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F 4883

In vitro haploid zygotic embryogenesis due to pollination with maize pollen and induced *in vitro* androgenesis in Czech wheat breeding genotypes

J. Vagera[1], Z. Nesvadba[2], P. Martinek[2], L. Ohnoutková[1]

[1]*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Olomouc, Czech Republic*
[2]*Agricultural Research Institute Kroměříž, Ltd., Czech Republic*

ABSTRACT

Induction of haploid zygotic embryogenesis and androgenesis was studied in 72 and 31 wheat genotypes, respectively. Green zygotic plants (haploids only) were obtained in 52 genotypes (72%), and green androgenic regenerants (haploids and spontaneous polyhaploids) were observed in 17 genotypes (55%). In zygotic embryogenesis embryos developed in 65 genotypes (90%), and in androgenesis embryos or microsporial calli were detected in 28 genotypes (90%). The highest frequency of haploid zygotic regenerants was 0.24 per pollinated flower, whilst that of androgenic regenerants was 0.08 per cultured anther (0.24 per three anthers in one flower). On average, in induced androgenesis the plants were produced by one out of three androgenic anthers. In induced haploid zygotic embryogenesis the plants were regenerated by every third flower (ovary) with induced embryos. In a set of selected genotypes the yield of induced haploid zygotic embryogenesis was compared with induced pollen embryogenesis. The productivity of both methods is discussed on the level of single gametes and in total.

Keywords: wheat genotypes; induced embryogenesis; maize pollen; haploid zygote

Recently it has become realistic to obtain adequate amounts of suitable haploid material in cereals by means of two methods, either through pollen embryogenesis from immature pollen grains or from zygotic embryogenesis induced by remote pollen, e.g. in wheat following pollination of its emasculated flowers by maize pollen. Subsequent dihaploidization (polyhaploidization) serves as an interactive model for germplasm enhancement. *In vitro* culture of unpollinated ovaries has not been practical owing to a low level of haploid induction. A wheat × maize method was studied by Laurie and Bennett (1986, 1988, 1989), Matzk and Mahu (1994), Macháň et al. (1995), and many others using different materials. In general, most of recent experiments have shown that the yield expressed as a total number of induced haploids and spontaneous polyhaploids obtained from pollen embryogenesis in androgenic responsive materials is higher than that from haploid zygotic embryogenesis induced by pollination of emasculated flowers with maize pollen. There were three anthers per flower in cereals and about three thousand pollen grains per anther, but only one ovum per ovary in each flower. At the level of one male gamete : one female gamete – the yield of induced embryogenesis was opposite (Darvey 1998). A method of wide hybridisation has been reported to be suitable for such genotypes where the frequency of androgenic plants is either very low or only albinos are regenerated. Nevertheless, in all the cases the induction of haploid or polyhaploid sporophytes within cereals strongly depends on a genotype of donor plants (Suenaga and Nakajima 1993). Genetic differences in the reaction were also demonstrated in the experiments with non emasculated wheat flowers bearing mature stigmas and immature anthers pollinated with maize pollen or after emasculation by heat treatment only (Suenaga et al. 1997).

The present study was focused on possible applications of the method of induced haploid zygotic embryogenesis to selected Czech breeding materials of wheat. Induced androgenesis was tested in some genotypes, too.

MATERIAL AND METHODS

Seventy-two *Triticum aestivum* L. genotypes were used as maternal plants and two genotypes of *Zea mays* L. (line 2001 and line Seneca 60) served as paternal ones. Wheat genotypes described in Table 2 represent a commercial intraspecific hybrids between raionized varieties (cultivars) or with some donors with favourable food quality of corn. Genotypes in Table 3 (and Table 4) represent the progenies derived from homogeneous donors. The all plant materials of wheat were obtained from Agricultural Research Institute Kroměříž.

The seeds were sown gradually under experimental field and greenhouse conditions. Wheat and maize anthesis was synchronized continually by means of different sowing dates and temperature control. Three to two

days before anthesis the youngest flowers were removed from the spikelets, and the two remaining basal flowers were emasculated (20 flowers per spike). In each plant, only the first two spikes were used. Parts of maize panicles containing fresh pollen were collected and immediately used for pollination of emasculated wheat flowers when the stigmas of their pistils reached the feathery receptive stage. The emasculated wheat flowers were wrapped in paper bags before and after pollination to prevent from desiccation and undesirable pollination. Embryo development in the flowers was enhanced by 2,4-dichlorophenoxyacetic acid (2,4-D) applied to the spikes or flowers that were sprayed with a solution of 10 mM 2,4-D. Enlarged ovaries were excised from the spikes about two weeks after pollination. The developing embryos were extirpated immediately and transferred onto the surface of nutritive solid media in Petri dishes (Table 1). Earlier embryos were placed onto medium 1/2, and later on they were transferred to medium 1/1 together with some pieces of the former medium (Iglesias et al. 1994). After four weeks' culture the growing embryos were inoculated on the standard MS medium in Erlenmeyer flasks. The plants were then transferred into garden soil after about eight weeks' culture depending on the ontogenetic development of the regenerants. In anther cultures the immature anthers containing uninucleate microspores extirpated from cold-treated spikes were cultured on a standard wheat medium according to the known procedures (Vagera and Ohnoutková 1993). The frequencies of androgenic anthers, albino and green regenerants were estimated as percentages per cultured anthers, and the green plants were grown under greenhouse conditions.

In wheat genotypes together responsive on androgenic and zygotic levels the standard statistical evaluation of ratios (Myslivec 1957) numbered per one experimental flower was realized.

Shoots of haploid zygotic regenerants at ontogenetic stage 2 (1st tillering stage) were washed in 0.5% solution of colchicine with 4% dimethylsulfoxide (4-hour-treatment), and regenerants were recovered in a greenhouse. The ploidy of zygotic or androgenic regenerants was evaluated by means of flow cytometric analysis (flow-cytometer PARTEC II) according to DNA content in the nuclei isolated from young wheat leaves (Doležel and Gohde 1995).

RESULTS

The induced zygotic embryogenesis was successful in 65 out of 72 genotypes under study (Tables 2 and 3). Green plants were obtained in 52 genotypes, and there were only seven genotypes that failed to produce any haploid zygotic embryos. The frequency of zygotic embryos and green plants was ranging between 0.00–0.45 and 0.00–0.24 per flower pollinated with maize pollen, respectively. In the first experiment with 41 wheat genotypes the mean frequency of induced zygotic embryos equalled about 0.16 per flower, in the second experiment involving 31 genotypes it was about 0.05 per flower. The mean frequency of green regenerants in the first and second experiment was about 0.06 and 0.05 plants per flower, respectively. Only some of the induced zygotic embryos gave rise to regenerants, i.e. around one in three flowers carrying embryos produced plants. All of the regenerated plants were green. Ploidy detection by means of flow-cytometric analysis of the nuclear DNA content in the plants originated from zygotic embryos revealed haploids only. There were no spontaneous polyhaploid plants. The habitus of immature and mature green regenerants remained unchanged as compared to the control. The polyhaploid (dihaploid) regenerants were obtained through zygotic embryogenesis only following colchicine treatment. Among zygotic haploids approximately 70% dihaploids were induced by colchicine (0.5% colchicine solution with 4.0% dimethylsulfoxide). There was no increase in the number of *in vitro* regenerants arisen either from additional embryogenic structures of the induced zygotic embryos or from *in vitro* originated bulbs on basal parts of the regenerants in induced androgenesis. It was impossible to find out a significant correlation between the frequency of induced zygotic embryos and those having given rise to plants.

Twenty-eight wheat genotypes out of 31 tested were androgenically responsive (Table 3). A total of 19 genotypes were able to produce plants, 17 of them green ones. There was no significant correlation between androgenic responsive genotypes and those with induced haploid zygotic embryogenesis. In pollen embryogenesis, albino and green regenerants were detected in 13 and 17 genotypes, respectively. Eleven genotypes consisted of a mixture of green and albino plants, two genotypes produced albinos only, whilst six of them green plants only.

Table 1. The key to media used

Medium	Macro-elements	Micro-elements	Thiamine	Pyridoxine	Nicotinic acid	Casseine hydrolysate	Inositol	Sucrose	Gerlite	Agarose	IAA	GA_3
1/1	MS	MS	0.1	0.5	0.5	200	100	30 000	–	8 000	–	–
1/2	MS	MS	0.1	0.5	0.5	200	100	150 000	–	20 000*	–	–
H2	MS	MS	1.0	0.5	0.5	–	100	20 000	3 000	–	0.2	0.2

The numbered values in mg/l
MS = according to Murashige and Skoog (1962)
* type VII – low gelling temperature
IAA = β-indolylacetic acid
GA_3 = gibberellic acid

Table 2. The frequency of induced haploid zygotic embryogenesis in wheat

Genotypes of wheat	Genotypes of maize pollen donors	The number of pollinated flowers	The number of induced embryos per pollinated flower	The number of induced plants per pollinated flower
Köin/HYB 92104	Seneca 60	100	0.25	0.04
Contra/Alka	Seneca 60	100	0.00	0.00
Estica/Köin	Seneca 60	100	0.05	0.00
HYB 92.104/Contra	Seneca 60	100	0.26	0.24
HYB 92.104/355-2	Seneca 60	100	0.15	0.09
BR 84/HYB 93.15	Seneca 60	100	0.16	0.01
BR 84/355-2	Seneca 60	100	0.15	0.02
BR 84/HYB 92.104	Seneca 60	100	0.27	0.03
BR 84/BU 45	Seneca 60	100	0.13	0.00
BR 84/HYB 92.104	Seneca 60	100	0.26	0.03
BR 84/HYB 91.10	Seneca 60	100	0.15	0.03
BR 84/HYB 93.15	Seneca 60	100	0.16	0.02
Brea/HYB 93.15	Seneca 60	100	0.17	0.03
SG-U 410/Ambras	Seneca 60	100	0.04	0.01
SG-U 410/Brea	Seneca 60	100	0.02	0.00
Alka/246-10	Seneca 60	100	0.21	0.17
Alka/Köin	Seneca 60	100	0.25	0.13
Alka/SG-S 352	Seneca 60	100	0.07	0.00
SG-S 352/Contra	Seneca 60	100	0.21	0.04
SG-S 352/BR94	Seneca 60	100	0.15	0.01
Ina/Brea	Seneca 60	100	0.11	0.01
Ina/Köin	Seneca 60	100	0.09	0.01
HYB 93.15//ZG K 3-82/Hana	Seneca 60	100	0.18	0.06
Livia/Köin	Line 2001	100	0.19	0.18
HE 341/355-2	Seneca 60	100	0.07	0.05
BU 45/HYB 92.10	Line 2001	100	0.26	0.20
BU 45/Köin	Seneca 60	100	0.05	0.00
BU 45/Köin	Line 2001	100	0.23	0.15
BU 45/355-2	Seneca 60	100	0.20	0.09
342-4 Köin	Seneca 60	100	0.08	0.06
363-94//ZG K 3-82/Köin	Seneca 60	100	0.20	0.16
Köin//ZG K 171-1-82/B.C.	Line 2001	100	0.09	0.08
HYB 92.104//ZG K 171-1-82/B.C.	Seneca 60	100	0.12	0.06
BR 94/ZG K 171-1-82/B.C.	Line 2001	100	0.16	0.09
ZG K 171-1-82/Mona	Seneca 60	100	0.25	0.09
ZG 171-1-82/HYB 92	Line 2001	100	0.07	0.04
MLU 3614/SG-S 352	Line 2001	100	0.09	0.07
Contra/Brea	Seneca 60	100	0.17	0.06
Asta/Brea	Seneca 60	100	0.42	0.08
KM 2912-1/HYB 92.05	Seneca 60	100	0.33	0.06
Ambras/KM 2912-1	Seneca 60	100	0.18	0.03

On the average, there was one plant originated from three anthers with microsporial calli or embryos.

Practical effectivity (on level of used flowers) of both methods of embryogenesis induction in such genotypes which responded in both cases is demonstrated according to ratios numbered per one experimental flower (Table 4). There are 22 (from 31 tested) bilateral responsive genotypes and nine genotypes in which the whole plants regenerated. Significant differences in responsivity benefit to androgenesis were demonstrated in 12 genotypes, one genotype benefited the haploid zygotic embryogenesis. On the level of whole plant regeneration the significant differences were registered in two genotypes only. They benefited the androgenesis. According to total responsivity the androgenesis was preferred more than according to regeneration of whole plants.

Table 3. The frequency of induced androgenesis and haploid zygotic embryogenesis in wheat

Genotypes of wheat	Anther culture				Haploid zygotic embryogenesis		
	the number of cultured anthers	the number of androgenic anthers (%)	the number of albino regenerants (%)	the number of green regenerants (%)	the number of pollinated flowers*	the number of embryos per pollinated flower	the number of plants per pollinated flower
S 88	172	0.00	0.00	0.00	100	0.01	0.01
S 92	384	15.36	2.86	0.78	100	0.06	0.02
S 102	312	13.14	1.92	7.37	100	0.03	0.00
S 105	196	0.00	0.00	0.00	100	0.02	0.02
S 113	267	3.74	0.00	0.00	100	0.00	0.00
S 202	660	0.30	0.00	0.00	100	0.03	0.03
S 206	225	3.11	0.00	0.88	100	0.03	0.00
S 215	219	5.93	0.00	0.00	100	0.00	0.00
S 217	596	5.87	0.16	0.00	200	0.04	0.02
S 218	705	14.60	2.12	1.13	100	0.02	0.00
S 219	122	9.01	0.00	0.00	80	0.11	0.06
S 220	720	19.02	7.77	0.27	100	0.00	0.00
S 221	420	13.57	1.42	0.23	40	0.01	0.12
S 223	662	2.26	0.00	0.90	100	0.01	0.01
S 224	476	5.67	0.00	0.21	100	0.02	0.00
S 225	856	17.87	0.70	2.80	100	0.00	0.00
S 227	596	3.52	0.16	1.51	100	0.03	0.03
S 228	401	1.49	0.00	0.00	100	0.10	0.00
S 229	835	6.70	0.00	1.43	100	0.20	0.00
S 230	438	18.49	1.14	3.42	100	0.20	0.00
S 231	458	2.83	0.00	0.21	100	0.05	0.02
S 232	590	27.45	1.35	3.05	100	0.00	0.00
S 233	669	3.58	0.14	0.44	100	0.05	0.02
S 238	234	4.70	0.00	1.28	100	0.01	0.01
S 245	385	4.41	2.33	0.77	100	0.00	0.00
S 249	390	1.28	0.00	0.00	100	0.02	0.02
S 253	450	7.55	2.22	0.00	100	0.01	0.01
S 254	177	6.21	0.00	0.00	100	0.03	0.03
S 256	142	0.00	0.00	0.00	100	0.18	0.02
S 258	514	9.33	0.00	0.00	100	0.07	0.01
S 259	440	5.00	0.00	0.00	100	0.13	0.00

* The pollination by the mixture of maize pollen from genotypes Seneca 60 and Line 2001 was realized

DISCUSSION

In the past years, the induction of haploid plants from female gametes pollinated with remote pollen has been realized in many cereals. Maize pollen was used in hexaploid wheat (Laurie and Bennett 1986; Inagaki and Muejeeb-Kazi 1995; Inagaki et al. 1997; Sarrafi et al. 1999; Marciňska et al. 1999, and others), and tetraploid wheat (Inagaki 1998). Pearl millet (*Pennisetum glaucum*) and sorghum pollen grains were applied experimentally (Ohkawa et al. 1992). Most of the authors reported successful hybridization of maize pollen with wheat egg cells and subsequent production of hybrid zygotes. Nevertheless, the paternal chromosomes were rapidly eliminated from hybrid zygotes during the first few cell division cycles.

As demonstrated by numerous papers, the frequency of induced zygotic embryogenesis is a very specific feature depending on pollen donors and maternal plant genotypes (Laurie and Snape 1990; Laurie and Reymondie 1991). Our as well as other experiments have clearly shown that the amounts of embryos and plants produced from them are not in a significant correlation with each other (Giura 1998; Snape 1998; Bauer and Schwarz 1999). A similar phenomenon was also observed in induced androgenesis (Maheshwari et al. 1983; Hu 1997; Inagaki 1998). It seems probable that embryo origin and complete plant regeneration are controlled by different genetic systems.

The induction of mere androgenic and/or haploid zygotic embryos inside the same genotypes was rather rare,

Table 4. Differences in ratios between responsive anthers and experimental flowers and ratios between responsive ovaries and experimental flowers and differences in ratios between androgenic plants and experimental flowers and ratios between haploid zygotic plants and experimental flowers in the same genotypes of wheat

Genotypes	Numbers of experimental flowers		Ratios in responsivity				Ratios in regeneration of whole plants			
	androgenesis	haploid zygotic embryogenesis	androgenesis	haploid zygotic embryogenesis	*t*-values	significance level	androgenesis	haploid zygotic embryogenesis	*t*-values	significance level
S 92	128	100	0.468	0.080	6.359	0.001	0.109	0.020	2.617	0.010
S 102	104	100	0.394	0.030	6.320	0.001				
S 202	220	100	0.009	0.060	2.713	0.010				
S 206	75	100	0.093	0.030	1.777	–				
S 217	199	200	0.176	0.060	3.599	0.001	0.005	0.020	1.920	–
S 218	235	100	0.438	0.020	7.547	0.001				
S 219	41	80	0.268	0.175	1.196	0.050				
S 221	140	40	0.407	0.150	3.006	0.010	0.050	0.125	1.682	–
S 223	221	100	0.068	0.020	1.778	–	0.027	0.010	0.975	–
S 224	159	100	0.170	0.020	3.728	0.001				
S 227	199	100	0.105	0.060	1.281	–	0.050	0.030	0.814	–
S 228	137	100	0.044	0.100	1.697	–				
S 229	278	100	0.194	0.200	0.128	–				
S 230	146	100	0.554	0.200	5.548	0.001				
S 231	153	100	0.084	0.070	0.403	–	0.006	0.020	0.973	–
S 233	223	100	0.107	0.070	1.044	–	0.018	0.020	0.179	–
S 238	78	100	0.141	0.020	3.079	0.010	0.038	0.010	1.269	–
S 249	130	100	0.038	0.040	0.077	–				
S 253	150	100	0.226	0.020	4.545	0.010	0.066	0.010	2.878	0.010
S 254	59	100	0.186	0.060	2.484	0.050				
S 258	171	100	0.280	0.080	4.491	0.001				
S 259	147	100	0.149	0.130	0.420	–				

but the differences in the induction of green regenerants were more frequent.

