars and landraces identified 14 characteristic profiles within 15 cultivars. Profiles of cv. Valtická osinatá

Table 1a. Description of wheat (*Triticum aestivum L.*) landraces and obsolete cultivars used in this study; thousand grain weight and quality parameters – microsedimentation test (*SDS*) values, protein dry contents according to Kjeldahl were undertaken from Michalová and Dotlačil (1992)

Cultivar/Landrace	Year of registration	Botanical name	Origin	Breeding method	Pedigree	A (%)	B (ml)	C (g)
Bezosinná červená perla	1935	*Triticum aestivum* L. var. *milturum* (ALEF.) MANSF.	Uničov	crossing	Dregerova BI 22/ Dobrovická přesívka (01C010124)	15.6	6.3	41.4
Červená perla	1935	*Triticum aestivum* L. var. *milturum* (ALEF.) MANSF.	Uničov	crossing	Dregerova BI 22/Dobrovická přesívka	15.7	7.1	33.6
Dobrovická červená 19	1926	*Triticum aestivum* L. var. *milturum* (ALEF.) MANSF.	Semčice u Dobrovic	crossing	non available	16.5	7.2	33.4
Hanácká osinatá	1939	*Triticum aestivum* L. var. *ferrugineum* (ALEF.) MANSF.	Přerov	crossing	Diosecká 777/Hanácká řídkoklasá M 20	16.6	6.7	39.3
Hořická	1934	*Triticum aestivum* L. var. *milturum* (ALEF.) MANSF.	Přerov	crossing	Diosecká 200/Šebkova česká červenka přesívka	15.0	5.6	42.4
Kelčanská holice	1935	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Kelčany	crossing	S – Kelčanská osinatka	14.7	5.9	38.5
Kelčanská Secalobastard	1935	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Kelčany	crossing	*T. aestivum*/*S. cereale*	15.8	6.6	34.9
Mandelíkova ratbořská Mara	1933	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Ratboř-Uhlířské Janovice	crossing	Rimpaus Frueher Bastard/ Strubes St. 56 Hadmersleben	14.7	5.2	36.9
Novodvorská Volha	1933	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Olomouc	crossing	Sv. Panzer/Ruská genealogická belka	14.9	5.6	40.7
Selecty Chrudimka	1940	*Triticum aestivum* L. var. *milturum* (ALEF.) MANSF.	Stupice	individual selection	LV – česká červenka, Chrudim	16.6	7.4	38.5
Selecty ZVb	1922	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Stupice	individual selection	S-LV, Dolní Rakousko	15.6	7.8	33.3
Slapská vouska	1940	*Triticum aestivum* L. var. *ferrugineum* (ALEF.) MANSF.	Slapy u Tábora	individual selection	S – Manitoba	14.1	7.2	40.6
Slovenská 1784	1947	*Triticum aestivum* L. var. *aestivum* [var. *erythrospermum* (KOERN.) MANSF.]	Diosek-Nový Dvor, Slovensko	crossing	Diosecká 124/Marquis	16.1	7.1	39.3
Valtická osinatá B	1927	*Triticum aestivum* L. var. *ferrugineum* (ALEF.) MANSF.	Lednice	crossing	S-LV, Uhersko	17.2	5.9	40.4
Židlochovická bezosinná km. 12	–	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Židlochovice	individual selection	S – Židlochovická podhorka	13.7	5.0	41.2

A = protein content in dry mass (%)

B = *SDS* test (ml)

C = thousand grain weight (g)

Table 1b. Description of wheat (*Triticum aestivum* L.) modern cultivars used in this study

Cultivar	Year of registration	Botanical name	Origin	Breeding method	Pedigree
Alka	1995	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Selgen, Uhřetice (CZE)	crossing	Hana/Mercia
Astella	1995	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Istropol, Slovakia (SVK)	crossing	Viginta/SO-80-2208
Hana	1985	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Plant Select, Hrubčice (CZE)	crossing	NS 984-1//Mironovskaya 808/Moisson
Šárka	1997	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Selgen, Uhřetice, VÚRV Ruzyně (CZE)	crossing	UH677/Miron.nizkorostlaya// Avalon/Miron.nizkorostlaya
Sparta	1988	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Selgen, Stupice (CZE)	crossing	Mironovskaya 808/Artois Desprez// A. Desprez/3/ M. Huntsman/Weihestephan 378-57
Vala	1980	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Plant Select, Hrubčice (CZE)	crossing	Moisson/Mironovskaya 808
Vlada	1990	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Monsanto ČR, Branišovice (CZE)	crossing	Mironovskaya 808/4/Kaštická osinatka/ *T. tomopheevi*//Harrachw./3/Harrachw./ /S.Pastore/Kavkaz
Vlasta	1999	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	Selgen, Uhřetice,VÚRV Ruzyně (CZE)	crossing	Hana/Brimstone/S13

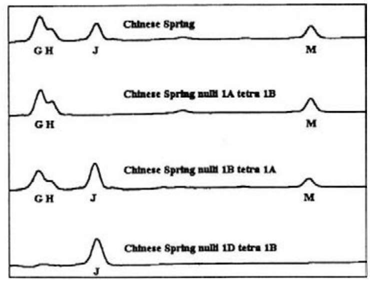

Figure 2. Amplification profiles of nulli/tetrasomic lines of cv. Chinese spring separated on capilary electrophoresis in ABI PRISM 310 Genetic Analyser; primer pair I was used for amplification; letters are corresponding to categories listed in Table 3

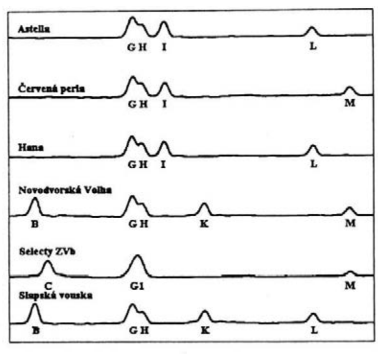

Figure 3. Amplification profiles of several cultivars separated obtained by primer pair I on capilary electrophoresis in ABI PRISM 310 Genetic Analyser; primer pair I was used for amplification; letters are corresponding to categories listed in Table 3