According to responsivity (induction of microsporial calli and pollen embryos or haploid zygotic embryos) numbered per one experimental flower in the same genotypes the androgenesis was benefited more than androgenesis evaluated according to regeneration of whole plants (Table 4). It again supports the differences between inductive and regenerative processes.

All of the regenerated zygotic plants were haploids, and no spontaneous polyhaploidization was observed. On the contrary, high percentages of spontaneous polyhaploids (dihaploids) of wheat are known from pollen embryogenesis (Figure 1).

The absence of spontaneous polyhaploidization in zygotic embryogenesis (Figures 2 and 3) can be affected by two factors. The first one is a different developmental stage of a female gamete during induction (i.e. after megagametophyte) contrary to pollen embryogenesis where the induction takes place before or within microgametophyte. The second reason is a presupposed short diploid unstable status of wheat female gametes after their fertilisation with maize pollen.

A list of the tested wheat genotypes (Table 3) points to the fact that in some androgenic responsive genotypes the development of haploid zygotic embryos was not induced at all, and in some genotypes responsive to haploid zygotic embryogenesis induction the process completely failed.

In our experiments with wheat only a third of the induced zygotic embryos produced complete plants. In the last experiment, the range of genotypes responsive to pollination with maize pollen was similar to those we could normally observe in experiments with induced androgenesis.

Having compared the numbers of the zygotic embryos induced in emasculated flowers and those pollinated with maize polen to the total number of ovaries contained in the same flowers we found out that the highest frequency of induced zygotic embryos and induced green plants (Figure 4) was 0.42 and 0.24 per flower, respectively. The mean number estimated in all the experiments equalled about 0.16 per flower for embryos and 0.05 per flower for green plants. In spite of the fact that the frequencies of the successful haploid zygotic embryo induction are controlled genetically, the average amounts of embryos per emasculated flower obtained in our experiments were not

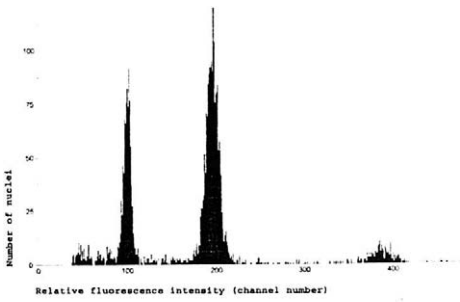

Figure 1. The histogram of relative nuclear DNA content obtained by analysis of cell nuclei isolated from leaves of haploid and spontaneously dihaploid androgenic plant in *Triticum aestivum* L. The nuclei of haploid plant were used as standard. The flow-cytometer was calibrated so that peak of haploid nuclei in G_1 phase was on channel 100. Peak of nuclei in dihaploid plant is on channel 200

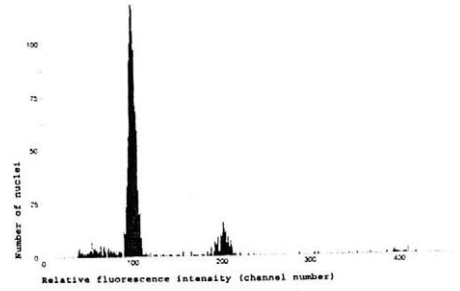

Figure 2. The histogram of relative nuclear DNA content in haploid plant from haploid zygotic embryogenesis. Peak corresponding to nuclei in G_1 phase appears on channel 100. Peak on channel 200 represents nuclei in G_2 phase

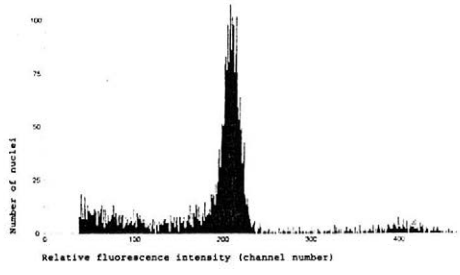

Figure 3. The histogram of relative nuclear DNA content in colchicine induced dihaploid plant from haploid zygotic embryogenesis. The flow-cytometer was calibrated so that peak of haploid nuclei in G_1 phase was on channel 100

strongly different from other authors (Kisana et al. 1993 – 0.1, Bauer and Schwarz 1999 – 0.07, Biesaga-Kościelniak et al. 1999 – 0.19, and others). In our trials every sixth female gamete being pollinated with maize pollen developed an embryo, and every twentieth gamete produced green plants. In anther cultures, at the same level of gametic responsivity (i.e. about 3000 male gametes per anther) one anther could produce theoretically 500 pollen embryos, and subsequently 150 green plants. Such a frequency has not been achieved in our experiments using standard anther culture procedures common for all cereals. It is evident that pollination with maize pollen is more efficient for inducing embryogenic development in wheat at the one-to-one gamete level contrary to *in vitro* culture of isolated anthers. For example, in wheat genotypes recently selected for high androgenic productivity, a total of 493 pollen embryos and 65 green plants per 100 cultured anthers (0.65 plants per anther) were obtained (Sarrafi et al. 1999). The highest frequencies detected in cereal model objects for androgenesis (cv. Igri in barley and cv. Florida in wheat) equalled about 12 green plants per anther (Jahne and Lörz 1995, and others).

Based on the number of the responsive gametes, the induced haploid zygotic embryogenesis is more efficient than induced pollen embryogenesis. Nevertheless, the procedure of induced zygotic embryogenesis is experimentally difficult and the ratio of a total number of female gametes to male gametes is rather poor in disfavour of female ones. This method can be successfully applied in androgenic non responsive genotypes and those containing large amounts of androgenic albinos within the scope of the Czech breeding materials.

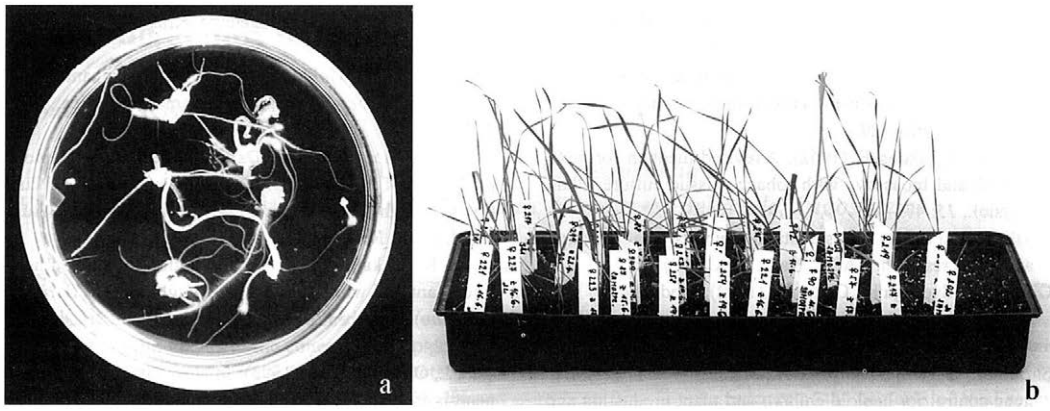

Figure 4. The samples of wheat plants from haploid zygotic embryos growing *in vitro* (a) and cultivated *in situ* (b)

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ABSTRAKT

Haploidní zygotická embryogeneze indukovaná pylem kukuřice a androgeneze *in vitro* u českých šlechtitelských genotypů pšenice

U českých šlechtitelských materiálů pšenice (*Triticum aestivum* L.) jsme testovali možnost indukovat haploidii a spontánní polyhaploidii jak sprášením emaskulovaných květů pšenice pylem kukuřice (haploidní zygotickou embryogenezí *in vitro*) u 72 genotypů, tak pomocí prašníkové kultury (androgenezí *in vitro*) u 31 genotypů. Raná pšeničná embrya exstirpovaná z vyvíjejících se ovarií byla kultivována *in vitro* a dopěstována *in situ*, androgenní regeneranti byli odvozováni standardní kultivací prašníků *in vitro* a dopěstováváni *in situ*. Ploidie všech regenerantů byla stanovována průtokovou cytometrií. Celistvé rostliny se podařilo odvodit haploidní zygotickou embryogenezí u 52 z testovaných genotypů (72%), androgenezí *in vitro* u 17 z testovaných genotypů (55%). Haploidní zygotická embrya se vytvořila u 65 z testovaných genotypů (90%), pylová embrya nebo mikrosporiální kalusy u 28 z testovaných genotypů (90%). Nejvyšší frekvence tvorby celistvých haploidních zygotických regenerantů byla 0,24 rostlin na jeden emaskulovaný, kukuřičným pylem sprášený květ pšenice, nejvyšší frekvence celistvých androgenních regenerantů byla 0,08 rostlin na jeden kultivovaný prašník pšenice (0,24 na tři prašníky v jednom květu). V androgenezi připadala v průměru jedna rostlina na tři reagující (androgenní) prašníky, v haploidní zygotické embryogenezi na tři emaskulované květy, ve kterých vznikla embrya. Absenci spontánně polyhaploidních rostlin a rostlin albikátních v procesu indukované haploidní zygotické embryogeneze v porovnání s indukovanou androgenezí vysvětlujeme odlišným ontogenetickým stadiem samčích a samičích gamet v okamžiku indukce jejich sporofytického vývoje. V práci jsou diskutovány možnosti obou metod (na úrovni jednotlivých gamet i celkového výnosu haploidie a polyhaploidie) při získávání izogenních regenerantů pšenice potřebných pro rostlinnou produkci.

Klíčová slova: genotypy pšenice; indukovaná embryogeneze; pyl kukuřice; haploidní zygota

Corresponding author:

Doc. RNDr. Jiří Vagera, CSc., Ústav experimentální botaniky AV ČR, Sokolovská 6, 772 00 Olomouc, Česká republika, tel.: + 420 68 522 85 21, fax: + 420 68 522 85 23, e-mail: vagera@risc.upol.cz

Content of active components in dependence on the number of lupulin glands in the hop cones

J. Sabo[1], J. Kišgeci[2], I. Ikić[1]

[1]*Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia*
[2]*Federal Institute for Plant and Animal Genetic Resources, Belgrade, Yugoslavia*

ABSTRACT

The work is concerned with the investigation of the dependence of total resins content and content of α-acids on the number of lupulin glands in the cones of 12 hop genotypes. It was found that the increase in the number of lupulin glands is accompanied by an increase in content of total resins and α-acids. The increase of lupulin glands in the cones by 100 corresponds to an increase of total resins by 3% and α-acids by 2%. The dependence of the contents of total resins and α-acids on the number of lupulin glands is significant ($r = 0.649$, $r = 0.641$).

Keywords: hops; total resins; α-acids, lupulin glands

Hop is cultivated for its female flower clusters – cones, which are indispensable in the brewery industry, as they lend to brewing the mellow bitterness and delicate hop aroma. Its components act as antiseptic agents, increase the biological value of beer, accelerate its clarification, and increase the foam stability. A cone consists of the stem, spindle, and two types of papery scales – bracts and bracteoles. The scales are covered with lupulin glands, whose secreta contain resinous matter and essential oils – basic active matter in the brewery. The spindle is a segmented axis of the fruit, partitioned by the nodes, with the internodes between two successive nodes. From each node are developed four bracteoles and two bracts.

The spindle and bracts, and especially the bracteoles, are covered with the lupulin glands, whose secreta are resinous lemon-yellow or gold-yellow matter. With the naked eye they look like granules and they are also called the lupulin granules or lupulin, and they contain the active matter imparting the beer desired characteristics. The inner part of bracteoles and the spindle are especially rich in lupulin glands.

The resinous secreta of lupulin glands are called total resins and they are composed of soft and hard resins and essential oils. Soft resins consists of α- and β-acids.

Content of active matter depends primarily on the hop genotypes, which, on the basis of the content of α-acids are classified as aromatic and bitter ones (Roberts 1961). Aromatic genotypes are traditional, originated from the local populations, with pronounced pleasant hop aroma and pleasant bitterness, and they are used in brewing of high-quality beer. Their main component is α-acids, whose content in the cones is up to 7%. Bitter genotypes have been obtained by crossbreeding, and they usually give higher yields than the aromatic ones, with the content of α-acids being in the range of 7–14%. As a rule, they have no pleasant aroma and impart the beer unpleasant bitterness. In the brewing process, they have to be mixed with the aromatic hops.

As intensive research has resulted in various new hop genotypes, the existing classification appeared to be inappropriate. Namely, many of the newly-created genotypes have aromatic characteristics but also a higher content of α-acids. For this reason a new classification has been proposed (Joh. Barth and Sohn 1999):

A) genotypes with fine hop aroma (content of α-acids to 5%)
B) aromatic genotypes (content of α-acids in the range of 5–6%)
C) aromatic-bitter genotypes (content of α-acids in the range of 6–8%)
D) bitter genotypes and genotypes with a high content of α-acids (above 8%)

According to this classification the first group consists of only several traditional genotypes giving high-quality brewed beverages, whereas to the second group belong the other traditional and newly-created aromatic hop genotypes. To the third group belong the new sorts having neither pronounced aromatic nor (unpleasant) bitter component, whereas the fourth group is made of the genotypes with a high content of α-acids, usually characterized by unpleasant bitterness.

Active matter content varies depending on the growing season and it is influenced by a number of factors. A reduced content of active matter is mainly due to: a) bad choice of seedlings in forming the hop field; b) non-uniform nutrition of plants due to the inappropriate choice of fertilizers and continuing use of mineral fertilizers, neglecting the organic ones; c) mechanized harvesting, i.e. unavoidable cutting of plants in the stage of technological ripeness of the cones before a full maturity of the plant itself; d) cone harvesting outside the optimal time of their technological ripeness; e) high content

of impurities in the harvested cones (Spevak, Kišgeci 1982; Kišgeci et al. 1984; Galović, Ikić 1992).

Weather conditions can also influence the content of α-acids, as our hop region is at the south border of the optimal growing conditions. Accumulation of active matter in the cones is favourably influenced by insolation, relative air humidity, and mean daily temperatures in the beginning of the plant growing (Kišgeci, Spevak 1982; Kišgeci et al. 1986).

Having in mind the previous findings on the dependence of total resins content on the number of lupulin glands (Poliščuk, Zagrafova 1986) and the dependence of the content of α-acids on the content of total resins (Sabo 1989), the aim of this work was to find out the potential dependence of the contents of total resins and α-acids on the number of lupulin glands in the cones of particular hop genotypes.

MATERIAL AND METHODS

The investigation encompassed 12 hop genotypes: Bačka, Htcl 2/23, Perle (quality group B), Aroma, Robusta, Cascade, College Cluster, Wye Target (group C), Brewer's Gold, Magnum, Chinook, and Eroica (group D) from the collection of hop genotypes of the Institute of Field Crops and Vegetables Novi Sad. The genotypes Bačka, Brewer's Gold, Robusta, and Htcl 2/23 are grown in our country (Mijavec 1969; Lačok 1978; Galović et al. 1995), Chinook, Eroica, and Cascade in the USA, Magnum and Perle in Germany, whereas College Cluster and Wye Target are grown in England (Joh. Barth and Sohn 1999).

The cones were picked up manually in the stage of their technological ripeness. Lupulin glands were counted immediately after harvesting. Of the representative samples of each genotype 10 cones were taken for the investigation. The number of glands was determined from the fourth node of the cone spindle (Poliščuk, Zagrafova 1986) and the counting was carried out with the aid of a binocular using 16× magnification.

The cone samples were also analyzed for moisture content. After drying the cones were assayed for the content of total resins, while α-acids were determined by the Wolmer method.

The dependence between the contents of total resins and α-acids on the number of lupulin glands in the cones of particular hop genotypes was established on the basis of regression and correlation analyses.

RESULTS

Moisture content and numbers of lupulin glands in the cones of particular hop genotypes are presented in Table 1. The moisture content in the range of 74–83% indicates the degree of technological ripeness of the cones. The high moisture content (above 80%) suggests that the cones were at the margin of their technological ripeness, although they were picked up in the middle of September. Judging from their appearance, the cones were technologically ripe, so that the observed higher moisture contents can be explained in terms of ample raining during the growing season.

Table 1. Moisture content and number of lupulin glands in the cones of particular hop genotypes

Variety	Moisture content (%)	Number of lupulin glands
Bačka	83.2	353
Brewer's Gold	80.3	477
Aroma	78.9	412
Robusta	79.4	424
Magnum	73.8	634
Chinook	80.8	520
Eroica	83.4	433
Cascade	78.6	382
College Cluster	82.7	270
Wye Target	82.6	435
Htcl 2/23	83.0	262
Perle	75.2	532
Average	80.2	429

The number of lupulin glands varied in the range from 262 to 634. The number of glands above average was found with the genotypes Magnum, Brewer's Gold, Chinook, Eroica, Wye Target, and Perle, while the smallest numbers were found with Htcl 2/23 and College Cluster.

The total resins content was in the range from 9.23 to 23.72 (Table 2), the highest content being observed with the genotype Magnum, while the values larger than average were observed for Brewer's Gold, Aroma, Robusta, Chinook, Eroica, and Cascade. The lowest content was found with the genotype Bačka, while the values below

Table 2. Content of total resins and α-acids in the cones of particular hop genotypes

Variety	Total resins (% water free)	α-acids (% water free)
Bačka	9.23	0.87
Brewer's Gold	20.37	8.91
Aroma	22.76	10.78
Robusta	20.38	7.94
Magnum	23.72	11.00
Chinook	22.31	12.55
Eroica	22.70	12.19
Cascade	17.98	5.79
College Cluster	13.46	5.64
Wye Target	13.76	4.75
Htcl 2/23	10.68	1.85
Perle	14.57	6.01
Average	17.65	7.36

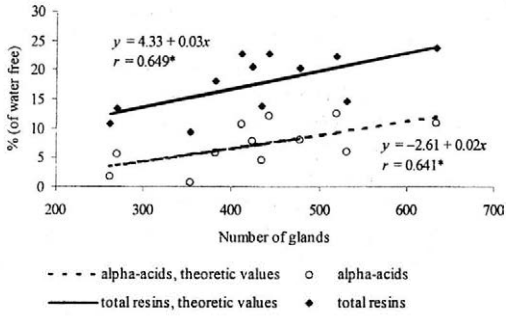

Figure 1. Dependence of the contents of total resins and α-acids on the number of lupulin glands

the average were observed with College Cluster, Wye Target, Htcl 2/23, and Perle.

Similar observations hold also for the content of α-acids (Table 2), the highest content being observed with Chinook. Contents higher than the average were found for those genotype having also a higher content of total resins, the exception being the genotype Cascade. The lowest content of α-acids was found for the genotype Bačka.

The dependence of the contents of total resins and α-acids on the number of lupulin glands is presented in Figure 1. It is evident that the increase in the number of lupulin glands is accompanied by the increase in the contents of total resins and α-acids. The constant b ($y = a \pm bx$) shows that the increase in the number of lupulin glands in a cone by 100 results in an increase of the contents of total resins by 3% and of α-acids by 2%. The increase in the content of total resins and content of α-acids as a function of the number of lupulin glands is significant at the level $P_{0.05}$, which is also evident from the high values of the correlation coefficients r.

DISCUSSION

As was mentioned above, the content of active matter in the hop cones depends on a number of factors. The content of α-acids is primarily a characteristic of the genotype, serving as the basis for classification. Besides, the content of α-acids depends on the weather conditions during the growing season (Zattle, Jehl 1962; Kralj 1996a, b). It has been found that the content of α-acids depends also on the number of lupulin glands (Lewis, Thomas 1982). High values of the coefficient of correlation between the number of glands and total resins content ($r = 0.649$) and the content of α-acids ($r = 0.641$) support the literature data. There are opinions that this can be explained by the fact that the influence of weather conditions on the content of active matter in the cones is especially pronounced in the blooming period (Park 1988) and during the growth and ripening of cones (Hautke, Petříček 1968). This is the time of formation and activation of lupulin glands in which resinous secreta are formed – total resins and α-acids.

A question arises as to the influence of weather conditions on the number of glands and the amount of their secreta. As the prospective male parents are determined on the basis of the number of glands in the male flower clusters (Brooks, Likens 1962; Kralj 1982) it can be supposed that the number of lupulin glands is a genotype-dependent quantity and that the amount of secreta – resins, varies from year to year, depending on weather conditions.

CONCLUSIONS

On the basis of the results obtained by studying the dependence of the contents of total resins and α-acids on the number of lupulin glands in the cones of particular hop genotypes, it is possible to draw the following conclusions:

The number of lupulin glands varied in the range from 262 to 634, the highest number of glands being found with the genotype Magnum and the lowest with Htcl 2/23.

Contents of total resins were in the range from 9.23 to 23.72%, the highest value being found for the genotype Magnum and the lowest for Bačka. The highest content of α-acids was observed with the genotype Chinook and the lowest with Bačka.

A relationship was established between the contents of total resins and α-acids on the number of lupulin glands in the cones. The increase in the number of lupulin glands is accompanied by the increase in the contents of total resins and α-acids: an increase in the number of lupulin glands in a cone by 100 caused increase in the content of total resins by 3% and the content of α-acids by 2%. The dependence of the contents of total resins and α-acids on the number of lupulin glands is significant, as the respective values of the correlation coefficients are 0.649 and 0.641.

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ABSTRAKT

Obsah aktivních složek v závislosti na počtu lupulinových žlázek v chmelových hlávkách

Byla sledována závislost obsahu veškerých pryskyřic a obsahu α-kyselin na počtu lupulinových žlázek v hlávkách 12 genotypů chmele. Bylo zjištěno, že zvýšení počtu lupulinových žlázek doprovází zvýšení obsahu veškerých pryskyřic a α-kyselin. Zvýšení počtu lupulinových žlázek v hlávkách o 100 odpovídá zvýšení obsahu veškerých pryskyřic o 3 % a α-kyselin o 2 %. Závislost obsahu veškerých pryskyřic a α-kyselin na počtu lupulinových žlázek je významná (r = 0,649, r = 0,641).

Klíčová slova: chmel; veškeré pryskyřice; α-kyseliny; lupulinové žlázky

Corresponding author:

Dr. Jan Sabo, Institute of Field and Vegetable Crops, M. Gorkog 30, 21000 Novi Sad, Yugoslavia, tel.: + 381 21 41 18 88, fax: + 381 21 41 38 33, e-mail: sabo@ifvcns.ns.ac.yu

The content and quality of organic matter in cambisol in a long-term no-tillage system

J. Horáček, R. Ledvina, A. Raus

Faculty of Agriculture, University of South Bohemia in České Budějovice, Czech Republic

ABSTRACT

A long-term field trial was conducted at Schwandorf (FRG) to determine some quality and quantity parameters of soil organic matter in soil profiles to a depth of 0.55 m, split to 0.05m sequences, under Horsch no-tillage system for about 30 years and parallelly under a tillage system used nearby. All other cultural practices on experimental plots (crop rotation, fertilizing, protection measures, etc.) were identical all the time. The values of the main parameters of soil organic matter in no-tillage variant decreased slowly and steadily from surface to deepest sequences. In tilled variant these values were steady from surface to sequence 0.3 m, then they fell steeply to a relatively low level. The contents of total carbon C_{ox} total, carbon of humic substances C_{ox} HS (the sum of humic acids HA and fulvic acids FA) and hot water-soluble carbon C_{hws} are comparable in both variants for the total of the sequences to 0.3 m, but they are lower in tilled variant for the total of the sequences 0.3–0.5 m. At sequences below 0.5 m the values of soil organic matter parameters are steady at a practically identical level. A similar trend was observed in the color quotient of humic substances Q4/6, which indicates increasing condensation of HS at deeper sequences in both variants, and it is also confirmed by a humic to fulvic acid ratio HA:FA. The presence and steady distribution of primary organic matters at deeper layers of no-tillage variant is documented by a degree of humification Dh. The experimental results demonstrate that the long-term use of no-tillage system led to an increase in the total content of organic matters in the soil, to their more favorable overall composition especially in the subsoil and more steady distribution in the soil profile than in tilled control.

Keywords: soil organic matter; soil tillage; no-tillage system; humus content and quality; soil profile sequences

No-tillage (or conservation-tillage) systems with their modifications have increasingly been introduced under an economic pressure also in less favorable soil and climatic conditions of the CR. These systems have been well-known for a long time; for the first time they were used at a larger scale in the USA in the fifties and sixties (Sprague and Triplett 1986), nowadays Michalson et al. (1999) report ca. 60% of farm land under this system in production regions of the USA and Canada in total. Nevertheless, no unambiguous opinion of the long-term continuous use of no-tillage technologies exists in the agricultural professional community. Many authors described some advantages of these systems in the past years (Sprague and Triplett 1986; Horsch 1990; Ledvina and Horáček 1996; Stach 1997; Kinsela 1998; Horáček et al. 1999; Šimon et al. 1999, etc.). Even though there have occurred great changes since Duchoň's era, who was a zealous advocate of a tillage system, some other authors underline certain risks of a long-term continuous plowless system (Danfors et al. 1992; Alblas et al. 1994; Hůla et al. 1997, etc.). The continuous omission of plowing is not recommended by Škoda and Cholenský (1993) or Šimon et al. (1999), either.

The breakdown of primary organic matter in the soil is an important and frequently discussed problem of no-tillage systems. If post-harvest residues and organic manure are shallow-incorporated into the soil or if they are partly left near the soil surface, there arise considerations about organic matter accumulation in the surface layer after long-time omission of tillage (Anger et al. 1993), and about the insufficient breakdown of organic matters to produce humus after shallow incorporation. But there is a lack of information on this problem both in Czech and in world agricultural literature. An absolute majority of studies was carried out to investigate only humus content and/or C_{ox} content in the surface layer or in topsoil (Sprague and Triplett 1986; Salinas-Garcia et al. 1997) or maximally at one or two other sequences. An exception is the paper by Dalal (1989), who reported the carbon content in carbon-rich fractions in the soil profile higher by 26% for no-tillage system than at the same layers after conventional tillage.

As part of Project CEZ: 1222 00002, a detailed profile study was carried out aimed at the content and quality of soil organic matter in a field trial under a long-term no-tillage system. Analyses were made of soil samples that were taken by courtesy of Mr. M. Horsch from lands of his firm at Sitzenhof where Horsch surface-tillage system has been used for 30 years except a demarcated control strip with conventional tillage.

The objective of the study was to determine the profile sequence distribution and quantitative and qualitative parameters of soil organic matter in a long-term continuous no-tillage system in comparison with the same profile under conventional tillage while other conditions were approximately identical.

MATERIAL AND METHODS

An experimental plot at Sitzenhof (Schwandorf, FRG) is situated at a height of 430 m above sea level; average annual temperature is 7°C, average annual precipitation sum 550–600 mm and the soil quality (soil productivity) scores according to a German scale (die Bodenzahl) amount to 40. The soil type is Cambisol (Braunerde) of medium-heavy to heavy texture (40.2% of textural class I < 0.01 mm and 20.8% of textural class II 0.01–0.05 mm in topsoil, and/or ca. 46% of textural class I and 25–30% of textural class II at sequences around 0.4 m), hence according to Novák's scale the soil type is on the boundary between loamy soil and clay-loam. The plot is owned by Horsch firm, and similarly like on all surrounding farm lands, a system of shallow tillage without plowing has continually been used there for 30 years. There is a control strip of land with conventional tillage, ca. 40 m in width, in the middle of the plot. All other cultural practices such as crop rotation, fertilizing, plant protection, etc., are identical every year.

Soil samples were taken in the fall 1998 from two pairs of soil pits at soil profile sequences of 0.05 m to a depth of 0.55 m. The pits of no-tillage variant (Horsch system) designated as SFH and control pits of plowed variant (designated SFK) were situated close to each other to avoid the effect of area heterogeneity of the plot. The sequences 0.10–0.25 m were omitted in the tillage system due to the expected minimum differences in soil properties in this part of the profile, hard sampling conditions (wet soil) and savings of analytical time.

The soil was air-dried and processed with a soil pulverizer Pulverisette 8 (Fritsch) to 2mm screenings. A part of homogenized samples separated by quartation were processed to 0.25mm screenings that were used to determine total oxidizable carbon and for fractionation of humus substances.

These parameters were determined to characterize organic matter in soil profiles: total oxidizable carbon C_{ox} total; oxidizable carbon of humus substances (acids) as the sum of humic acids and fulvic acids in soil alkaline extract C_{ox} HS; oxidizable carbon of humic acids C_{ox} HA; oxidizable carbon of fulvic acids C_{ox} FA; humic to fulvic acid ratio HA:FA and degree of humification Dh (Dh = C_{ox} HS.100/C_{ox}). A direct method was used to determine color quotient Q4/6 of humus substances by measuring the absorbance of their alkaline extract at 465 and 665 nm in a Jenway spectrometer; hot water-soluble (easily metabolizable) carbon C_{hws} was determined by a method according to Kubát (1994). Total carbon and its fractions were determined by a commonly used method, i.e. wet oxidation by an excess of potassium dichromate in an acid medium and re-titration of unused dichromate by addition of ferrous salt. A shortened procedure of humus fractionation (Hraško 1962; Valla et al. 1980, etc.) introduced by this university (Horáček 1995) was modified so that 0.25mm screenings were used and an extract of humus substances was separated from the soil by centrifugation instead of by filtration, and oxidation of total solids of HS, HA and FA was used instead of oxidation of aliquot parts of their solutions; finally, an equivalence point of terminal reductometric re-titration was determined in an automatic titrator DL 50 Mettler-Toledo.

All analyses were made in three replications, and excel program was used for mathematico-statistical processing of data acquired for the different parameters. As three sequences were omitted in tilled control, significance of differences in the means of the parameters was evaluated at three steps. First, the whole data sets (all profile sequences) were compared, omitting the incriminated three sequences in both variants. Secondly, the layer 0–0.30 m was evaluated separately (omitting again the above-mentioned sequences in both variants), thirdly, a data set from sequences 0.30–0.55 m was compared.

RESULTS AND DISCUSSION

Figure 1 shows that the total carbon content of organic matter in no-tillage variant ranged from ca. 1.55% (or ca. 2.70% of humus after multiplication by the factor 1.724) in surface layer 0.0–0.10 m to 0.22% C_{ox} total at the deepest sequence of sampling 0.5–0.55 m. The values of C_{ox} total decrease quite steadily at deeper sequences when slight increases can be observed at sequences 0.20–0.25 m and 0.35–0.40 m. In tilled control, the total carbon content at a layer 0.0–0.30 m was about 1.30%, C_{ox} total made 0.18% at the deepest sequence while its expectedly relatively steady content in the profile of 0.0–0.3 m with conventional tillage falls very steeply to quite low values at sequences below 0.3 m. These values are statistically significantly lower to sequence 0.55 m in comparison with no-tillage profile. A comparison of the entire

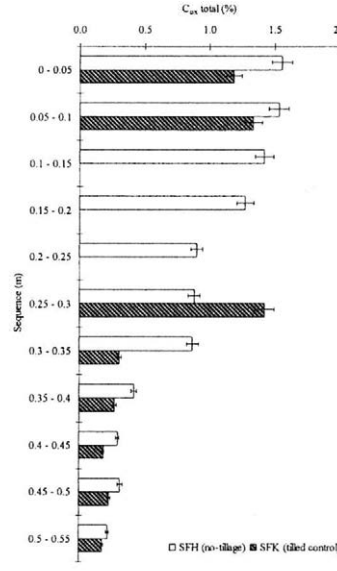

Figure 1. Total oxidizable carbon content in soil profile

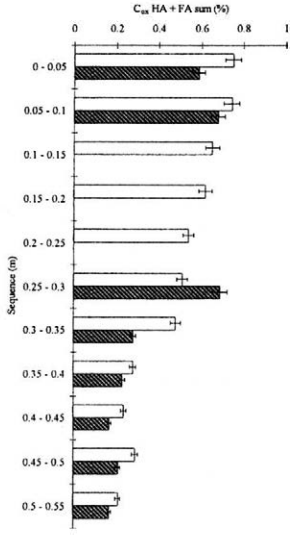

Figure 2. Oxidizable carbon of the humic and fulvic acids sum in soil profile

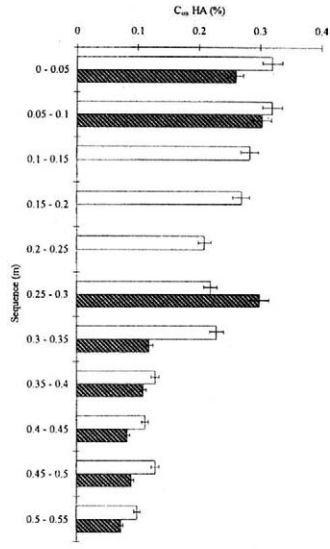

Figure 3. Oxidizable carbon of humic acids in soil profile

profiles of both variants makes it possible to state that C_{ox} total contents are comparable to sequence 0.3 m (on condition that the values of the three omitted samples from tilled control are around the mean of three determinations, i.e. 1.32% in comparison with the average content 1.27% of the first six sequences of no-tillage variant), but mainly as a result of higher values of C_{ox} total at the lower sequences of unplowed profile with long-time and continuous surface tillage only the sum of total carbon is higher in this variant than in a tillage system. Unlike the opinions of Sprague and Triplett (1986), Hendrix et al. (1989) or Salinas-Garcia et al. (1997) it is proved by these results that neither primary nor secondary (see below) organic matters are accumulated in the soil surface layer in a long-term continuous no-tillage system.

Figure 2 shows a profile diagram of humus substances C_{ox} HS. In no-tillage variant, there is quite a steady decrease from soil surface to deepest sequences when the values of HS at a layer 0.25–0.35 m are somewhat atypical. In tilled control, following the expected relatively steady HS content at a layer 0.0–0.3 m (in agreement with C_{ox} total) the content steeply falls at sequence 0.3–0.35 to low values that are statistically significantly lower at every corresponding sequence than in surface tillage. This documents a more steady and generally higher content of humus substances and/or humic acids in the unplowed profile in comparison with control. Similar results were reported e.g. by Sprague and Triplett (1986) or Kinsella (1998), even though some authors (Stevenson 1982) believe that the content of humus substances in the soil is hardly influenced by tillage.

A profile diagram of the content of humic acids in both experimental variants is shown in Figure 3. The unplowed profile is characterized by a continually decreasing trend of C_{ox} HA while the decrease is somewhat steeper at sequence 0.35–0.40 m and a slight increase was observed at sequence 0.45–0.50 m similarly like in humus substances as well as in the case of fulvic acids. In tilled control, C_{ox} HA steeply falls from almost steady values in the profile sequences by 0.3 m to low values below this layer that are lower at the corresponding sequences than in no-tillage variant.