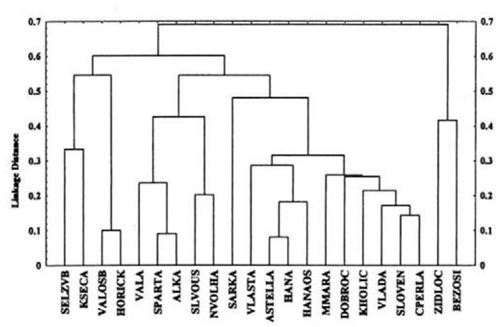

Figure 4. Association among wheat landraces and obsolete cultivars revealed by cluster analysis performed on genetic similarity estimates (Nei and Li 1979) calculated from 40 polymorphic products amplified by three LMW-GS specific PCR primers

Table 2a. HMW-GSs identified in evaluated set of wheat (*Tritium aestivum L.*) landraces and obsolete cultivars detected by separation of extracted HMW-GSs on SDS-PAGE

Cultivar/Landrace	Glu-1 loci			
	Abbreviation	Glu-A1 subunits	Glu-B1 subunits	Glu-D1 subunits
Bezosinná červená perla	Bezosi	0	20	2 + 12
Červená perla	Cperla	2*	7 + 9	5 + 10
Dobrovická červená 19	DobroC	1	7 + 9	2 + 12
Hanácká osinatá	Hanaos	1	22	2 + 12
Hořická	Horick	0	7 + 8	2 + 12
Kelčanská holice	Kholic	2*	7 + 8	2 + 12
Kelčanská Secalobastard	Kseca	0	7 + 8	2 + 12
Mandelíkova ratbořská Mara	Mmara	0	7 + 9	2 + 12
Novodvorská Volha	NVolha	1	7 + 9	2 + 12
Selecty Chrudimka	SelChr	1	6 + 8	2 + 12
Selecty ZVb	SelZvb	1	7 + 8	5 + 10
Slapská vouska	Slvous	1	7 + 9	2 + 12
Slovenská 1784	Sloven	1	7 + 9	2 + 12
Valtická osin. B	Valosi	0	22	2 + 12
Židlochovická bezosinná km. 12	Zidloc	2*	7 + 8	5 + 10

and Selecty Chrudimka were identical. Even less variability was found in modern Czech wheat cultivars. Profiles were evaluated according to the categories into which amplification products were divided. The primer pair I amplified products 390–462 pb long. Amplification products were divided into 13 distinct categories. The primer pair II amplified shorter products ranging from 298 to 492 divided into 13 categories. The amplification with the primer pair III amplified the shortest fragments (355–418 pb long) and the fragments were divided into 14 categories. The less variability was found in elite modern cultivars. (Table 3, Fig. 3). The category III/D, the category III/E, the category III/L and the category III/M were amplified by primer pair III and occurred in all accessions. Categories I/J, II/K, III/C, III/H, III/I were chromosome A specific. The category III/M was chromosome B specific and categories I/G, I/H, I/M, II/G, II/M, IIID, III/K and III/O were chromosome D specific as it was certified by analysis of NT lines of cv. Chinese Spring (Fig. 2). Categories III/E and III/L were not chromosome specific. The other categories were not present in Chinese spring, so their chromosome specificity is uncertain.

Table 2b. HMW-GSs identified in evaluated set of wheat (*Tritium aestivum L.*) modern cultivars detected by separation of extracted HMW-GSs on SDS-PAGE

Cultivar		Glu-1 loci		
		Glu-A1 subunits	Glu-B1 subunits	Glu-D1 subunits
Alka	(CZE)	0	6 + 8	5 + 10
Astella	(SVK)	0	7 + 9	5 + 10
Hana	(CZE)	0	7 + 8	5 + 10
Šárka	(CZE)	0	7 + 8	5 + 10
Sparta	(CZE)	0	6 + 8	5 + 10
Vala	(CZE)	0	7	2 + 12
Vlada	(CZE)	1	7 + 9	5 + 10
Vlasta	(CZE)	1	7 + 8	5 + 10

Table 3. List of categories into which products amplified by each of the primer pairs were divided according to their sizes. Sizes are indicated in basepairs

Category	Primer pair I	Primer pair II	Primer pair III
B	390–392 bp	298–300 bp	355–356 bp
B1			357 bp
C	393–394 bp	318–320 bp	362–364 bp
D	402–403 bp	321–323 bp	
D1			368 bp
E	403.5–405 bp	330 bp	
F	405.5–407 bp		379–381 bp
G	412–414 bp	333–335 bp	383–384 bp
G1		333 bp	
H	414–415 bp	339–342 bp	391–393 bp
H1	414 bp		394 bp
I	419–421 bp	342–344 bp	395–397 bp
J	423–424 bp	347–348 bp	398–399 bp
K	428–431 bp	349–350 bp	400–402 bp
K1			403 bp
L	453–454 bp	355–356 bp	
M	461–462 bp	387–388 bp	
N		492 bp	413–414 bp
O			417–418 bp

Table 4. PCR patterns specific for each of the accessions (*Triticum aestivum* L.) amplified by primer pair I (pattern I), primer pair II (pattern II), primer pair III (pattern III); presence or absence of specific amplification product is indicated by 1 or 0, resp.

Cultivar/Landrace	Pattern I	Pattern II	Pattern III
Bezosinná červená perla	0010100010010	0001100010000	01100010100010
Červená perla	0000011010001	0000011010010	01100010001001
Dobrovická červená 19	0000111010001	1000101110010	01100010001001
Hanácká osinatá	0000111010010	0000011010001	01100010101001
Hořická	0100000100001	0010011000010	00000000101001
Kelčanská holice	1000011010101	0100011010010	01100010101001
Kelčanská Secalobastard	0000010110001	1001001000010	10100100011001
Mandelíkova ratbořská Mara	0011011010011	0000111010010	01100010101011
Novodvorská Volha	1000011000101	0100011000110	00000000100001
Selecty Chrudimka	0100000100001	0010011000010	00000000101001
Selecty ZVb	0000000100010	1000011000000	10000100011001
Slapská vouska	1000011000110	0100011000101	00000000101001
Slovenská 1784	0000011010001	1000011010010	00100010101011
Valtická osinatá B	0100000100001	0010011000011	00000000100001
Židlochovická bezosinná km. 12	1010110010111	0101100000100	00000010101010
Hana	0000011010010	0000011010000	10101010001001
Alka	1000011001010	0100011001000	00000001010001
Astela	0000011010010	0000011010001	11101010001001
Šárka	0000011010010	1000011010000	00010001000100
Sparta	0000011001010	0100011001000	00000001000001
Vala	0000011001001	0100011001010	00000001000000
Vlada	0000011010001	0000011010010	10101010011001
Vlasta	0000011010010	0000011010000	00100000100000

LMW-GS PCR patterns differentiated all the accessions except Selecty Chrudimka and Valtická osinatá B. The PCR analysis shows the important variability in the LMW loci with the average diversity index 0.425. This value is considered appropriate for markers used for similarity/diversity studies (Dahleen 1998).

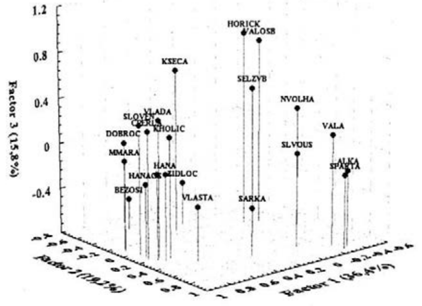

Figure 5. Association among wheat landraces and old cultivars revealed by principal component analysis performed on genetic similarity estimates (Nei and Li 1979) calculated from 40 polymorphic products amplified by three LMW-GS specific PCR primers

The relative genetic distances based on 40 polymorphic amplification products were corresponding to LMW GSs coding sequences ranged from 0.071 (Astella vs. Hana) to 1.000 (Bezosinná červená perla vs. Vala) with an average mean 0.488. The cluster analysis divided the accessions into five groups (Fig. 4). The cluster one included Selecty ZVb., and cv. Kelčanská Secalobastard. Selecty ZVb is a selection out of regional variety from Austria. Kelčanská Secalobastard is a product of wide

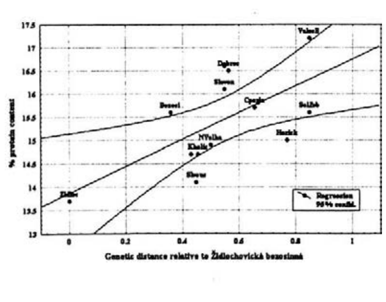

Figure 6. Relationship between genetic similarity and protein content in grains of wheat landraces and old cultivars in comparison to the cultivar Zidlochovicka jubilejní with poor bread-making quality; data for protein content taken from Dotlačil and Michalová (1996); correlation coefficient $r = +0.641^{*}$

hybridisation between *T. aestivum* and *S. cereale*. The cluster two included Valtická osinatá B – a local cultivar from Valtice and cv. Hořická. The cluster three included Czech modern cultivars – Vala, Sparta and Alka and two old cultivars Slapská vouska and Novodvorská Volha. The cluster four is the largest one and it includes modern cultivars Šárka, Vlasta, Astella, Hana and Vlada. Five landraces and obsolete cultivars are also included in this cluster – Hanácká osinatá, Mandelíkova ratbořská Mara, Dobrovická červená, Kelčanská holice, Slovenská 1784 and Červená perla. The largest group included all investigated wheat botanical varieties – *milturum, ferrugineum* and *lutescens* from different regions indicating a dispersion of different LMW-GS alleles in all of them. The fifth cluster is composed of landraces Židlochovická bezosinná and Bezosinná červená perla with a lower value of *SDS* test. The clustering based on LMW-GS did not fully correspond to clustering based on gliadins analysis. The principal component analysis (PCA) explained 42.3% (PC1), 23.2% (PC2) and 18.1% (PC3) (Fig. 5) of the total variance. The PCA shows better genetic dissimilarity of landraces and old cultivars with rare alleles, which are not present in modern cultivars as compared with the cluster analysis.

DISCUSSION

The emphasis on the preservation of crops genetic diversity and the potential of landraces for crop improvement has led to a growing interest in cultivated germplasm pools throughout the world. In the present paper we estimated the diversity of seed storage proteins in obsolete cultivars and landraces used in the Czech region. Gliadins and HMW GSs compositions were studied in a common way using A-PAGE and SDS-PAGE as well. Bulked samples containing mixture of 100 grains were used to detect the total variability of each accession. According to our study obsolete cultivars and landraces contained only one main HMW glutenin line which is in an agreement with analysis of Černý and Šašek (1996b), who found the most of the Czech old cultivars to consist only of one HMW glutenin line. Not all the materials studied here were included in their investigation. The comparable representation of HMW alleles was found also in our set of old cultivars and landraces. There was a higher frequency of rare HMW GS alleles (*Glu-B1e* – subunit 20, *Glu-B1k* – subunit 22) in the set of Czech accessions as compared to the set of European landraces and old cultivars (Gregová et al. 1999).

LMW-GSs were studied on the basis of PCR patterns. The bulked sample here should cover all the variability of the samples, because only a single molecule can be amplified by PCR. Each possible band was considered as a different locus with two alternative alleles (presence or absence) using PCR system. The presence of one band or another band could represent the alternative forms of the same locus differentiated by deletion or mutation of the DNA tract with the same two primers. Although our analysis should not to be strictly correct in terms of loci and alleles, it provides good estimates of the variability of LWM GSs present among obsolete cultivars and between sets of obsolete cultivars and modern materials. The three primers were able to differentiate all but one of the analysed cultivars and landraces and were able to reveal the diversity. Together 40 polymorphic fragments of different sizes were amplified. It shows the potential of PCR to characterise the diversity of LWM-GSs coding sequences. PCR is therefore an alternative way, how to detect specific LMW-GS alleles presence in breeding material. There is no necessity to purify DNA for PCR reaction, piece of leaf tissue or part of endosperm can be used as a direct template (Martynková et al. 1997). Therefore PCR could be a fast way for LMW-GS evaluation.