The content of fulvic acids shown in Figure 4 truly copies the profile content of HA, and the interpretation of FA values would practically be identical except the fact

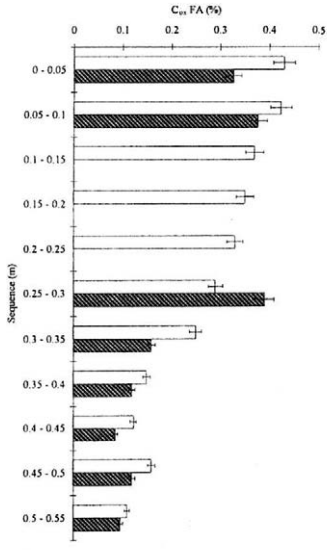

Figure 4. Oxidizable carbon of fulvic acids in soil profile

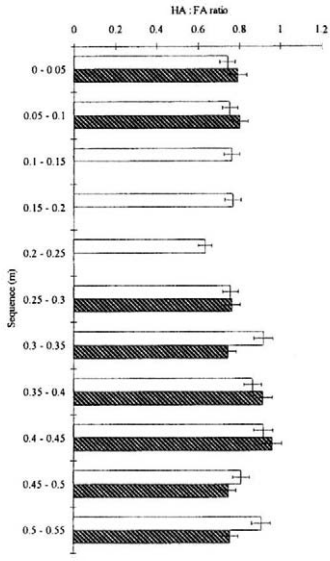

Figure 5. Humic acid to fulvic acid ratio in soil profile

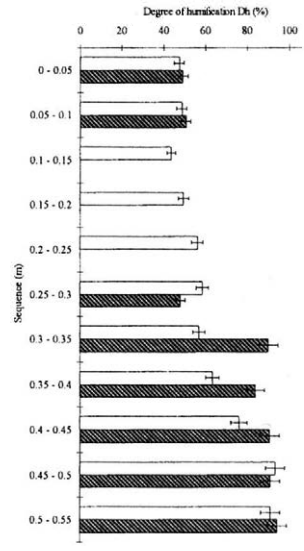

Figure 6. Degree of humification in soil profile

that at all sequences of both combinations C_{ox} FA is higher than C_{ox} HA, that means the HA:FA ratio will be lower than one as illustrated in Figure 5.

Profile evaluation of a humic to fulvic acid ratio as the most frequently used quality criterion of soil organic matter and/or humus substances is rather complicated in this case. The value of this ratio increases at deeper sequences both in no-tillage variant and in tilled control, which would demonstrate an improvement of humus quality. But the actual reason is that there are hardly any primary organic matters at deeper layers (see the following evaluation of humification degree) and the remaining humus substances present at a relatively small amount are condensed to a larger extent. If the soil tillage systems are compared, better quality of humus substances is indicated by an HA:FA ratio for tillage to sequence 0.45 m where this trend turns in favor of no-tillage variant, but neither of the trends is statistically significant.

The condition of soil organic matter in the investigated soil profiles and illustrative differences between both systems are described by a degree of humification Dh represented in Figure 6. It is comparable for both tillage systems at layers that can be taken as topsoil (0.0–0.3 m), at the same time it can be evaluated as high because humus substances account for about 50% of oxidizable carbon of organic matter. In no-tillage variant, Dh is slowly increasing at sequences below 0.3 m, that means primary organic matters also occur at deeper layers of the soil profile although their amount steadily decreases at deeper sequences. On the contrary, in tilled control the degree of humification steeply rises to values around 90% at sequences below 0.3 m, which indicates the actual absence of primary organic matter, and parallelly a reduction of energy sources not only for the edaphon but also for humification processes in the soil. Graphical representation of the degree of humification confirms the above-mentioned fact that primary organic matter is not accumulated in the surface soil layer in a no-tillage system.

The values of color quotient Q4/6 shown in Figure 7 confirm higher condensation of aromatic nuclei of humus substances at deeper sequences in both experimental variants that was demonstrated by interpretation of the HA:FA ratio, but in general, the numerical data are relatively high and/or higher (especially in the topsoil) than those usually reported for the soil type concerned (Pospíšil 1980; Pavel et al. 1984), so they would indicate the

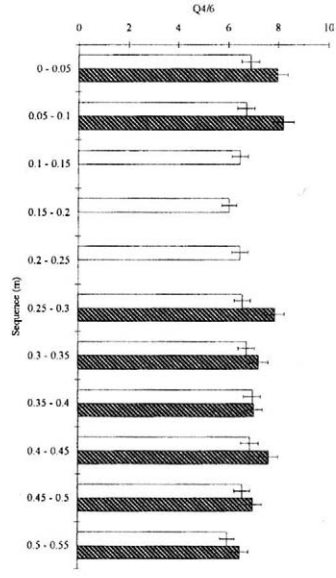

Figure 7. Colour quotient of (HA's + FA's) extract in soil profile

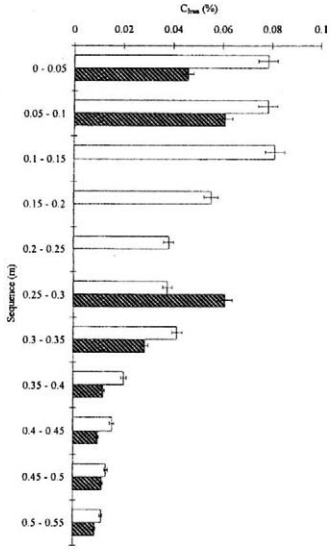

Figure 8. Content of hot water-soluble carbon in soil profile

lower quality of humus substances in both variants, and particularly in tilled control, confirmed also statistically.

Figure 8 shows the content of hot water-soluble carbon as quite a frequently used characteristic of soil organic matter in recent time.

The first three sequences of no-tillage variant had the highest content of C_{hws}, then there is a more or less steady decrease from surface to deepest sequences. In tilled control, its steady content of topsoil falls to low values very steeply, at sequence 0.35–0.4 m this time; but the contents of C_{hws} are in fact lower at all sequences than in Horsch no-tillage system. This indicates not only better soil structure [according to Haynes et al. (1991) C_{hws} is in higher correlation with soil structure than C_{ox} total] but also, on the basis of the relationship determined by Weigel et al. (1998), a higher amount of so-called potentially mineralizable nitrogen determined by incubation time, both in topsoil and at deeper sequences of the soil with surface tillage and without plowing.

Let us mention some minor anomalies in the investigated profiles at the end of discussion. First, the surface layer of tilled control (0.0–0.05 m) shows lower values of most investigated parameters than other two sampling sequences (0.05–0.10 and 0.25–0.30 m). It is possible to state that the topsoil layer was likely to be increased by subsoil incorporation or the breakdown of organic matters after turning the topsoil is faster. Some values determined for sequence 0.30–0.35 m are straying from the other ones, mainly in no-tillage variant; it may be ascribed to residual effects of previous conventional tillage as mentioned also in the monograph by Sprague and Triplett (1986). Finally, there are some departures of the values of soil organic matter parameters at sequence 0.45–0.50 m that are mostly higher than in the preceding (as well as in the subsequent) sequence and might be explained by different chemistry of this soil profile sequence.

CONCLUSION

A detailed sequence study of the content and quality of soil organic matter in a field trial at Sitzenhof in which conventional tillage was compared with a long-term continuous no-tillage system has provided these results:

- the entire profile (0–0.55 m) of soil under a long-term no-tillage system showed higher contents of organic matter and humus substances including easily metabolizable carbon than the tilled control while at the sequences below 0.3 m this difference is statistically significant except FA content,
- the quality of soil organic matter expressed by the evaluated parameters in total is comparable in both systems, and the distribution of organic matters including humic acids within the soil profile is more favorable in the no-tillage system than in tilled control while they do not accumulate in the surface layer.

Acknowledgment

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ABSTRAKT

Obsah a kvalita organické hmoty v kambizemi zpracovávané dlouhodobě bez orby

V dlouhodobém polním pokusu ve Schwandorfu (SRN) byly v půdních profilech do hloubky 0,55 m, dělených po 0,05 m, stanoveny vybrané kvalitativně-kvantitativní parametry půdní organické hmoty ve variantě ca 30 let zpracovávané bezorebnou technologií Horsch a v paralelně založené orané kontrolní variantě. Všechna ostatní agrotechnická opatření na pokusných plochách (osevní postup, hnojení, ochrana apod.) byla shodná. Hodnoty hlavních parametrů půdní organické hmoty v bezorebné variantě klesají od povrchu do hloubky pozvolna a rovnoměrně. U orby jsou tyto hodnoty od povrchu až do 0,3 m vyrovnané a pak klesají skokově na poměrně nízkou úroveň. Obsahy uhlíku celkového C_{ox}, uhlíku humusových látek C_{ox} HS (součet huminových kyselin HA a fulvokyselin FA) a uhlíku za horka vodorozpustného C_{hws} jsou v součtu vrstev do 0,3 m u obou variant srovnatelné, v součtu vrstev 0,3 až 0,5 m jsou u orby nižší. V hloubkách pod 0,5 m se hodnoty jednotlivých ukazatelů půdní organické hmoty vyrovnávají na prakticky stejnou úroveň. Podobný trend má i barevný kvocient humusových látek Q4/6, který zároveň ukazuje na větší kondenzaci HS s hloubkou u obou variant, což potvrzují i hodnoty poměru huminových kyselin k fulvokyselinám HA : FA. Stupeň humifikace Dh dokládá přítomnost a rovnoměrnější rozložení primárních organických látek i v hlubších vrstvách bezorebné varianty. Výsledky pokusu ukazují, že dlouhodobé uplatňování bezorebné technologie má za následek zvýšení celkového obsahu organických látek v půdě, jejich souhrnné příznivější složení zvláště v podorničí a rovnoměrnější rozmístění v půdním profilu než v kontrolní orané variantě.

Klíčová slova: půdní organická hmota; zpracování půdy; bezorebné technologie; obsah a kvalita humusu; sekvenční profilový průběh

Corresponding author:

Doc. Ing. Jan Horáček, CSc., Zemědělská fakulta, Jihočeská univerzita v Českých Budějovicích, Studentská 13, 370 05 České Budějovice, Česká republika, tel.: + 420 38 777 24 08, fax: + 420 38 530 01 22, e-mail: horacek@zf.jcu.cz

The effect of time of weed removal on maize yield

Z. Martinková, A. Honěk

Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

ABSTRACT

The effect of the duration of weed competition on maize aboveground biomass was investigated in the Central Bohemia, in 1994–1999. Each year a maize crop was grown together with a concordantly established weed stand [largely *Echinochloa crus-galli* (L.) P. Beauv.]. In the experimental stand we established series of four 2 × 5 m plots where weeds were mechanically removed. Three series of plots were established successively, the first about 10 days after maize emergence, the two latter in 10–20-day intervals. The plots were kept weed-free until the end of the experiment. On control plots weeds were not removed. We compared the final mass, initial growth and changes of leaf chlorophyll content in plants liberated from and stressed by weed competition. The final shoot mass (measured in early October) decreased proportionately to the length of weed competition. The regression of standardized plant mass on duration of weed competition (biological time) revealed that maize mass decreased until approximately 415 day degrees dd (above 10°C temperature threshold) from sowing. The competition then reduced plant size to about 20% of shoot mass predicted for plants growing without competition. During this period the rate of growth (log scale) of plants free of weed competition was by about half greater than in plants stressed by weed competition. Leaf chlorophyll content of plants free of competition remained stable until late July, then gradually decreased. In weedy controls leaf chlorophyll content decreased since mid-June. After liberation from weed competition chlorophyll content slowly increased to the level of non-competing plants. The extent of damage caused by competition lasting < 250 dd from maize sowing varied largely between years, but extended competition had similar detrimental effects in all years.

Keywords: critical period of weed removal; maize; weed; *Echinochloa crus-galli*; plant mass; growth rate; chlorophyll content; weather

A great attention has been paid to the study of interference between weed species and maize growth. As in other crops, the effect of weeds on growth and yield of maize depends on the intensity of competition as well as its length and timing. The intensity of competition increases with the ratio of weed to crop abundance or biomass (Ferrero et al. 1996). The models assume a hyperbolic relationship between decreasing maize yield and increasing weed density (Spitters et al. 1989; Kropff and Spitters 1991). The effects of weeds on maize are often caused by underground competition for nutrients and water (Ito 1990; Ognjanovic 1990; Varshney 1991; Martinková and Honěk 1998), in some cases also by aboveground competition for light. The timing of competition relative to crop development is very important. It is generally known that early weed competition is most detrimental to maize yield (Sandhu and Gill 1973; Chandrasagar 1983; Bridgemohan et al. 1992; Ghosheh et al. 1996). The importance of competition in early period of crop development for timing of protection measures led to definition of the critical period which is a span of time between that time period after seeding or emergence, when weed competition does not reduce crop yield, and the time period after which weed competition will no longer reduce crop yield (Zimdahl 1988). The length of critical period is investigated by two complementary techniques: (a) The crop stands are kept weed-free for varying intervals after seeding, then weeds are allowed to grow. (b) Weeds are allowed to grow for varying intervals after crop seeding followed by a weed-free period until the end of growing season (Zimdahl 1988; Welsh et al. 1999). The first approach investigates the length of period after which competition of newly established weeds is unimportant. The second approach indicates the length of period for which the competition of concurrently emerged weeds may be tolerated without a significant decrease of crop yield. The periods specified by such studies are usually called critical weed-free period and a critical time of weed removal (Bedmar et al. 1999). With respect to a given yield loss, critical weed-free period is longer than critical time of weed removal.

Barnyard grass, *Echinochloa crus-galli* (L.) P. Beauv. is among the most important weeds of maize stands. The studies of maize competition with barnyard grass or weed mixtures where this species was an important component revealed that critical weed-free period recommended for agriculture practice was 2–3 weeks (in some cases up to 6 weeks) after sowing (Nayital et al. 1989; Ferrero et al. 1991; Varshney 1991; Al-Kathiri 1994; Song et al. 1997). The length of this period depends on the accepted magnitude of yield decrease, and is influenced by weather and agriculture practices (Adzgauskiene and Jakstaite 1997). Some studies investigated also the duration of the critical time of weed removal (Sharma and Nayital 1991; Ferrero et al. 1996; Bedmar et al. 1999). The length of critical periods varied annualy (Hall et al. 1992; Ferrero et al. 1996; Bosnic and Swanton 1997; cf. Spitters et al. 1989).

Since 1989, we studied the effects of weed competition on maize growth and herbivore and predator performance in weed-stressed and weed-free stands (Honěk and Martinková 1991). The dominant weed species in our experiments was *E. crus-galli* whose stands naturally established after maize sowing. Relative abundance of barnyard grass was probably influenced by temperature and humidity variation in the uppper soil layer (Martinková and Honěk 1993) and termination of seed dormancy (Honěk et al. 1999). The changes of host plant vigour caused by weed competition (growth rate, leaf nitrogen, sugar and chlorophyll content) affected the rate of growth and maximum abundance of aphid populations (Honěk 1994; Honěk et al. 1998). Since 1994, the experiments, primarily aimed at investigating tritrophic plant-insect relationships, had a uniform design. Weeds were removed from experimental plots at different times after maize sowing and the plots were then kept weed-free until the autumn. Maize plant mass, growth rate and changes of leaf chlorophyll were examined. These experiments were evaluated to reveal critical time of weed removal in climatic conditions of Central Bohemia.

MATERIAL AND METHODS

Crop stand. The experiments were conducted in 1994–1999, at the locality of Dolínek (50°12' N, 14°25' E, altitude 270 m a.s.l.), 15 km north of Prague. Experiments were established in crop stands grown in two fields of 30 × 60 m (1994–1996) and 20 × 40 m (1997–1999) area, situated in roughly 150 m distance, with identical soil quality, fertilized each year with 30 $t.ha^{-1}$ manure. Silage maize hybrids were sown in late April to early May (Table 1), densities (60 cm spaced rows, plants thinned to 7–9 per 1 m of the row, about 125 000 $plants.ha^{-1}$, about 10–15 days after emergence) were maintained according to agricultural practices accepted in the Czech Republic (Vrzal et al. 1995). Early or mid late maize hybrids were sown whose selection was determined by the owner of the experimental ground. No cultivation or pesticide treatments were made after sowing. Weeds germinated simultaneously with maize plants and then grew concurrently until the autumn. Experimental treatment consisted in establishing small plots where weeds were hoed, and maize was liberated from weed competition. Every year (except 1998) experimental plots were established in three terms (Table 1). The first term of weed eradication (treatment 1) was set within 10 days following weed germination, next two treatments (treatment 2 and 3) were established in 10–20-day intervals, according to weather conditions. On each date we established four plots (replicates) of 2 × 5 m area (5 m section of three rows). The plots established on later dates were situated next to the earlier established plots. After weed extermination all plots were hoed in frequent intervals and thus kept free of weeds until harvest. One series (control) consisted of plots where weeds were allowed to grow until harvest. The plants were harvested at the end of vegetational season when the growth was terminated by both early and mid-late hybrids. The area of control and first treatment plots was doubled in 1996 and 1997, when samples of plants were removed in May to July to measure plant growth (see below).