It is generally supposed that there is much higher variability in obsolete cultivars and landraces as compared to modern ones. Several authors confirmed this finding by the use of RFLP, HMW-GSs analysis of wheat landraces and obsolete cultivars from different regions of the world (Ciaffi et al. 1993; Autrique et al. 1996; Gregová et al. 1999). Our investigation also corroborates this finding. The average genetic distance among landraces and old cultivars evaluated in our study was 0.464, range (min.–max.) 0.917, whereas the average genetic distance among modern cultivars was only 0.369, range (max.–min.) 0.680. On the other hand, Cooke and Law (1998) did not find significant changes in the seed storage protein diversity over time in UK germplasm. They found also more narrow genetic basis of UK wheats in comparison with European (continental) germplasm. We detected several LMW-GSs categories, which were specific for the obsolete cultivars and landraces. However, three signals appeared solely in modern cultivars. They could be introgressed from cultivars and lines of Soviet and French origin used in the breeding in last decades. They were not included in our study.

It is apparent from the results (Table 4) that some LMW-GS alleles are seldom in modern cultivars. The short fragments from pattern I were absent in modern cultivars. Cv. Alka was the only exception out of evaluated modern materials with one short fragment in the PCR pattern I (category B). Some categories from pattern II were also missing in modern cultivars. Patterns I and II reflect mostly the length variability of the central repetitive domains of the protein. The length of repetitive domains of LMW-GSs may be important in the similar way, as it was proved for the function of repetitive domain of HMW glutenins. (Shewry et al. 1989; Belton 1999). The contribution of individual alleles upon bread-making quality has to be proved. The possibility of their identification on the DNA level can aid to solve this task.

Regression analysis based on two years' evaluation of protein content in landraces and obsolete cultivars (Michalová and Dotlačil 1992) and the relative genetic distance to cv. Židlochovická bezosinná shows a significant correlation between these parameters $r = 0.641^{*}$

(Fig. 6). Židlochovická bezosinná has got a very low value of the sedimentation test. The PCR profile with respect to LMW GSs of Židlochovická bezosinná includes several fragments typical for old materials only and does not contain some fragments present in all modern cultivars.

We have investigated the correlation between gliadins and LMW-GSs patterns. Singh and Shepherd (1988) proved, that there is a close linkage between *Glu-3* and *Gli-1* same *Gli-1* alleles together with one *Glu-3* alleles. The genetic distance between *Glu-B3* and *Gli-B1* loci was assessed to be 1.2–1.5 cM (Cassidy et al. 1998). However, the low relationship was found between clustering based on gliadin and LMW-GSs patterns evaluation (Figs. 1, 4). Only a few of the accessions was grouped in the same way by the both approach. Valtická osinatá and Selecty Chrudimka identical according to the LMW GSs analysis and very similar by gliadin patterns analysis. However, Valtická osinatá and Selecty Chrudimka were differed substantionally in HMW-GSs. Also Slapská vouska and Novodvorská Volha were grouped together in LMW and gliadin based clustering. The other accessions were clustered in a different way. Probably, the materials should be evaluated with respect to the both gliadins and the LMW-GSs glutenins on the same level. Kubánek et al. (1999) showed, that HMW-GSs lines represented under 5% were not detectable in bulked samples. A situation can be similar for gliadins. The PCR, on the other hand, should amplify all the alleles presented in the sample. Therefore more knowledge will be necessary to prove, whether gliadins, which are easy to be analysed, could be markers for specific LMW-GSs alleles, especially when DNA level is considered on the other side.

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Abbreviations

GS = glutenin subunit; dNTP = deoxynucleotide; DNA = deoxyribonucleic acid; PCR = Polymerase Chain Reaction; LMW = low molecular weight glutenin

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ABSTRAKT

Variabilita zásobních proteinů ve vybraných českých krajových, starých a některých moderních odrůdách pšenice (*Triticum aestivum* L.)

Hodnotili jsme variabilitu *Gli-1, Glu-1* a *Glu-3* lokusů v souboru starších krajových odrůd. Pro hodnocení nízkomolekulárních (LMW) gluteninů byla použita technika založená na PCR. Bylo potvrzeno, že PCR je vhodná pro sledování variability LMW lokusu. Byla zjištěna vyšší variabilita v hodnoceném souboru starších a krajových odrůd pšenice než u kontrolního souboru moderních odrůd. V moderních odrůdách chybí zejména krátké amplifikační produkty, které odpovídají zkrácené centrální repetitivní doméně genu. Starší a krajové odrůdy mohou být využity jako zdroj alternativních alel nízkomolekulárních gluteninů. Alelomorfismus gliadinů a vysokomolekulárních gluteninů, který byl hodnocen na úrovni proteinových spekter, je srovnatelný s výskytem alel nalezených u dalších souborů krajových a starších odrůd. Nebyly zaznamenány významné souvislosti mezi výsledky shlukové analýzy genetických vzdáleností založené na analýze LMW lokusů a gliadinových spekter, i když lokusy *Gli-1* a *Glu-3* jsou v těsné vazbě.

Klíčová slova: nízkomolekulární (LMW) gluteniny; vysokomolekulární (HMW) gluteniny; gliadiny; diverzita; pšenice; krajové odrůdy; genové zdroje

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SHORT COMMUNICATION

Determination of phytotoxic effects of pre-emergence herbicides using a technique of very rapid fluorescence induction

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ABSTRACT

The following herbicides were applied in spring 1996 to 13 selected potato varieties before emergence: 3 and 6 kg.ha^{-1} Topogard 50 WP (35% terbutryn + 15% terbuthylazine); 1.5 kg.ha^{-1} Sencor 70 WP (70% metribuzin). Part of the site was not treated (control). To evaluate the effect of herbicide residues, 12 varieties of winter wheat were sown in the autumn after harvesting chemically treated and untreated potatoes. A PEA instrument (Plant Efficiency Analyser) was used to determine the differences in photoanalysis of chemically treated and untreated plants. After the application of 3 kg.ha^{-1} Topogard 50 WP, no important changes in parameters compared with the untreated control were observed. After application of 6 kg.ha^{-1} Topogard 50 WP, photosynthesis decreased. Marked and statistically significant deviations in parameters were found in the Kobra, Korela and Krasa varieties. After application of Sencor 70 WP, the decrease in photosynthesis was statistically significant in all varieties. Based on percentage deviations of parameters of fluorescence induction, potato varieties could be ranked according to their sensitivity to the active ingredient (a.i.) metribuzin: Kobra (the most sensitive variety), Keřkovské rohlíčky, Krista, Kréta, Krasa, Korneta, Kora, Korela and Krystala, Koruna, Impala, Karin, Ukama (the least sensitive variety). During investigations of residual effects of the preparations in spring 1997, no negative reaction of photosynthesis of selected winter wheat varieties to residues of active ingredients of Topogard 50 WP and Sencor 70 WP applied in the previous year was found. The differences in levels of parameters of very rapid fluorescence induction suggested that the applied method was sufficiently accurate as was the usability of the PEA analyser for determinations of the sensitivity of varieties to herbicides.

Keywords: potatoes; winter wheat; terbutryn; terbuthylazine; metribuzin; very rapid chlorophyll fluorescence induction

Effects of pre-emergence herbicides Topogard 50 WP (35% terbutryn a.i. and 15% terbuthylazine a.i. from a group of triazines) and Sencor 70 WP (metribuzin a.i. from a group of substituted triazinones) on selected potato varieties and effects of residues of these herbicides on subsequently grown varieties of winter wheat were studied in field trials. The active ingredients of the applied herbicides inhibit photosynthesis of photosystem II (PSII, Cremlyn 1985).

These preparations have been applied in potato stands for a long-time period with good results. Vokál et al. (1985) and others studied these problems.

Growers have been afraid of residual effects of Topogard 50 WP applied at higher doses on subsequent winter wheat. Relationships between a level of residues of persistent herbicides in the soil (e.g. triazines) and the crop are variable and depend on a large number of factors (soil texture and type, course of temperatures, soil tillage, and others, Buryšková 1988). Maximum acceptable (limit) values of residues were assessed using bioassays in model pot experiments. These limits are 0.25 to 0.40 mg.kg^{-1} in terbutryn and terbuthylazine and 0.15 to 0.30 mg.kg^{-1} in metribuzin for winter wheat. Lower and higher values have been assessed for light and heavy soils, respectively (Buryšková and Dvořák 1990). The mentioned active ingredients are degraded during the growing season, so subsequent crops are not damaged if recommended doses are applied. The residues do not reach a level of limit values.

The objective of the presented paper is to evaluate usability of parameters of very rapid fluorescence induction at measuring phytotoxic effects of herbicides applied to the soil.

MATERIAL AND METHODS

The trial was established at Žabčice in the field of the School Experimental Farm of Mendel University of Agriculture and Forestry in Brno. The location is 176 m above sea level. Soil substrate is formed by non-calcareous alluvial deposits. The topsoil humus horizon is visible to the depth of 35 cm, the content of size fractions to 0.01 mm is 66.3%.

In spring 1996, the below-given potato varieties were planted each in two rows at 75-cm row spacing (very early varieties: Impala, Kora, Koruna, Krasa, Krystala, Ukama; early varieties: Karin, Kobra, Kréta, Korneta; midearly varieties: Keřkovské rohlíčky, Korela, Krista). On 2nd May 1996, about four days before emergence of potato plants, Topogard 50 WP at doses of 3.0 and 6.0 kg.ha^{-1} and Sencor 70 WP at a dose of 1.5 kg.ha^{-1} were applied crosswise to the planted varieties. The preparations were applied in 300-cm-wide strips; one strip was not treated (a control). On 4 October 1996, 12 winter wheat varieties (Hana, Vlada, Bruta, Boka, Bruneta, BR 442, Brea, Samanta, Siria, Astella, Soldur and Blava) were sown crosswise to treated strips of potatoes and the untreated control.

Effects of the applied herbicides on photosynthesis of individual varieties of potatoes and winter wheat were investigated. A status of PSII in the leaf was determined using a fluorimeter PEA (Plant Efficiency Analyser). The instrument measures fluorescence signals emitted by chlorophyll. If the photosynthetic process is inhibited, chlorophyll fluorescence rises.

Parameters of very rapid fluorescence induction (vrFI) were measured with a fluorimeter PEA (Strasser and Govindjee 1991, 1992). Intensive exposure of the leaf area adapted to the dark using a red light (max. 650 nm) is absorbed well by chlorophyll. Fluorescence rapidly rises from a low level Fo to a peak level Fp and then declines through several transient maxima to a level close to the initial low level (the higher fluorescence, the higher Fo). The difference between Fp and Fo indicates a variable fluorescence component, Fv. Based on these values, the ratio Fv/Fp (variable fluorescence/maximum fluorescence) is calculated. The lower the level of photosynthesis (and fluorescence is higher), the lower is this parameter. A level of the rFj parameter is determined from an internal vrFI curve from a level Fo to Fp (the wave designated J). The level of this parameter increases along with rising the fluorescence. Another parameters may be also used to assess chlorophyll fluorescence. However, the parameters Fo, Fv/Fp and rFj are the most suitable to express a status of investigated photosynthetic responses (Matoušková et al. 1996). Fluorescence intensity is expressed by deviations in parameters (in %) determined on treated variants vs the untreated variant (Nauš and Melis 1992).

The vrFI parameters were assessed in potatoes on top leaves at the growth stage 31, beginning of elongation growth, on the 27th day after herbicide application, i.e. on 29 May 1996. Measurements in wheat were carried out on blades of third leaves (growth stage BBCH 23–24) on 11 April 1997.

Since earlier studies showed that there was no generally valid normal distribution in vrFI parameters, the results are presented using medians and quartiles. The measurement was carried out in all cases in ten replicates.