Plant growth. Growth and quality of maize were evaluated by three criteria: (i) Final plant mass at maturity (Table 1) was established every year. Ten or 20 maize plants were cut 5 cm above the ground level in each experiment plot (treatment replicate) and their wet shoot mass was determined. Since it was impracticable to establish dry mass of each plant, the samples of fresh biomass of each treatment (about 0.3 kg plant material cut into small pieces) were weighed, dried at 105°C to constant weight, and weighed again, with 0.1 g precision. The dry mass of plants was then calculated by multiplying the fresh weights by a coefficient C = (sample dry mass)/(sample fresh mass). Since the average size of maize plants varied among years (Table 2), the data were standardized by dividing the average mass of the plants of weed-free treatments by average mass of the controls. This quantity, further called standardized plant mass (SM), was calculated for each replicate as SM = (average replicate plant mass)/(average control plant mass). Using standardized plant mass enabled to compare data of all experiment years. (ii) Growth of maize plants in the early period of their development was compared in control plants and plants of weed-free treatment established first in the season. In May to July 1996 and 1997, samples of plants were removed in weekly intervals. Seven (before mid-June) or

Table 1. Details of experimental design; maize hybrids, terms of maize sowing and harvest, and terms of weed exterminations (treatments 1–3), calendary dates and biological time (day degrees dd above 10°C threshold) elapsed since the time of sowing

Year	Maize hybrid	Sowing date	Treatment 1		Treatment 2		Treatment 3		Harvest	
			dd	date	dd	date	dd	date	dd	date
1994	CE 330	7. 5.	164.5	10. 6.	240.3	22. 6.	392.3	4. 7.	1154.2	5. 10.
1995	CE 330	2. 5.	49.8	25. 5.	130.5	8. 6.	199.6	25. 6.	928.0	6. 10.
1996	KW 212	9. 5.	198.3	10. 6.	300.8	28. 6.	366.7	10. 7.	829.8	10. 10.
1997	TAO 220	10. 5.	102.6	22. 5.	155.5	8. 6.	305.2	2. 7.	966.7	7. 10.
1998	TAO 220	27. 4.	143.1	14. 6.	244.5	29. 6.	–	–	972.2	7. 10.
1999	CE 240	1. 5.	88.2	25. 5.	219.8	10. 6.	280.1	23. 6.	1100.1	7. 10.

sTable 2. The aboveground dry biomass of weeds and individual dry mass (average ± *SE*) in maize plants liberated from weed competition on different dates (treatments) and in the control grown under competition until the harvest

Year	Weed biomass ($g.m^{-2}$)	Maize plant mass (g)			
		treatment 1	treatment 2	treatment 3	control
1994	287	133.8 ± 9.2	65.7 ± 6.6	32.6 ± 3.3	23.6 ± 2.3
1995	303	108.1 ± 10.8	80.8 ± 8.4	58.2 ± 7.2	20.0 ± 2.8
1996	318	148.0 ± 13.5	108.3 ± 13.5	84. 8 ± 12.4	49.4 ± 7.5
1997	380	85.1 ± 12.4	78.0 ± 12.1	39.9 ± 6.6	31.7 ± 5.4
1998	442	174.3 ± 33.5	130.3 ± 14.2	–	72.9 ± 11.5
1999	485	118.1 ± 35.7	108.7 ± 30.2	65.0 ± 17.3	22.7 ± 3.4

three plants were removed from each treatment and control replicate (the number of plants decreased due to increasing plant size), dried at 105°C and weighed. (iii) Changes in chlorophyll content of maize leaves were measured in weekly intervals, in 1997. Chlorophyll content of leaves was measured optically by Chlorophyll Meter SPAD-502 (Minolta). The measurement of chlorophyll content (indicated in SPAD units) is based on the amount of light transmitted by the leaf in two wavelength zones, in which the absorbance of chlorophyll is different from carotenoids and other leaf components (peak wavelength approximately 650 and 940 nm). Values indicated by SPAD units are closely correlated with chlorophyll and nitrogen content of the leaves, and (in winter wheat) with crop yield (Barraclough 1998). Of each replicate we took 30 measurements, on the upper leaves which already completed the growth. In plants at eight and more leaf stage this was the 4th to 6th leaf from the top of the plant. Two measurements were taken on each leaf, one at quarter, the other at three quarters of its length.

Each year, weed (all species together) aboveground dry biomass was determined after reaching its maximum (July) (Table 2). Weed biomass per area was used as an index of weed abundance, since numbers of weed plants per area varied between years together with their individual size. Four 0.25 m^2 plots were harvested from intact weed stands near the edge (at about 1 m distance) of replicate series. The samples were dried at 105°C to constant weight, and weighed with 1 g precision. Average dry aboveground weed biomass.m^{-2} was calculated using data of all replicate series. Dominant weed species were recorded each year. Barnyard grass *E. crus-galli* dominated in weed stands in all years of observation, except 1995, when the stand was dominated by *Galinsoga parviflora*, and 1996 when dominant species was *Amaranthus retroflexus*. These species together represented each year more than 95% of the aboveground weed biomass.

Data elaboration. When investigating the effects of competition, calendary dates were transformed to biological time (Table 3). This was measured in day degrees (dd) above the threshold temperature of 10°C at which maize growth ceases (Belej et al. 1982; Vrzal et al. 1995). Day degrees were calculated as the sum of the differences between average daily temperature and 10°C threshold temperature, accumulated from the day of maize sowing. This method of measuring biological time is well established in ecological studies (Honěk 1999) and convenient for between season or between species comparisons. Meteorological data were obtained from the meteorological station of the Research Institute of Crop Production in Prague-Ruzyně, about 15 km from the locality where experiments were carried out. A comparison of the Prague-Ruzyně data with data of a local meteorology station (Sedlec, about 4 km from the experimental site, data available only before 1995) revealed that the differences between average daily temperatures were mostly less than 1°C. Linear regressions ($y = ax + b$) and correlations were calculated to establish the relationship between standardized maize plant mass (y) and length of weed competition in dd (x). Exponential regressions ($y = be^{ax}$) were calculated to establish the relationship between log dry maize plant mass and time elapsed since maize sowing in dd (rate of maize growth).

Table 3. Temperature sums (day degrees dd above threshold temperature 10°C) during the period of maize growing; the figures for the first and the last months are calculated from the date of sowing and until the date of harvest, respectively

Year	April	May	June	July	August	September	October	Total
1994		115.7	227.3	386.7	278.1	135.7	10.7	1154.2
1995		103.9	131.9	321.1	244.7	102.0	24.4	928.0
1996		96.1	212.0	214.4	252.9	36.9	17.9	829.8
1997		105.9	198.7	229.8	279.3	117.0	36.0	966.7
1998	25. 6.	150.6	227.6	227.6	236.9	99.5	4.4	972.2
1999		148.4	184.1	297.8	241.8	217.1	10.9	1100.1

RESULTS

Length of weed competition

Final maize plant mass decreased with the length of initial weed competition (Figure 1). The common regression ($a = -0.010$, $b = 5.220$, $R2 = 0.476$, $F = 13.612$, $df = 15$, $p < 0.005$) of standardized plant mass on length of weed competition (biological time) indicated that damage, which decreased plant mass to the size of control, was inflicted after 417 dd elapsed since maize sowing. The regressions calculated separately for data of particular years indicated average. The regression predicted the standardized plant mass 5.2 for a maize plant never suffering from competition. Permanent weed competition thus will reduce potential plant mass by 81%. The scatter of data was greatest in early period of about 250 dd following maize sowing (Figure 1). In this period the outcome of competition differred between years and was apparently influenced by environment conditions. As the length of competition increased, negative effects were important regardless of annual variation of experimental circumstances and the data were then close to regression line.

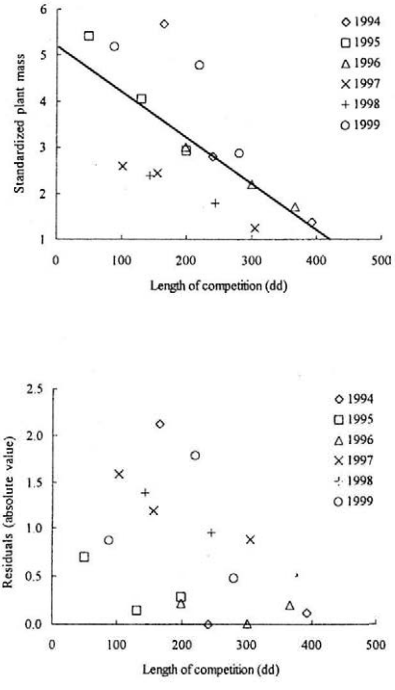

Figure 1. The effect of the length of weed competition (biological time in day degrees dd above 10°C threshold summed from the time of sowing) on the aboveground mass of maize plants; standardized plant mass (above) and residuals of regression of standardized plant mass on biological time (below) plotted against time (dd) elapsed from maize sowing

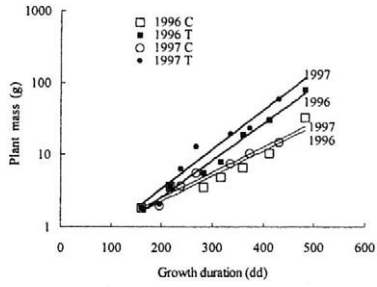

Figure 2. Growth of control (C) maize plants and plants liberated from weed competition at the earliest term (treatment 1, T); regression lines of log dry plant mass on duration of maize growth (biological time in day degrees dd above 10°C threshold summed from the time of sowing) in T (heavy line) and C (thin line) plants

Plant growth

The growth of maize plants between 150 and 450 dd from crop sowing (period when damage was inflicted) was compared in controls and plants of treatment 1 liberated from competition at the earliest term (Figure 2). In both 1996 and 1997 the growth rate of plants liberated from weed competition ($a = 0.0117$ and 0.0125, resp.) was by half greater than in control plants ($a = 0.0080$ and 0.0081, resp.). Final differences in plant mass (Table 2) originated due to variation of growth rate during the early period of plant life. The dry matter yields thus decreased from 10.6–21.7 t.ha^{-1} in treatment 1 to 2.5–9.1 t.ha^{-1} in controls.

Leaf chlorophyll content

The changes in leaf chlorophyll content of controls and treatments liberated from competition were checked in 1997 (Figure 3). Chlorophyll content was highest shortly after emergence, before the onset of intensive competition (2-leaf stage, 45 SPAD units) but quickly decreased to about 40 SPAD. In controls, chlorophyll content was between 30–35 SPAD until mid-June, then decreased quickly to roughly 20 SPAD after July 15, and remained on this level until the end of August. In plants liberated from weed competition on May 25 (treatment 1) chlorophyll content remained at approximately 40 SPAD until late July, thereafter it decreased to about 30 SPAD at the end of August. In plants of treatment 2 (liberated from competition on June 10) chlorophyll content increased slightly and paralleled the development in plants of treatment 1. In plants of treatment 3 (liberated from competition on July 10) chlorophyll content increased slowly above the level of control, but only matched the SPAD of plants of the treatments 1 and 2 at the end of August, when SPAD was generally low. The differences (Figure 3) between SPAD of the control and treatments 1 and 2 thus increased to up to 25 SPAD units, in early July. By

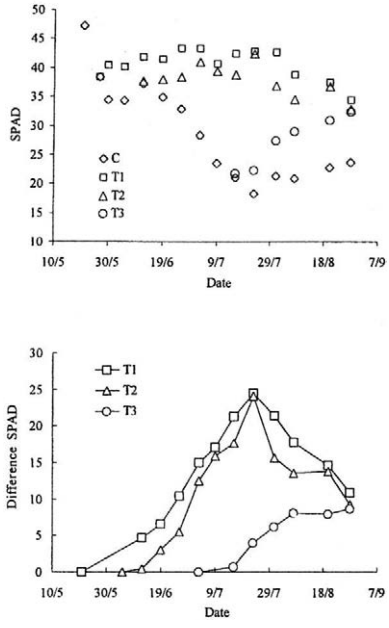

Figure 3. Chlorophyll content of maize leaves (SPAD units) in 1997 season (calendary dates) changes of chlorophyll content in control plants (C) and plants of treatments 1–3 liberated from weed competition at three terms (T1–T3); seasonal course of changes of leaf chlorophyll content (above) and differences (smoothed curve) between leaf chlorophyll content of plants of treatments 1–3 and control (below)

contrast, the differences between control and plants of treatment 3 increased slowly and remained below 10 SPAD units, because of the general decrease of leaf chlorophyll content towards the end of the vegetational season.

DISCUSSION

The effects of timing of weed removal

As expected from results of earlier works, the final maize yield decreased with timing of removal from weed competition. The increase of weed damage with the increasing length of competition could be approximated by a non-linear sigmoid function (e.g. Gompertz), which reflects the low effect of early competition and the large increase of its importance at the time, when weed stand becomes dense (Hall et al. 1992; Ferrero et al. 1996; Bedmar et al. 1999; Welsh et al. 1999). This type of approximation requires several treatments made during the same season to prevent the effect of scatter caused by conditions of different years. Our data were not convenient to establish detail relationship for particular years due to small number of treatments performed each year. We therefore preferred linear approximating of the data. These approximations enabled to estimate (i) the length of period during which negative effects of weed competition increased, and (ii) the extent of relative damage which weed competition inflicts to maize plants when competition extends over the whole season.

Using a common regression smoothed the differences which may exist in the response of early and mid late hybrids. In fact, regressions calculated for results of particular years (each calculated from 2 or 3 points only) indicated that the period during which negative effects of weed competition increased was shorter in mid late cultivars (410 ± 35 dd) than in early cultivars (543 ± 42 dd). The effects of large annual variation in the outcome of competition revealed by the data was also smoothed. This variation may be caused by differences in growth rates of weeds and crop which have different threshold temperatures for growth as well as temperature sums needed to complete development until mature stage (Hall et al. 1992). It is difficult to compare our results with data of authors investigating competition effects on grain yield. Nevertheless, the published results indicated similar variation, apparently due to correlation which exists between competition effects on grain yield and green matter. Ferrero et al. (1996) found a 12.7% yield loss after competition until 4-leaf stage in 1992, and a 8.3% yield loss after competition lasting until 9-leaf stage in 1993, Bedmar et al. (1999) established that critical time of weed removal to prevent yield losses of 2.5% ranged from 128 to 261 dd (above 0°C threshold). The overal magnitude of the negative effects on maize growth was similar as found by other authors (Nayital et al. 1989; Adzgauskiene and Jakstaite 1997; Song et al. 1997).

Origin of variation in crop growth

In our experiments the mechanism of differences between competing and weed-free crops consisted in decreasing maize growth rate during the early stage of its development. This retardation of growth reduced stem length and aggravated the effect of aboveground competition, since weeds grew nearly as tall or even taller than maize plants. The aboveground competition is not very important when weeds establish later than maize crop (Martinková and Honěk 1998).The development of differences in plant size was paralleled by variation in leaf chlorophyll content which decreased earlier in weed-stressed than weed-free plants.

The effects of early competition were compensated. Liberation from weed competition was always followed by increase of chlorophyll content and plant growth. However, chlorophyll content increased only up to the level typical for non-competing plants of the same age. The high leaf chlorophyll content similar to that of young non-competing plants was never attained. Thus, there exist two causes of negative effects of early weed competition on maize growth: (a) small plant size attained at the time of weed extermination (a consequence of slow

growth under competition) and (b) incomplete compensation of photosynthetic capacity following liberation from competition.

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ABSTRAKT

Vliv termínu odplevelení na výnos kukuřice

V letech 1994 až 1999 byl studován v podmínkách středních Čech vliv délky trvání konkurence plevelů na biomasu nadzemních částí kukuřice. Pokusy byly konány v porostech kukuřice, v nichž byl ponechán přirozený vývoj zaplevelení rostlinami vzešlými po zasetí kukuřice, převážně ježatkou kuří nohou *Echinochloa crus-galli* (L.) P. Beauv. V pokusných porostech byly každý rok zřízeny tři série po čtyřech pokusných parcelkách o rozloze 2 × 5 m. Série byly zakládány ve třech termínech, první asi 10 dní po vzejití kukuřice, další v 10- až 20denních intervalech. Pokusné parcelky byla mechanicky odpleveleny a takto udržovány až do sklizně. V další sérii kontrolních parcelek byl umožněn nerušený růst plevelů. Na těchto parcelkách byla sledována hmotnost nadzemních částí kukuřice při sklizni (počátek října), rychlost růstu v počátečním období a změny obsahu chlorofylu v listech během sezony. Hmotnost nadzemní části kukuřice klesala úměrně délce trvání zaplevelení. Regrese standardizované hmotnosti prýtu kukuřice v závislosti na termínu odplevelení (vyjádřeném v biologickém čase jako suma denních stupňů nad prahovou teplotou 10 °C) ukázala, že hmotnost kukuřice klesala až do doby 415 denních stupňů od zasetí. Po delším trvání konkurence by hmotnost rostlin poklesla na velikost trvale zaplevelené kontroly, která představovala asi 20 % předpokládané hmotnosti rostlin rostoucích bez konkurence plevelů. Během období exponenciálního růstu hmotnosti kukuřice (asi 450 denních stupňů) byla rychlost růstu rostlin zbavených konkurence plevelů asi o polovinu vyšší než u rostlin z trvale zaplevelených parcelek. Obsah chlorofylu v listech rostlin bez konkurence se udržoval na přibližně setrvalé úrovni do konce července, pak zvolna klesal. Naproti tomu obsah chlorofylu v listech u kontrolních variant počal rychle klesat již od poloviny června. Pozdní odplevelení mělo za následek postupné zvýšení obsahu chlorofylu až na úroveň rostlin z raně odplevelených parcelek. V odplevelení v raném období (do zhruba 200 denních stupňů od vysetí kukuřice) se účinek na biomasu prýtu kukuřice značně lišil mezi jednotlivými lety, delší zaplevelení však mělo podobné negativní účinky ve všech sledovaných letech. Výsledky potvrdily rozhodující význam zaplevelení v raném období vývoje kukuřice pro snížení jejího výnosu.