To assess significant differences in tested fluorescence parameters Kolmogorov-Smirnov test with Lilliefor's procedure was used in order to determine if the data were normally distributed. Then dispersion equality was tested in individual data sets using a Leven median test. If the data are normally distributed, then *t*-test is used to assess whether a statistically significant difference exists between the two sets of data. In case the data are not normal or they do not show equal dispersions, Mann-Whitney test is used instead of the *t*-test.

RESULTS AND DISCUSSION

If Topogard 50 WP was applied at a dose of 3 kg.ha^{-1} (Fig. 1), the Fv/Fp parameter increased by up to 4% (Ko-

Table 1. The effect of a pre-emergence application of 6 kg.ha^{-1} Topogard 50 WP on parameters of very rapid fluorescence induction (changes of medians as compared with untreated control in %, control = 100%)

Variety	Parameter		
	Fo (+)	Fv/Fp (–)	rFj (+)
Impala	10	4	–3
Karin	20	4	15
Keřkovské rohlíčky	8	4	2
Kobra	28	7	19
Kora	14	5	7
Korela	18	9	14
Korneta	18	3	12
Koruna	19	5	2
Krasa	17	9	15
Kréta	13	5	9
Krista	8	1	1
Krystala	15	4	5
Ukama	15	5	1

Table 2. The effect of a pre-emergence application of 1.5 kg.ha^{-1} Sencor 70 WP on parameters of very rapid fluorescence induction (changes of medians as compared with untreated control in %, control = 100%)

Variety	Parameter					
	Fo (+)		Fv/Fp (–)		rFj (+)	
	%	ranking	%	ranking	%	ranking
Impala	21	12	11	5–8	9	13
Karin	23	11	5	13	16	9–10
Keřkovské rohlíčky	44	3	21	1	25	5–6
Kobra	58	1	15	2–3	27	3–4
Kora	29	9–10	11	5–8	19	7
Korela	31	7–8	11	5–8	16	9–10
Koruna	31	7–8	6	11–12	13	12
Korneta	39	5	9	9–10	27	3–4
Krasa	29	9–10	13	4	28	2
Kréta	40	4	11	5–8	30	1
Krista	45	2	15	2–3	26	5–6
Krystala	35	6	9	9–10	18	8
Ukama	20	13	6	11–12	15	11

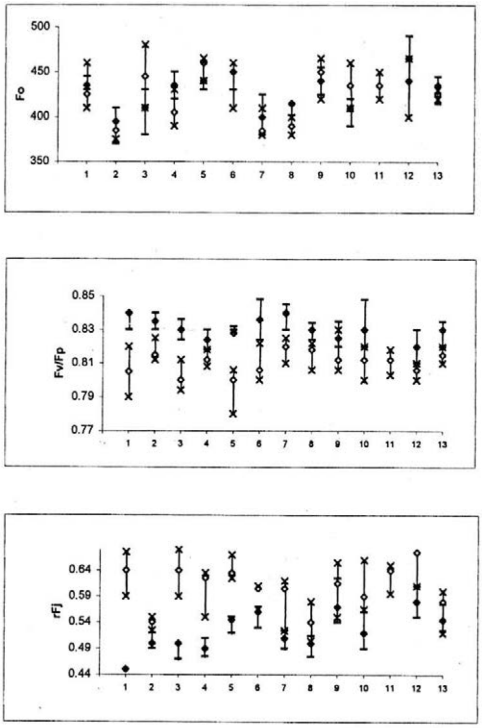

Figure 1. Values of the vrFI parameter after pre-emergence application of 3 kg.ha[-1] Topogard 50 WP (medians, quartiles, n = 10)

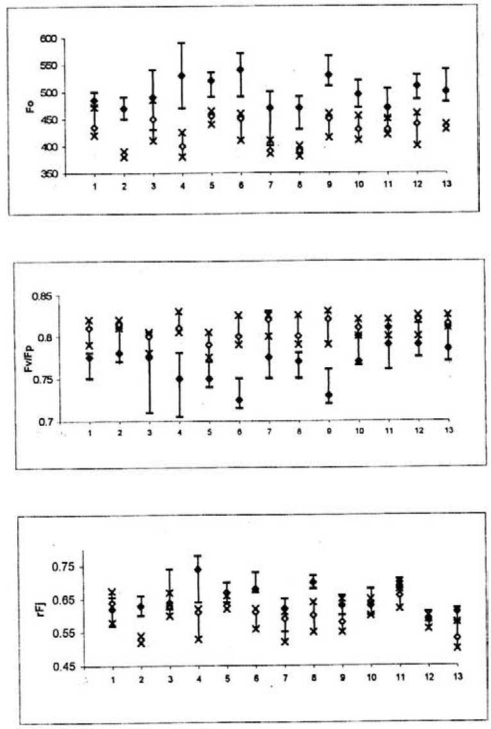

Figure 2. Values of the vrFI parameter after pre-emergence application of 6 kg.ha[-1] Topogard 50 WP (medians, quartiles, n = 10)

1. Impala, 2. Karin, 3. Keřkovské rohlíčky, 4. Kobra, 5. Kora, 6. Korela, 7. Koruna, 8. Korneta, 9. Krasa, 10. Kréta, 11. Krista, 12. Krystala, 13. Ukama

◆ treated ◇ untreated

rela), the rFj parameter decreased by up to 29% (Impala). Changes of Fv/Fp and rFj parameters exhibited inverse trends than those present in photosynthesis reduction.

After application of Topogard 50 WP at a dose of 6 kg.ha[-1] (Fig. 2), the Fo parameter increased in all studied varieties in comparison with control ones (by up to 28% in the Kobra variety, Table 1), the Fv/Fp parameter decreased (by up to 9% in Krasa and Korela) and the rFj parameter increased (by up to 19% in Kobra). The rFj parameter decreased by 3% in the Impala variety.

Clear changes in parameters were assessed after application of Sencor 70 WP at a dose of 1.5 kg.ha[-1] (Fig. 3, Table 2). Values of the Fo parameter vs untreated variants were apparently higher (by 20 to 58%). The Fv/Fp parameter decreased; the highest differences were found in the varieties Keřkovské rohlíčky (–21%), Kobra and Krista (–15%). The rFj parameter in treated variants increased by up to 30% (Kréta). In some varieties effects on the studied parameters were not balanced. In the Krasa variety, for instance, the difference of the Fo parameter vs the control ranked 9th and 10th among studied varieties, and the difference of the Fv/Fp parameter ranked 4th and rFj 2nd. In some varieties differences in parameters were balanced in all cases, i.e. on a high (Kobra) or low level (Ukama). The cases where responses of individual parameters are different in a certain subject indicate some failure of this method.

No visual phytotoxicity was found (using the scoring scale of the EWRC) in any variety evaluated three and seven weeks after pre-emergence applications of Topogard 50 WP and Sencor 70 WP.

Residual effects of recommended and double application doses of Topogard 50 WP (3.0 and 6.0 kg.ha[-1]) and a recommended dose of Sencor 70 WP (1.5 kg.ha[-1]) applied pre-emergently to potatoes did not induce changes in the vrFI parameter that would have resulted in adverse effects on photosynthetic activity of subsequently grown varieties of winter wheat. That is confirmed by the rFj parameter in the Vlada and Samanta varieties (Fig. 4 where medians, upper and low quartiles, and maxima and minima are presented). Some increase vs the control was assessed after application of 6 kg.ha[-1] Topogard 50 WP in these varieties only.

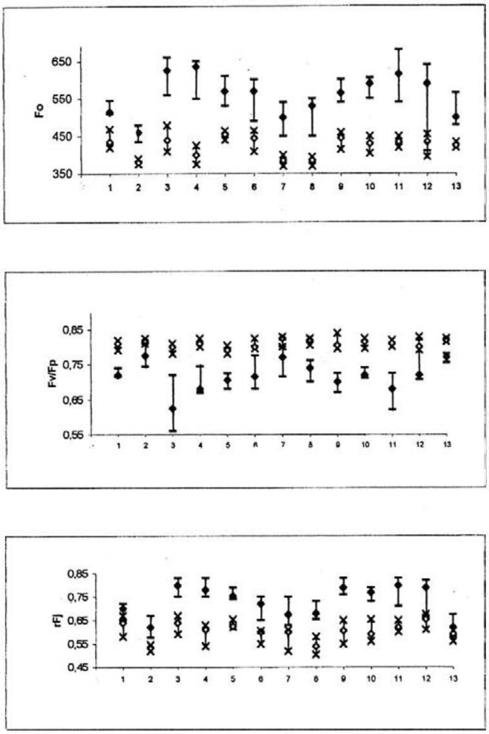

Figure 3. Values of the vrFI parameter after pre-emergence application of 1.5 kg.ha^{-1} Sencor 70 WP (medians, quartiles, n = 10)

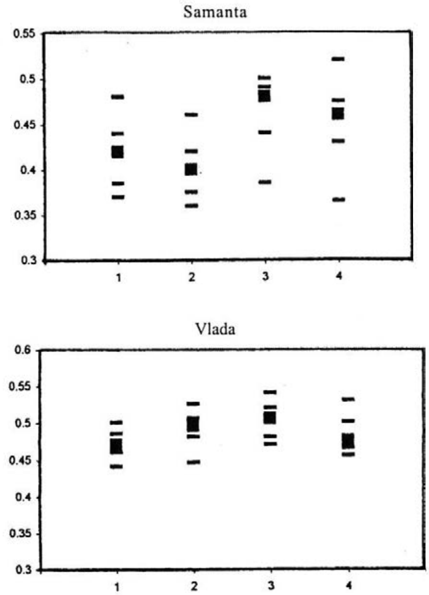

Figure 4. Changes of rFj parameters in selected varieties of winter wheat (1997)

1 = untreated
2 = 1.5 kg.ha^{-1} Sencor 70 WP
3 = 6 kg.ha^{-1} Topogard 50 WP
4 = 3 kg.ha^{-1} Topogard 50 WP

Previous experiments (Lazár and Nauš 1998) revealed that rFj was the most sensitive among all the studied fluorescence parameters. So if this parameter does not vary, photosynthesis can be assumed to be stable.

Terbutryn and terbuthylazine exhibit high selectivity to potatoes (Vokál et al. 1985). The recommended dose (Topogard 50 WP, 3 kg.ha^{-1}) did not decrease photosynthesis in any variety. By contrast, a stimulation effect of the mentioned application dose can be assumed. At a double application dose, changes in vrFI parameters indicated decrease in photosynthesis. However, more serious damage of potatoes cannot be supposed. Based on evaluation of all three parameters, photosynthesis was considerably decreased in the varieties Kobra, Korela and Krasa, and the lowest effect was found in Krista. It is interesting that in the variety Impala the rFj parameter decreased most at a dose of 3 kg.ha^{-1} Topogard 50 WP vs the control. It was also the only variety in which this parameter decreased even at a dose of 6 kg.ha^{-1} (Figs. 1 and 2).

Large varietal differences were found in post-emergence application of Sencor 70 WP at a dose of 0.75 kg.ha^{-1} (Remešová and Dvořák 1998). If Sencor 70 WP was applied at a recommended dose of 1.5 kg.ha^{-1}, there were changes in vrFI parameters in comparison to controls which express decrease in photosynthesis in all examined varieties. Based on percentage differences in vrFI parameters, the varieties Kobra and Keřkovské rohlíčky exhibited the highest and Ukama and Karin the lowest responses.

The difference in a level of vrFI assessed on the 27th day after pre-emergence application of the preparation in comparison to untreated controls shows sensitivity of the used method and its predicating ability. Differences among varieties, especially after application of Sencor 70 WP, indicate a possibility of using this method to determine varietal sensitivity.

Significant differences in vrFI parameters were found after application of 6 kg.ha^{-1} Topogard 50 WP in the varieties Kobra, Korela and Krasa, and after application of 1.5 kg.ha^{-1} Sencor 70 WP in all of 13 investigated varieties. In all above-presented cases, the applied herbicides induced significant differences in all tested fluorescence parameters.