Klíčová slova: kritická perioda pro odplevelení; kukuřice; plevele; ježatka kuří noha; hmotnost rostlin; rychlost růstu; obsah chlorofylu v listech; počasí

Corresponding author:

Ing. Zdenka Martinková, CSc., Výzkumný ústav rostlinné výroby, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika, tel.: + 420 2 33 02 22 88, fax: + 420 2 33 31 06 36, e-mail: martinkova@vurv.cz

The impact of fertilizing on fenugreek yield (*Trigonella foenum-graecum* L.) and diosgenin content in the plant drug

A. Zupančič[1], D. Baričevič[1], A. Umek[2], A. Kristl[2]

[1]*Biotechnical Faculty, University of Ljubljana, Slovenia*
[2]*Faculty of Pharmacy, University of Ljubljana, Slovenia*

ABSTRACT

The impact of fertilizing on the seed yield and the diosgenin content in four cultivars (Margaret, Ionia, Barbara, Paul) of fenugreek (*Trigonella foenum-graecum* L.) was observed in a three-year field experiment (1995–1997) on two diverse climatological and pedological localities in Slovenia (Rakican, Vinjole). The seed yields and their diosgenin contents were compared in all four cultivars after various fertilizing schemes that had been applied (organic fertilization with no addition of nitrogen fertilizers, optimum fertilization with mineral nitrogen, high dosage of nitrogen fertilizers and control treatment without fertilizers). When compared to the control treatment, high nitrogen fertilizing (170 kg/ha) had no impact on the seed yield. Application of 30 kg N/ha (optimal nitrogen fertilization dosage) or of organic matter (43 t R-substrate/ha) increased the seed yield on poor soil. The content of diosgenin, measured with GC, was not significantly influenced by different fertilizing schemes ($a = 0.01$). The lowest content of diosgenin was observed in cv. Ionia, the highest in cv. Margaret and in cv. Paul, which had simultaneously the highest absolute weight of seed. The yield of diosgenin per hectare can be increased only with selection of a cultivar with a high seed yield (Ionia, Barbara).

Keywords: field experiment; saponins; medicinal plants; secondary metabolites; *Leguminosae*

More and more attention is being placed on increasing the yields of aromatic and medicinal plants in agrosystems through augmented biomass production. Consequently, the extending and merging of ecological analyses with up-to-date techniques is assuming ever greater importance. Although considerable information on model production systems is available, the principles and practices characterizing the primary plant production cannot be directly applied to the production of secondary plant products. Namely, the appearance of special products (i.e. secondary metabolites) in ecological systems postulates the presence of special circumstances beyond general production regularities (Bernath, 1986). The accumulation level of secondary products depends on the physio-ecological background of the accumulation of secondary compounds influenced by environmental factors.

Nowadays, the industry, especially the pharmaceutical industry, needs sufficient amounts of standardized raw materials with high and uniform contents of special compounds for profitable processing. Because of the low and variable content of active compounds in native (non-selected) populations, plants collected from nature cannot meet the demand from the processing industries. As an interesting alternative, cultivation of medicinal and aromatic plants has became an important way of optimizing the quality of raw materials.

Our study was aimed at determining optimal fertilization practices in the sustainable cultivation of fenugreek (*Trigonella foenum-graecum* L.). Fenugreek is a plant with a good agricultural potential, also for the colder areas of Europe and of North America. This plant is due to its short vegetation period cultivated in the Mediterranean and Indian areas as well as in the colder areas of Europe (Elujoba 1987).

It is used in the following forms: as a condiment (Sharma 1986), as a food additive (Mital and Gopaldas 1985), medicinal plant (Bhati 1986; Sharma 1986; Wichtl 1989; Javan et al. 1997), forage (Mir et al. 1993), biotic protective (Afifi et al. 1988; Panadey et al. 1993), mixed crop (Mosaad and Abdel Shafi 1990; Sharma and Khanna 1991; Makai and Pecsi 1993), nitrogen fixation crop (Desperrier et al. 1985; Nikolai et al. 1990). It is mainly used as a raw material for isolation of diosgenin, a well known starting material in pharmaceutical semi-synthesis of steroid hormones. Many pharmacopoeias comprise the monograph of *Foenugraeci semen* (European Pharmacopoeia III, Suppl.1999, Deutsche Arznei Buch 10, Östereiche Arznei Buch 1990, Pharmacopoea Helvetica VII, British Herbal Pharmacopoea 1983); the drug plant has also been approved by the German Commission E Monograph (BAnz. No. 22a. 1. 2. 1990). Ground fenugreek seeds are known for their health benefits with respect to reduction of cholesterol (Madar and Shomer 1990; Sharma et al. 1990) and sugar levels in the blood stream.

The objective of the present research work is to study the production potentials of fenugreek in a continental climate compared to that of a Mediterranean area, with respect both to the yield capacity and to the quality of the crude drug. In our experiment the influence of fertilization and selection of the cultivar on the seed yield and the diosgenin content were studied. These parameters are very important for field cultivation of fenugreek for the pharmaceutical industry.

MATERIAL AND METHODS

Field experiment

Three-year (1995–1997) field experiment was plotted on two climatologically and pedologically different localities in Slovenia. In Rakican (46°40' N, 16°10' E, continental area, mean year temperature 9.4°C, mean annual precipitation 814.2, sandy loam soil with pH 5.9, P_2O_5 (mg/100 g soil) 12.2, K_2O (mg/100 g soil) 20.6, organic matter 1.7%, C 1.0%), and in Vinjole (45°32' N, 13°34' E, Mediterranean area, mean year temperature 13.5°C, mean year precipitation 1046.5 mm, loamy soil with pH 7.2, P_2O_5 (mg/100 g soil) 108.0, K_2O (mg/100 g soil) 37.4, organic matter 2.4%, C 1.4%). The decades of meteorological data are shown in Figures 1–3). Four different cultivars were examined: cv. Barbara and cv. Paul (intersubspecies crossbreed between subsp. *foenum-graecum* and subsp. *indica*); cv. Margaret (subsp. *foenum-graecum*); and cv. Ionia (listed on OECD List of varieties/Schemes for varietal certification of seed moving in international trade). Experiments were repeated on the same site during the three years of study. The effects of three different fertilizing methods (organic, mineral with optimal nitrogen supply of 30 kg N/ha, mineral with high nitrogen supply of 170 kg N/ha) and of control treatment on the seed yield and seed quality (diosgenin content) were observed. Because fenugreek does not tolerate fresh manure, the R-substrate (producer Agro Ruse, 43 t/ha) was applied in organic fertilization treatment. This substratum contains approx. 0.6% N, 0.07% P_2O_5, 0.03% K_2O and between 60 and 70% of organic matter. The R-substrate was mixed up before sowing. For the other two treatments we used mineral fertilizers (producer INA Kutina). Our optimal fertilization treatment (30 kg N/ha) was based on the observation of Parek and Gupta (1981), who reported that the highest seed yield and diosgenin content in seeds of fenugreek were achieved with a dosage of 30 kg N/ha. Nitrogen fertilizer was applied in two periods, one half of the total amount before sowing and the other half after three weeks. The second treatment was called intensive fertilizing, in which 170 kg N/ha were applied. This amount is the highest quantity of nitrogen, which is allowed to be applied on water protection areas in Slovenia.

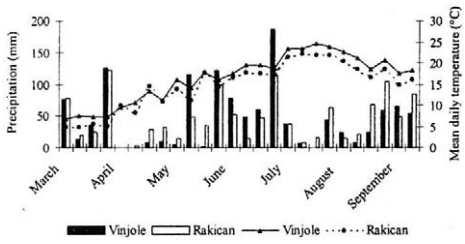

Figure 1. Mean daily temperature and precipitation in Vinjole and in Rakican in the year 1995

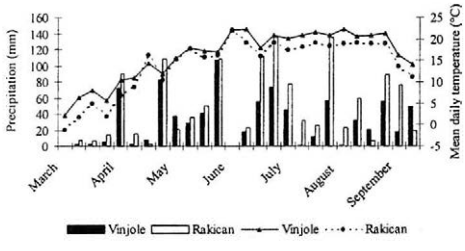

Figure 2. Mean daily temperature and precipitation in Vinjole and in Rakican in the year 1996

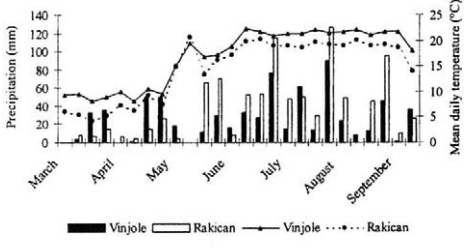

Figure 3. Mean daily temperature and precipitation in Vinjole and in Rakican in the year 1997

In the basic fertilization treatment (before sowing period) the combined fertilizer (N:P:K = 15:15:15) was used. In additional nitrogen fertilizations we also used a commercially available fertilizer (nitrochalk, KAN-Kutina), containing equal amounts of nitrogen in nitrate and in ammonium forms (soluble mineral forms, available to plants). Nitrogen in the intensive fertilizing method was applied three times during a three-week period, with first application before sowing. The crop field was manually cultivated without the use of pesticides. The distance between rows was 25 cm and inside the row 10 cm. The sowing and harvesting periods are presented in Table 1. The plants were harvested after full legume development. Seeds were dried at 40°C.

The field plot was designed as a split plot experiment (Hadzivukovic 1969; Mead and Curnow 1990) on the assumption that the overall soil growing conditions were homogenous. The field area was divided into four strips. Each of the strips represented one of the treatments (con-

Table 1. Growing periods of fenugreek

	Vinjole	Rakican
Sowing	5. 4. 1995	19. 4. 1995
Harvest	11. 7. 1995	18. 7. 1995
Growing period	97 days	90 days
Sowing	26. 3. 1996	9. 5. 1996*
Harvest	22. 7. 1996	13. 9. 1996
Growing period	118 days	127 days
Sowing	27. 3. 1997	14. 4. 1997
Harvest	16. 7. 1997	5. 8. 1997
Growing period	112 days	113 days

* Sowing was late because of the bad weather conditions in spring

trol, organic – R-substrate, optimal and intensive). The strips were divided into five blocks (representing five repetitions), where the cultivars were randomized. The size of parcels within the experimental plot was 3 m². ANOVA, the standard errors of two average differences and Critical values of two average differences were used in statistical evaluation (Stratgraphic program) of plant samples.

Chemical analyses

Chemicals. The following chemicals of analytical grade (p.a.) were used in the extraction procedure: methanol, dichloromethane (Merck, Darmstadt, Germany), sodium hydroxide (Kemika Zagreb), hydrochloric acid (37%) (Merck, Darmstadt, Germany). As a reference, the commercially available standard diosgenin (25 R-spi-rost-5-en-3 b-ol) (98%) (Sigma Chemical Co., Germany) was used.

Glycoside hydrolisis and extraction of diosgenin. Diosgenin is an aglicon (sapogenine) of saponin glycoside dioscin. Saponin glycosides are regarded as a chemically heterogeneous group of secondary metabolites, due to the differences in their sugar moiety as well as in their aglycon functional groups. Therefore, there is no universal method of determining the quantity of saponins in plant material. Among the different methods the most common are high performance liquid chromatography (HPLC), gas chromatography (GC) and thin layer chromatography (TLC). All of these methods define saponin's aglykon. To determine the seeds' diosgenin content in our study, hydrolysis of glycoside dioscin was necessary. Hydrolysis and successive extraction of fenugreek seeds followed a procedure described by Sanchez et al. (1972). 1.00 g of whole seeds of fenugreek was hydrolyzed with 30 ml of 3M HCl in a 100ml flask for two hours under reflux. After neutralization with 10 M NaOH, the samples were filtered with a vacuum pump. The filter papers with seeds were dried up in a vacuum dryer (VS-50 S, Vakuumska tehnika Kambic) under negative pressure at 60°C. The dried seed samples (1.0 g) were extracted with dichloromethane (50 ml) in Erlenmayer flasks on a shaker (Kinetor M, Elektromedicina) for four hours. The extracts were filtered and the solvent was evaporated under reduced pressure (Büchi Rotavapor, Switzerland) at 40°C. Before GC analysis, the dried extracts were dissolved in 5 ml dichloromethan and samples (1 µl) injected into a gas chromatograph.

Gas chromatography. A 5890 Series II model gas cromatograph (Hewlett Packard) equipped with a flame ionization detector and coupled with an integrator (HP 3396 Series II) was used. The diosgenin determinations were carried out on an Ultra 2 capillary column (25 m × 0.32 mm inner diameter; (5%)-diphenyl-(95%)-dimethylsiloxane phase – 0.52 µm), with temperature programming from 230°C to 310°C at 1°C/min. The carrier gas (helium) and hydrogen flow rates were 1.50 ml/min and 66.7 ml/min, respectively, and the air-flow rate was 286 ml/min. The injector and detector were thermostated at 280°C and 320°C, respectively. The overall chromatographic analysis took 25.4 min.

Table 2. Average seed yield (kg/ha) and their diosgenin content (% dw) in fenugreek cultivars in Vinjole (Slovenia) in the years 1995, 1996 and 1997

		1995		1996		1997	
		yield (kg/ha)	diosgenin (%)	yield (kg/ha)	diosgenin (%)	yield (kg/ha)	diosgenin (%)
Margaret	control	277	0.24	432	0.32	179	0.30
	organic	339	0.20	513	0.36	365	0.30
	optimal N	307	0.20	661	0.33	145	0.28
	intensive N	230	0.18	753	0.31	137	0.23
	average	288	0.21	590	0.33	206	0.28
Ionia	control	518	0.22	243	0.26	325	0.23
	organic	441	0.21	614	0.28	416	0.24
	optimal N	376	0.20	834	0.29	499	0.23
	intensive N	418	0.17	804	0.25	359	0.24
	average	438	0.20	623	0.27	400	0.24
Barbara	control	417	0.19	378	0.29	287	0.29
	organic	419	0.19	570	0.31	306	0.26
	optimal N	352	0.19	618	0.30	348	0.31
	intensive N	347	0.19	701	0.29	344	0.26
	average	384	0.19	567	0.3	321	0.28
Paul	control	356	0.22	404	0.31	266	0.29
	organic	423	0.23	572	0.35	249	0.27
	optimal N	222	0.27	569	0.30	270	0.27
	intensive N	214	0.20	800	0.37	158	0.21
	average	304	0.23	586	0.33	236	0.26

The determination of diosgenin was carried out by the external standard method with diosgenin as the external standard. The calibration curve for diosgenin was measured by analysing the standard solutions (0.1–1.0 mg/ml), six times for each standard. The signal was linear in the whole range of measured concentrations (regression coefficient, 0.9998; standard error for diosgenin, 0.377). The relative standard deviation of the peak areas was 3.168%. The quantification limit was 36.2 µg/ml. The concentration (mg/ml) of diosgenin in each of the samples was calculated by comparing the areas under the peaks of the analytes with those of the external standard.

RESULTS

Seed yield

In the year 1995 the yield was higher in Rakican than in Vinjole, which could be the consequence of the dry Mediterranean climate in Vinjole. The interaction between cultivar and fertilizing was not statistically significant ($\alpha = 0.05$). In Rakican and in Vinjole there were statistically significant differences between the cultivars; in Vinjole the fertilizing differences were also statistically significant. In Vinjole, cv. Ionia had the highest yield (438 kg/ha), followed by cv. Barbara (384 kg/ha), cv. Margaret (288 kg/ha) and cv. Paul (304 kg/ha). Between the last two there was no statistically significant difference ($\alpha = 0.05$). The control and R-substrate treatments did not differ significantly, and were better than optimal and intensive fertilizing. In Rakican the best yield was obtained with cv. Margaret (1810 kg/ha) and cv. Paul (1624 kg/ha). The quantities of seed yield are shown in Tables 2 and 3.

The interaction between cultivar and fertilizing in the year 1996 was statistically significant ($\alpha = 0.05$) in both places. The yield rose with increased nutrition. Most cultivars had the same yield on R-substrate and optimal fertilizing, worse under control treatment, and the best with intensive fertilizing.

In Vinjole, in the year 1997, the interaction between cultivar and fertilizing was statistically significant ($\alpha = 0.05$). Most cultivars had an equal yield on R-substrate and optimal fertilization. However, it is not yet clear what influence the fertilizers have on the seed yield. The only statistically significant difference in Rakican was between the cultivars.

Diosgenin content

The diosgenin content was not influenced by fertilizing in the years 1995, 1996 and 1997 in Rakican. It was influenced only in Vinjole in 1995, when the statistically significant ($\alpha = 0.05$) lowest yield was observed in intensive treatment. The differences were noted only between the cultivars. Cv. Paul (0.23–0.35%) and cv. Margaret (0.21–0.35%) had the highest average diosgenin content. Cv. Barbara had 0.19–0.30% of diosgenin, and cv. Ionia

Table 3. Average seed yield (kg/ha) and their diosgenin content (% dw) in fenugreek cultivars in Rakican (Slovenia) in the years 1995, 1996 and 1997

		1995		1996		1997	
		yield (kg/ha)	diosgenin (%)	yield (kg/ha)	diosgenin (%)	yield (kg/ha)	diosgenin (%)
Margaret	control	1723	0.29	75	0.29	422	0.34
	organic	1753	0.26	124	0.31	462	0.35
	optimal N	1710	0.27	146	0.31	420	0.30
	intensive N	2054	0.29	303	0.32	435	0.31
	average	1810	0.28	162	0.31	435	0.33
Ionia	control	1224	0.25	94	0.27	478	0.31
	organic	1381	0.24	99	0.29	451	0.30
	optimal N	1647	0.23	130	0.24	384	0.28
	intensive N	1780	0.24	92	0.25	438	0.24
	average	1508	0.24	104	0.26	438	0.28
Barbara	control	1304	0.28	56	0.26	651	0.32
	organic	1234	0.29	86	0.33	649	0.35
	optimal N	1430	0.31	86	0.30	1041	0.33
	intensive N	1673	0.30	159	0.29	780	0.30
	average	1410	0.3	97	0.3	780	0.33
Paul	control	1298	0.34	79	0.33	381	0.38
	organic	1544	0.28	152	0.29	558	0.34
	optimal N	2171	0.27	211	0.32	392	0.36
	intensive N	1482	0.31	163	0.33	444	0.32
	average	1624	0.30	151	0.32	444	0.35

contained the lowest quantity of diosgenin (0.20–0.27%). The content was higher in Rakican than in Vinjole in 1995 and 1997. Presumably this is a consequence of humid weather during the ripening period in Rakican. The contents of diosgenin are shown in Tables 2 and 3.