Due to a short time of this study, the obtained results do not allow us to rank the tested varieties according to the sensitivity to pre-emergence application of applied preparations. The results can be considerably affected by the course of weather conditions in the growing season. Therefore, ranking the varieties would need conducting experiments for a longer time. In the case of Topogard 50 WP, however, such investigations would not be useful.

In given soil conditions, no adverse responses of photosynthesis in tested varieties of winter wheat to residues of active ingredients of Topogard 50 WP and Sencor 70 WP applied in the preceding years were assessed.

The results confirm the earlier published data (Vokál et al. 1985). No adverse responses were determined in consequently grown winter wheat varieties even at double doses of Topogard 50 WP.

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ABSTRAKT

Stanovení fytotoxických účinků preemergentních herbicidů metodou velmi rychlé fluorescenční indukce

Byla ověřována použitelnost stanovení parametrů velmi rychlé fluorescenční indukce při hodnocení fytotoxických účinků preemergentních herbicidů. Pokusnou plodinou byly brambory a následně po nich pěstovaná ozimá pšenice. Na jaře 1996 byly na brambory (13 odrůd) preemergentně aplikovány přípravky Topogard 50 WP a Sencor 70 WP. Na podzim téhož roku bylo po takto ošetřených bramborách vyseto 12 odrůd ozimé pšenice. Účinné látky obou herbicidních přípravků inhibují fotosyntézu. Pomocí fluorimetru PEA (Plant Efficiency Analyzer) byly 27. den po aplikaci měřeny fluorescenční signály emitované chlorofylem (při poklesu fotosyntézy fluorescence vzrůstá). Po aplikaci 3 $kg.ha^{-1}$ Topogardu 50 WP bylo zjištěno mírné zvýšení fotosyntézy oproti neošetřené kontrole, po aplikaci 6 $kg.ha^{-1}$ bylo zaznamenáno její snížení (průkazné na odrůdách Kobra, Korela a Krasa). Po aplikaci Sencoru 70 WP 1,5 $kg.ha^{-1}$ byl u všech odrůd naměřen statisticky významný pokles fotosyntézy. Na následně pěstovaných odrůdách ozimé pšenice nebyl zjištěn (jaro 1997) žádný pokles fotosyntézy.

Klíčová slova: brambory; ozimá pšenice; terbutryn; terbuthylazin; metribuzin; velmi rychlá fluorescenční indukce chlorofylu

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RECENZE

Koncentrace CO_2 a rostliny

L. Nátr

Praha, ISV 2000.

Rostlina jako živý organismus představuje obdivuhodně harmonický celek. Také rostliny jsou sladěny v celek s prostředím na Zemi. Člověk, zejména během uplynulého století, bezohledně stupňoval necitlivé zásahy do tohoto celku a řádu v přírodě. Autor publikace o tom podává velmi poutavě a názorně svědectví, pokud jde o vzestup koncentrace CO_2 v atmosféře Země a možné důsledky tohoto vzestupu. Oxid uhličitý je hlavním z tzv. skleníkových plynů. Zvyšuje jako sklo ve skleníku absorpci záření, a tak se zvyšuje i teplota vzduchu a výpar vody. Kniha podrobně popisuje nepříznivé dopady, které s tím souvisejí, a kriticky přitom rozlišuje fakta nezpochybnitelná od fakt pouze pravděpodobných. Autor je rostlinný fyziolog, jenž patří k našim nejzkušenějším pracovníkům v oblasti fyziologie fotosyntézy. Je proto logické, že těžiště jeho knihy tkví ve shrnutí účinků zvýšení koncentrace CO_2 na fotosyntézu. Rozlišil přímé účinky zvýšené koncentrace CO_2 na rostliny od účinků nepřímých. K prvým patří zejména zvýšená rychlost fotosyntézy, snížení fotorespirace a vodivosti průduchů, k druhým zvláště interakce s teplotou, minerální výživou rostlin, účinností využití vody apod. Kapitola věnovaná metodické stránce studia koncentrace CO_2 na rostliny je doložena cennými barevnými obrázky. Také grafy velmi názorně dokumentují popisované výsledky. Z příslušných číselných údajů, které autor získal ve vědeckých publikacích, sestavil velký počet originálních grafů.

Z textu je zřejmé, že relativně méně informací existuje o vztahu rostoucí koncentrace CO_2 k růstu a vývoji rostlin. Není např. známo, jak tato koncentrace ovlivňuje obsah rostlinných hormonů. Ze skutečnosti, že zesiluje větvení a odnožování rostlin, by se dalo usuzovat, že pravděpodobně snižuje hladinu auxinů a zvyšuje hladinu cytokininů. Velmi hodnotné jsou údaje týkající se zásahu CO_2 do orgánových korelací, zejména korelací mezi prýtem a kořenem. Autor podrobně rozvádí, jak zvýšená koncentrace CO_2 působí na přednostní ukládání sušiny do kořenů.

Předností monografie je i kapitola zaměřená na speciální fyziologii rostlin. Byliny reagují na CO_2 často jinak než dřeviny a mezi bylinami zaujímají zvláštní pozornost autora obilniny, zejména pšenice. Nezapře zde své bohaté zkušenosti dlouholetého pracovníka v obilnářském výzkumu.

Velká množství důležitých informací podává kniha nejen rostlinným fyziologům, agronomům a lesníkům, ale i ekologům. Týká se to např. vztahu koncentrace CO_2 k migraci organismů, vodnímu stresu, atmosférickým polutantům apod. Také vlivům CO_2 na procesy probíhající v půdě je věnována zvláštní pozornost ve vztahu nejen k půdním mikroorganismům, ale např. i k produkci metabolitů dešťovek nebo k ovlivnění mykorhizy.

Publikace vyniká přehledností a komplexností, s níž je sledován vztah koncentrace CO_2 k rostlinám. Shrnuje nejnovější vědecké poznatky a aktuálně doplňuje současné učebnice fyziologie a ekologie rostlin. Bude tak cennou pomůckou nejen pro studenty biologických a zemědělských oborů vysokých škol, ale i pro ochránce přírody a pro všechny, kdož se zajímají o charakter možných antropogenních vlivů na podnebí.

Prof. RTDr. Ing. Jiří Šebánek, DrSc.

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