Diosgenin content and seed yield

Diosgenin yields per hectare are presented in Tables 4–9. The interactions between fertilizing and cultivar were significantly different for all three years in Vinjole, but only in 1996 in Rakican. Cv. Barbara and cv. Ionia had high seed yield and low diosgenin content. The difference between the diosgenin content in better and worse cultivars was not so markedly pronounced as the difference in seed yield. Consenquently, it is better to use the cultivars with high seed yield than those with high diosgenin contents (cv. Margaret, cv. Paul). Cv. Paul had the highest yield of diosgenin (5 kg/ha) with optimal fertilization, and cv. Margaret with intensive fertilization in Rakican in 1995. The lowest yield (0.15 kg/ha) was observed with control treatment in Rakican in 1996.

The yield of diosgenin was higher in Rakican than in Vinjole. The statistically significant differences are shown in Tables 4–9.

DISCUSSION

Our hypothesis that the content of diosgenin depended on fertilizing proved to be incorrect. There was no evidence that R-substrate or mineral fertilizers do increase the diosgenin content.

The field experiment embraced a three-year period, and also three different weather conditions in two pedologically different places. As mentioned in the introduction, ecological factors also influenced secondary metabolite production. The oscillations in seed yield and the contents of diosgenin were mainly influenced by climatological conditions, and special by water availability. A particularly unfavorable year (with respect to precipita-

Table 4. Diosgenin yield (kg/ha) and statistically significant difference in Vinjole in 1995

	Control	5%	Organic	5%	Optimal N	5%	Intensive N	5%
Margaret	0.66	a	0.68	a	0.61	a	0.42	a
5%	a		a		ab		b	
Ionia	1.14	b	0.93	b	0.75	a	0.71	b
5%	a		ab		b		b	
Barbara	0.80	a	0.80	ab	0.67	a	0.66	b
5%	a		a		a		a	
Paul	0.78	a	0.97	b	0.60	a	0.43	a
5%	ab		a		b		b	

Table 5. Diosgenin yield (kg/ha) and statistically significant difference in Vinjole in 1996

	Control	5%	Organic	5%	Optimal N	5%	Intensive N	5%
Margaret	1.38	a	1.83	ab	2.18	a	2.33	a
5%	a		b		c		c	
Ionia	0.68	b	1.72	a	2.42	a	2.10	b
5%	a		b		c		b	
Barbara	1.10	c	1.77	ab	1.86	b	2.03	b
5%	a		b		b		b	
Paul	1.25	c	2.00	b	1.71	b	2.96	c
5%	a		b		b		c	

Table 6. Diosgenin yield (kg/ha) and statistically significant difference in Vinjole in 1997

	Control	5%	Organic	5%	Optimal N	5%	Intensive N	5%
Margaret	0.54	a	1.09	a	0.41	ac	0.31	a
5%	ab		a		b		b	
Ionia	0.75	a	0.97	ab	1.15	ab	0,86	ab
5%	a		a		a		a	
Barbara	0.83	a	0.80	ab	1.08	b	0,89	b
5%	a		a		a		a	
Paul	0.77	a	0.68	b	0.86	c	0,52	a
5%	a		a		a		a	

Table 7. Diosgenin yield (kg/ha) and statistically significant difference in Rakican in 1995

	Average	5%	Margaret	Paul	Barbara	Ionia
Margaret	5.03	a	0	0.23	0.86*	1.42*
Paul	4.80	a		0	0.63*	1.19*
Barbara	4.17	b			0	0.56
Ionia	3.61	c				0

tion) was 1996, with high rainfall (Figure 2) in the maturation period. The seed yield and the contents of diosgenin in Rakican were very low in that year. The conditions were better in Vinjole, which can be explained by the earlier sowing and more favorable weather conditions during the ripening period. In the Mediterranean area intensive fertilizing increased the seed yield only in 1996, when precipitation in growth seasons was higher than in the other two years.

The contents of diosgenin with regard to fertilizing were significantly different only in Vinjole in 1995. This could be attributed to the dry second part of the growing period. Growth was increased by intensive application of nitrogen during the first part of the growing period. Taller plants are more sensitive to drought; they need more water, due to higher evaporation (Larcher 1995). Our results are in accordance with those of Bhati et al. (1980) and Randwana (1996), who reported that the quantity of applied nitrogen does not influence the contents of diosgenin.

The absence of a statistically significant difference in seed yield with different fertilization in Rakican was probably a result of the high contents of organic matter in the soil (2.3–3%), leading to the conclusion that fenugreek cultivated on soil rich in organic matter does not need any additional fertilizing.

The yield of diosgenin was not higher when more nitrogen was applied. This is probably because the diosgenin does not include nitrogen. The surplus of nitrogen (NO_3^-) is probably converted into other secondary metabolites.

We may, therefore, conclude that high nitrogen fertilizing (170 kg/ha) does not affect the seed yield or the diosgenin contents. With the addition of mature organic manure or 30 kg N/ha in the form of mineral fertilizer, we can increase the yield of fenugreek seeds. Fertilization with a high dosage of nitrogen can also halt biological fixation with *Rhizobium meliloti* (Desperrier et al. 1985), thus increasing the production costs and presenting the danger of pollution of the groundwater.

The study indicates that the water and its interaction with fertilization has a significant impact on the production of fenugreek and the contents of diosgenin. This is in accordance with the finding, that water availability increases the seed yield of fenugreek (Elujoba 1987). The results of a recent study have also confirmed that the optimal irrigation regime is essential for the high yield and quality of the crude drug (*Semen Foenugraeci*).

CONCLUSION

Cv. Ionia and cv. Barbara were proved to be most convenient cultivars for growing fenugreek in open fields irrespective of seed yield or diosgenin yield. The fertilizing with a high dosage (170 kg/ha) of nitrogen has no influence on the seed yield or on the diosgenin contents.

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Table 8. Diosgenin yield (kg/ha) and statistically significant difference in Rakican in 1996

	Control	5%	Organic	5%	Optimal N	5%	Intensive N	5%
Margaret	0.22	a	0.39	a	0.45	a	0.97	a
5%	a		a		a		c	
Ionia	0.25	a	0.29	a	0.31	ab	0.23	b
5%	a		a		a		a	
Barbara	0.15	a	0.33	a	0.26	b	0.45	c
5%	a		bc		ab		c	
Paul	0.26	a	0.43	a	0.68	c	0.54	c
5%	c		a		c		b	

Table 9. Diosgenin yield (kg/ha) and statistically significant difference in Rakican in 1997

	Average	5%	Margaret	Paul	Barbara	Ionia
Barbara	2.60	a	0	1.02*	1.16*	1.3*
Paul	1.58	b		0	0.14	0.28
Margaret	1.44	b			0	0.14
Ionia	1.30	b				0

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ABSTRAKT

Vliv hnojení na výnos pískavice řecké seno (*Trigonella foenum-graecum* L.) a na obsah diosgeninu v rostlinné droze

V tříletém polním pokusu na dvou klimatologicky a pedologicky odlišných lokalitách ve Slovinsku (Rakican, Vinjole) jsme sledovali vliv hnojení na výnos semene a na obsah diosgeninu u čtyř odrůd (Margaret, Ionia, Barbara, Paul) pískavice řecké seno (*Trigonella foenum-graecum* L.). U všech čtyř odrůd jsme porovnávali výnosy semene a obsah diosgeninu po použití různých režimů hnojení (organického hnojení bez přídavku dusíkatých hnojiv, optimálního hnojení minerálním dusíkem, vysokých dávek dusíkatých hnojiv a kontrolní varianty bez hnojení). Ve srovnání s kontrolní variantou výnos semene nijak neovlivnily vysoké dávky dusíkatého hnojení (170 kg/ha). Na chudé půdě se výnos semene zvýšil po použití dávky 30 kg N/ha (optimální dávka dusíkatého hnojení) nebo organické hmoty (43 t/ha hnojiva R-substrat). Různé režimy hnojení neměly významný vliv na obsah diosgeninu zjišťovaný plynovou chromatografií ($\alpha = 0{,}01$). Nejnižší obsah diosgeninu jsme zjistili u odrůdy Ionia, nejvyšší u odrůdy Margaret a u odrůdy Paul, která zároveň vykazovala nejvyšší absolutní hmotnost semene. Hektarový výnos diosgeninu lze zvýšit pouze selekcí odrůdy s vysokým výnosem semene (Ionia, Barbara).

Klíčová slova: polní pokus; saponiny; léčivé rostliny; sekundární metabolity; *Leguminosae*

Corresponding author:

Alenka Zupančič, Ms.C.Agr., University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, 1111 Ljubljana, Slovenia, tel.: + 386 1 423 11 61, fax: + 386 1 423 10 88, e-mail: Alenka.Zupan@uni-lj.si

Variability of chemical properties of sage (*Salvia officinalis* L.)

S. Dražić, D. Brkić

Institute for Medicinal Plant Research, Dr. Josif Pančić, Belgrade, Yugoslavia

ABSTRACT

The content of essential oil and its more important constituents (α-thujone, β-thujone, camphor, 1,8 cineole, α-pinene, camphene, β-pinene, β-caryophyllene, α-humulene, viridiflorol, manool) was monitored during two years. These properties varied from 4.7% (α-thujone) to 28% (β-pinene). Effects of a year were very significant on observed properties. Different values of interrelationship were detected among chemical properties.

Keywords: sage; chemical properties; variability; interrelationship

Usability of sage as a row material is an expression of genetic constitution of the plant itself and impacts of environmental factors. The studies showed that there was a spectrum of genetic variation of principal constituents of essential oil. This could be a source of germplasm to be used in the development of improved cultivars (Franz 1990; Dražić 1999).

Proportion of hereditary and non-hereditary component on the amount of essential oil is different. Studies of relations between yield and amount of essential yields, on one hand, and growing conditions on the other, point out to essential oil content as more stable property, meaning that its variability is more regulated by genetic factors (Dražić and Šurlan 1991).

MATERIAL AND METHODS

Primorska, this domestic population is derived on the basis of the autochtonous material (origin: Monte Negro and south Herzegovina) that expressed wide adaptability, hence it is successfully grown in northern regions of Serbia (Dražić 1999). The plant height of the population is 50–70 cm. Comparing with sage indigenous to these regions, leaves of these populations are larger (6–8 × 1.5–2.5 cm) with poorer hair density and more intensive green colour. The flower corollas are pale violet (Stepanović 1999).

The crop development was performed by either planting of 1–2 kg seeds.ha^{-1} (graded seed is recommended) at the beginning of April or by planting of seedlings in October. The duration of a growing season amounts to approximately 60 days. Flowering starts at the end of May and runs on the for about 30 days. This population can be grown on different types of soil and is tolerant to drought and low temperatures. Lack of light and warmth during the growing season is unfavourable for the essential oil content. The yield of dry herb, dry leaves and essential oil amounts to 4–6 t.ha^{-1}, 2–3 t.ha^{-1} and 20–30 kg.ha^{-1}, respectively. A portion of leaves in the herb amounts to about 60% (Dražić 1997, 1998, 1999).

The trial with the Primorska and four replications was carried our in the experimental filed of the Institute in Pančevo during 1995 and 1996 (Dražić 1999). The elementary plot size was 26 m^2. Planting was done in October with a spacing of 70 × 30 cm. Cutting was performed in the 3rd decade of July and the 2nd decade of September (two harvests). Common sage growing practices was applied. Qualitative traits were determined in samples of dry leaves. Oil content (%) was done after Clevenger in accordance with Ph. Jug. IV (1984). Chemical content of essential oil was gas-chromatographically determined by the application of GC-FID and GC-MS techniques. The following oil constituents were analysed: α-thujone, β-thujone, camphor, 1,8 cineole, α-pinene, camphene, β-pinene, β-caryophyllene, α-humulene, viridiflorol and manool.

Furthermore, the following parameters were estimated: mean (x), standard error of arithmetic mean (S_x), standard deviation ($s.d.$) and coefficient of variation (CV%). Results were processed by the factorial analysis of variance. The interrelation of the observed components was determined by simple correlations.

Conditions under which the trial was conducted. – Pančevo is situated under conditions of semi-arid climate. Experimental plot soil belongs to marshy black soils. The average annual temperature amounts to 11.3°C, while the vegetation temperature (the temperature of the period April to September) amounts to 18.2°C. The precipitation sum amounts to 380 mm, i.e. 664 mm, for the vegetation period, i.e. the whole year, respectively.

RESULTS AND DISCUSSION

According to parameters of variability, standard deviation and coefficient of variation, the greatest variation (CV = 28%) and variation range (0.41–2.4%) was detected in the proportion of (β-pinene). On the other hand, the lowest values of these parameters were obtained for α-thujone (CV = 4.7%; 33.93–38.89%) (Table 1).

The average essential oil content in sage leaves amounted to 1.15%, which is not in accordance to the

Table 1. Mean values and variability of observed traits

No.	Components	$x \pm S_x$	min	max	s.d.	CV(%)
1.	essential oil	1.15 ± 0.68	1.02	1.26	0.1	8.7
2.	α-thujone	36.00 ± 0.36	33.93	38.89	1.68	4.7
3.	β-thujone	4.00 ± 0.28	3.02	5.31	0.87	22.0
4.	camphor	12.8 ± 0.33	12.01	14.10	0.7	5.5
5.	1,8-cineole	9.4 ± 0.04	8.59	10.80	0.81	8.6
6.	α-pinene	1.63 ± 0.16	0.88	3.12	0.1	19.4
7.	camphene	1.64 ± 0.17	1.01	2.78	0.38	23.8
8.	β-pinene	1.47 ± 0.13	0.41	2.40	0.41	28.0
9.	β-caryophyllene	1.28 ± 0.43	0.56	1.96	0.33	26.0
10.	α-humulene	4.94 ± 0.5	3.54	6.18	1.05	21.2
11.	viridiflorol	7.9 ± 0.8	6.3	9.24	1.18	15.0
12.	manool	7.1 ± 0.05	4.47	10.46	1.94	27.4

requirements (the lowest percentage of essential oil is 1.5%) of the standard Ph. Jug. IV (1984). Obtained values for a small range of variation of essential oil are in accordance with literature data (Dražić and Šurlan 1991; Dražić 1997; Aiello et al. 1998). Monitoring proportion of essential oil Dražić (1997), Adamović (1998), Stoeva and Bosseva (1998), Aiello et al. (1998) detected contents higher than the values obtained in the present study. The analysis of variance shows that the effects of a year were very significant on this trait (Table 2).

By observing a possibility for establishing quantitative relations between meteorological factors and essential oil amounts Dražić (1997) found out that temperature contribution amounted to 21.8–44.3% of variation. Precipitation mostly adversely affected essential oil amounts. A small proportion of their effects (5.2–22.7%) indicates more expressed contribution of all other factors.

Based on data for variability, high values of coefficient of variation were detected for the content of β-caryophyllene, manool, camphene, β-thujone and α-humulene (Table 1). These constituents of oil had large variation range. A year significantly affected the proportion of 1,8 cineole, camphene, β-pinene, and manool (Table 2), which is in accordance with the data stated by Adamović (1998).

According to the standard Ph. Jug. IV (1984) sage oil should contain at least 40% of thujone ketones. The proportion of thujone in these studies was equal to the standard. Based on parameters of variation this proportion is much greater (4%) for β-thujone (Table 1). Based on long-term investigations of thujone proportion in several sage genotypes Adamović (1998) and Aiello et al. (1998) determined a variation range of 27–54% and 30–45%, respectively. Stoeva and Bosseva (1998) point out that the average proportion of thujone in sage (*Salvia officinalis* L.) was 47%, which is a higher value than the one obtained in this study (Table 1).

The analysis of correlation coefficients (r) shows that α-pinene and camphene, as well as α-pinene and β-pinene were positively and very significantly correlated (0.995** and 0.915**). Furthermore, high significant interrelation was detected between β-pinene and camphene (0.949**). Interrelation between essential oil content and β-caryophyllene, 1,8 cineole and α-pinene and between 1,8 cineole and camphene was positive and significant, offering possibility to selection required genotypes (Table 3).

A very significant and negative correlation was determined between β-thujone and α-humulene, as well as between β-thujone and manool. Moreover, highly significant and negative correlation was detected between viridiflorol, on one hand, and α-pinene, camphene and α-humulene, on the other hand.

A positive, but statistically insignificant interrelation prevailed among other observed properties (Table 3).

Table 2. Two-factorial analysis of variance

Source of variation	d.f.	MS											
		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Components	11	0.0002	1.281**	0.196	0.161	0.06*	0.01	0.006	0.0024	0.057	0.43**	0.06	0.82
Year	1	0.0077**	0.033	0.09	0.234	3.03**	4.63**	3.53*	2.34**	0.34	0.11	6.87	5.67*
Interaction	11	0.0013	0.248	0.21	0.081	0.03	0.14	0.7	0.02	0.13	0.12	0.07	0.74
Total	23												

* and ** significant at 0.05 and 0.01 probability level

1. essential oil, 2. α-thujone, 3. β-thujone, 4. camphor, 5. 1,8 cineole, 6. α-pinene, 7. camphene, 8. β-pinene, 9. β-caryophyllene, 10. α-humulene, 11. viridiflorol, 12. manool

Table 3. Correlation coefficients (r) between analysed traits

No.	Trait	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
1.	essential oil	0.024	−0.140	−0.053	0.281	0.121	0.098	0.140	0.801*	0.020	−0.265	−0.196
2.	α-thujone	–	0.426	−0.755	0.072	−0.061	−0.013	0.101	0.571	−0.572	0.171	−0.561
3.	β-thujone	–	–	0.239	0.460	0.385	0.346	0.133	0.027	−0.903**	−0.190	−0.875**
4.	camphor	–	–	–	0.521	0.516	0.444	0.192	−0.522	0.049	−0.511	−0.097
5.	1,8-cineole	–	–	–	–	0.822*	0.810*	0.693	0.237	−0.205	−0.782	−0.634
6.	α-pinene	–	–	–	–	–	0.995**	0.915**	0.048	0.001	−0.973	−0.618
7.	camphene	–	–	–	–	–	–	0.949**	0.073	0.035	−0.968	−0.601
8.	β-pinene	–	–	–	–	–	–	–	0.208	0.206	−0.923**	−0.408
9.	β-caryophyllene	–	–	–	–	–	–	–	–	0.213	−0.111	−0.396
10.	β-humulene	–	–	–	–	–	–	–	–	–	−0.179	0.746
11.	viridiflorol	–	–	–	–	–	–	–	–	–	–	0.484
12.	manool	–	–	–	–	–	–	–	–	–	–	–

* and ** significant at the 0.05 and 0.01 probability level

CONCLUSION

Performed investigations show high variability of chemical properties in sage. The variation range, i.e. large maximum and minimum limits, detected for: β-pinene, β-caryophyllene, manool, camphene, β-thujone and α-humulene, could be of a practical importance in sage breeding and selection. Besides, a significant effect of a year on the expression of chemical parameters of oil quality was observed. Prevalence of the negative and weak correlation aggravates selection of more qualitative genotypes of this medicinal plant.

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ABSTRAKT

Variabilita chemických vlastností šalvěje (*Salvia officinalis* L.)

Po dobu dvou let jsme sledovali obsah silice a jejích nejdůležitějších složek (α-thujonu, β-thujonu, kafru, 1,8 cineolu, α-pinenu, kamfenu, β-pinenu, β-karyofylenu, α-humulenu, viridiflorolu, manoolu). Obsahy těchto složek kolísaly od 4,7 % (α-thujon) do 28 % (β-pinen). Vliv ročníku na sledované vlastnosti byl velmi významný. Mezi chemickými vlastnostmi jsme zjistili rozdílné hodnoty vzájemných závislostí.

Klíčová slova: šalvěj; chemické vlastnosti; variabilita; vzájemné závislosti

Corresponding author:

Dr. Slobodan Dražić, Institut za proučavanje lekovitog bilja Dr. Josif Pančić, Tadeuša Košćuška 1, 11000 Belgrade, Yugoslavia, tel.: + 381 11 30 31 656, fax: + 381 11 30 31 649, e-mail: iplb@sezampro.yu

Regeneration of *Rumex obtusifolius* L. after cutting

Z. Martinková, A. Honěk

Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

ABSTRACT

The time of spring sprouting of fertile shoots in individual plants of *Rumex obtusifolius* L. from a population at Prague-Ruzyně varied by more than 30 days. We investigated the effect of this variation on regrowth and reproduction after cutting of rosette leaves and fertile shoots in June or July. The number of plants which regenerated fertile shoots and the number of shoots regenerated per plant increased with the original (before cutting) date of sprouting. In uncut plants, the achenes matured in early August. Although sprouting date between these plants differred by one month, the average date of maturation varied only by about 12 days. Cutting postponed achene maturation by roughly one month and eliminated the significant covariation between sprouting and maturation date. Individual differences in seasonality persisted between years, but cutting advanced sprouting of fertile shoots in the following year. The individual variation in seasonality may vary susceptibility of *R. obtusifolius* plants to grassland and pasture management regimes.

Keywords: *Rumex obtusifolius*; seasonality; cutting; regrowth; plant size; reproduction; seed maturation

In the Czech Republic, the broadleaved dock *Rumex obtusifolius* L. is among three most important weeds of pastures and grasslands, together with *Cirsium arvense* (L.) Scopoli and *Taraxacum officinale* agg. (Mikulka et al. 1996). Increased abundance of broadleaved dock is favoured by extensive farming methods which often caused gradual deterioration of grassland quality. The converting of arable land into pastures with intensive cattle grazing has promoted the spread of broadleaved dock. Reproduction of *R. obtusifolius* takes place mostly by seeds and rarely by regeneration from split roots (Dierauer and Thomas 1994; Pino et al. 1995). Germinating plants may withstand intraspecific competition as well as competition with surrounding weeds or grass stand (Kobayashi et al. 1989; Nashiki et al. 1989, 1992; Nashiki 1995; Pino et al. 1995). Removing or destroying achenes before maturation thus substantially decrease the chance of *R. obtusifolius* spreading. Although viable *Rumex* achenes may ripe also on cut fertile shoots left in the field (Maun 1974), cutting significantly contributes to decreasing of achene production and dispersal (Hughes et al. 1993; Pino et al. 1994). Removal of fertile parts before reproduction together with manipulating nutrient content of soil (Novák 1995a) and sowing competitive plants (Novák 1995b) are the principal non-chemical means of preventing *R. obtusifolius* spreading.

The effect of cutting on *Rumex* performance was extensively studied. It generally decreases plant fitness and performance and, in combination with competition of surrounding grass, may reduce *Rumex* abundance (Hughes et al. 1993; Niggli et al. 1993). The negative consequences of cutting are greater in young plants established in the same year than in old plants. However, cutting also promotes branching (Hughes et al. 1993; Pino et al. 1995) and in absence of competition may even increase dock abundance (Aquilina and Clarke 1994). Biochemical composition of intact and regrown shoots is different (Nashiki et al. 1998).

Clearly, cutting progressively decreases size and achene production of the regrown plants as its date is postponed. However, even in simultaneously cut plants there is a large variation in the intensity of regrowth. The causes of this individual variation were not explained. Our studies (Martinková and Honěk 2000) revealed an important effect of seasonality on several parameters of *R. obtusifolius* life history. These effects include also the intensity of regrowth after cutting. In this work we report the study made in an experimental *R. obtusifolius* stand at Prague-Ruzyně, in 1998–1999. Its protected position enabled two year investigation of seasonality of individual plants and their response to cutting. We deal with the effects of seasonality on immediate and delayed consequences of cutting for *R. obtusifolius* growth and maturation.

MATERIAL AND METHODS

Experimental plants

The experimental area was established in 1998 at Prague-Ruzyně (50°06' N, 14°16'E, altitude 340 m a.s.l.) in a natural *R. obtusifolius* stand persisting at the locality for about 10 years. The 20 × 20 m area with roughly 300 *R. obtusifolius* plants was fenced and removed from ordinary grassland management. Previously, the sward was cut once or twice per year, last time on May 20, 1997. In the experiment plot grass and broad leaved weeds (except *Rumex*) were cut in approximately three weeks intervals, dry remnants of *Rumex* plants were removed in late February. In 1998 and 1999, 218 experimental dock plants were marked by wooden labels and included into two experiments.

Table 1. Two-way ANOVA of the effect of early and late sprouting and date of cutting on number of fertile shoots in regrown plants of experiment (i)

	Effect			Error			F	p
	SS	*df*	*MS*	*SS*	*df*	*MS*		
Sprouting	28.650	1	28.650	224.053	40	5.601	5.115	0.0292
Cutting	21.678	2	10.839	224.053	40	5.601	1.935	0.1577
Interaction	11.717	2	5.859	224.053	40	5.601	1.046	0.3608

Experiment (i). In 1998, experimental plants were divided into two groups: plants whose fertile shoots appeared before May 20 (early sprouting) and plants whose fertile shoots appeared after this date (late sprouting). We investigated the effect of cutting on number of regrown fertile shoots. Ten early and 10 late sprouting plants were cut on each of following dates, June 15, June 30, and July 21. The numbers of regrown shoots were counted on January 9, 1999. The control consisted of 15 early and 15 late sprouting plants grown intact, whose shoots were counted on September 25, 1998. The experiment was continued in 1999 by weekly recording (see below) the date of sprouting of cut and intact plants.

Experiment (ii). In 1999, sprouting was recorded in weekly intervals. Experimental plants were assorted according to the time of sprouting into five groups, plants that sprouted before May 10, on May 11–17, May 18–24, May 25–31, and June 1–7. Ten plants of each sprouting date were cut on June 21. The control was a group of 78 control intact plants, where 13 to 23 plants were labelled on each date, when sprouting was recorded. The course of maturation of fertile shoots in both intact and regrown plants was recorded in 14-day intervals starting from July 27. The maturity was evaluated according to seven degree scale (Martinková and Honěk unpubl.), where full maturity was marked by brown seed testa, dark brown and desiccated perianth, dead leaves of fertile shoots, and stems dried at full length. The number of mature fertile shoots was recorded, in control plants on September 22, in cut and regrown plants on October 5, 1999.

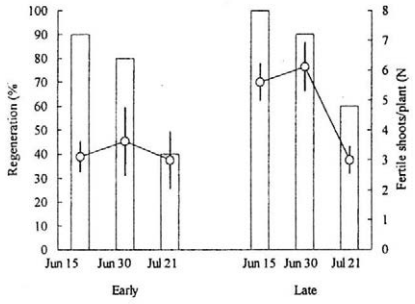

Figure 1. The proportion of plants with regenerated fertile shoots (bars, left ordinate) and average numbers (±*SE*) of fertile shoots (symbols, right ordinate) in early sprouting (before May 20) and late sprouting plants (after May 20); 10 early and 10 late plants were on each of three dates indicated on the abscissa

Data elaboration

The differences between cut and control plants were evaluated according to (a) frequency of plants which did not regrow fertile shoots after cutting and (b) number of mature fertile shoot per individual in plants which regenerated shoots. Means ± standard errors (*SE*) are indicated throughout the text. For statistical analysis the data were not transformed. The significance of differences between means was tested by one-way or two-way ANOVA, with date of sprouting and/or date of cutting as factors and data of individual plants as replicates. All calculations were made using STATISTICA® for Windows (StatSoft 1994).

RESULTS

Regrowth and seed maturation

Experiment (i). In 1998, plants cut late produced less regrowth (Figure 1). The proportion of plants that regenerated fertile shoots as well as average number of shoots per regenerated plant were smaller in early sprouting (70% regenerated, 3.3 ± 0.5 shoots) than late sprouting plants (83% regenerated, 8.9 ± 0.5 shoots). The two-way ANOVA revealed only a significant ($p = 0.029$) effect of time of fertile shoot sprouting on the variance of final shoot number (Table 1). In control plants the average number of shoots was 11.5 ± 0.7 and the difference between early and late sprouting plants was not significant (one-way ANOVA: $df_{effect} = 1, F = 2.316, p = 0.1401$). With increasing cutting date decreased the proportion of plants which regenerated reproductive growth (from 95% following cutting on June 15 to 50% following cutting on July 21) as well as number of fertile shoots per regenerated plant (Figure 1). In early sprouting plants average number of fertile shoots per regrown plant was not significantly affected by cutting date. In late sprouting plants average number decreased from 5.6 ± 0.6 following cutting on June 15 to 3.0 ± 0.5 following cutting on July 21 (one-way ANOVA: $df_{effect} = 2, F = 3.9775, p = 0.0335$). The overall effect of cutting date on number of fertile shoots was, however, not statistically significant (Table 1).

Experiment (ii). The proportion of plants with regenerated fertile shoots increased with date of shoot sprouting (Figure 2) from 40% in plants sprouting on May 10 to 100% in plants sprouting on May 24 and June 7. The number of

Table 2. One-way ANOVA of differences between shoot number of plants sprouting on May 10 and 17 and plants sprouting on May 24 to June 7 in experiment (ii); ANOVA was calculated separately for plants cut on June 21 (cutting) and those left intact (control)

Plants	Effect			Error			F	p
	SS	*df*	*MS*	*SS*	*df*	*MS*		
Control	22.4	1	22.4	4259.0	75	56.79	0.395	0.5314
Cutting	152.2	1	152.2	1001.4	37	27.06	5.623	0.0230

Table 3. One-way ANOVA of the variation in the date of fertile shoot maturation as a function of the date of sprouting; ANOVA was calculated separately for plants cut on June 21 (cutting) and those left intact (control)

Plants	Effect			Error			F	p
	SS	*df*	*MS*	*SS*	*df*	*MS*		
Control	1266.4	4	316.6	2491.9	72	34.61	9.1477	0.0000
Cutting	677.7	4	169.4	2928.4	29	100.98	1.6778	0.1821

shoots regrown in early (May 10 and 17) sprouting plants (3.2 ± 0.6) contrasted with numbers regrown by late (May 24 to June 7) sprouting plants (7.7 ± 1.1). The difference between plants sprouting before May 17 and after May 24 was significant in cut plants, but not in controls (Table 2), where average shoot number was 15.1 ± 2.7. Average shoot numbers in groups of control plants sprouting on particular dates varied between 13.3 ± 2.7 and 19.1 ± 2.2 with a maximum in plants sprouting on May 24. However, the differences between sprouting dates were not significant due to a large variability of shoot numbers (one-way ANOVA: $df_{effect} = 4, F = 1.3306, p = 0.2669$). The average date of maturation in groups of control plants increased with the date of their sprouting (Figure 3) and the differences were highly significant (Table 3). However, the magnitude of original differences in seasonality between groups of control plants was decreased during the period of shoot development and maturation. While the sprouting dates in the spring varied by approximately 28 days, the average dates of maturation (July 31 to August 12) varied by only 12 days. Cutting postponed the time of maturation by roughly one month (Figure 3). Although all plants were cut on the same day (June 21), average dates of maturation for groups of plants sprouted on particular dates also differed by 12 days (August 29 to September 11). There was a tendency to mature earlier in plants which sprouted later on, but the differences between groups of plants sprouting on particular days were statistically not significant (Table 3).

Delayed effects on shoot sprouting

Experiment (i). In 1999, the differences between early and late sprouting plants persisted in 1999 (Figure 4). The time of sprouting varied also according to whether the plants were cut or left intact in 1998. The differences were significant (Table 4) in the early sprouting plants, where plants cut in 1998 sprouted in the average by six days earlier (May 13) than the control plants (May 19). The difference in late sprouting plants was only two days, being not statistically significant.

DISCUSSION

Regrowth. In control plants of this study timing of spring sprouting did not significantly affect the number of

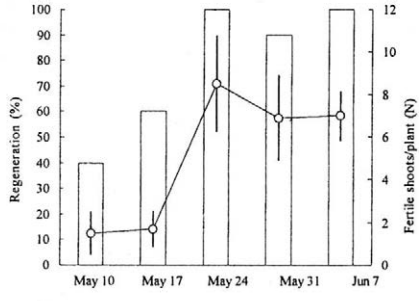

Figure 2. The proportion of plants which regenerated fertile shoots (bars, left ordinate) and average numbers (±*SE*) of fertile shoots (symbols, right ordinate) in cut plants (squares) and intact controls (circles); the abscissa indicates the date of sprouting of the plant cohorts

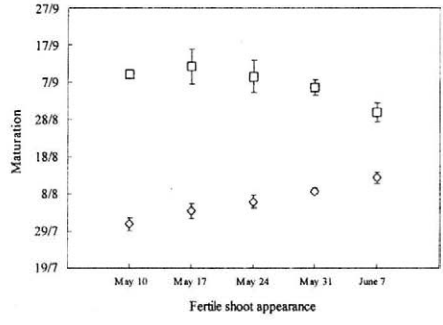

Figure 3. The average date (±*SE*) of maturation of intact control plants (circles) and plants cut on June 21 (squares); the abscissa indicates the date of sprouting of the plant cohorts

Table 4. One-way ANOVA of the effect of cutting in 1998 on the time of fertile shoot sprouting in 1999; the effect was tested separately for early (before May 24, 1998) sprouting and late (after this date) sprouting plants

Sprouting	Effect			Error			*F*	*p*
	SS	*df*	*MS*	*SS*	*df*	*MS*		
Early	348.5	1	348.5	1729.1	38	45.50	7.6589	0.0087
Late	11.8	1	11.8	752.6	38	19.81	0.5938	0.4457

fertile shoots. This contrasts with other *Rumex* species, where number of shoots decreased in late sprouting cohorts (Sman et al. 1992). The timing of sprouting, however, affected the extent of regrowth and maturation after cutting. Earlier studies revealed that intensity of regrowth in *R. obtusifolius* depends on several factors including the time and frequency of cutting, competition with surrounding plants and position at which the cutting was performed (Nilsson and Halgren 1991; Dierauer and Thomas 1994; Novák 1995a; Hopkins et al. 1997). The influence of seasonality on the process of regrowth was perhaps demonstrated for the first time. The early sprouting plants regenerated less fertile shoots and were more seriously affected by cutting than the late sprouting plants. The moment of transition between early sprouting plants which suffered deleterious effects and late sprouting plants which suffered benign effects was around May 20. The negative effect of cutting increased with the time elapsed between the sprouting and date of cutting.

The variation in timing of spring sprouting of fertile shoots had caused relatively small (though significant) difference in timing of plant maturation. The extent of variation in seasonality thus decreased with the course of season. Curiously, the order of maturation was reversed after cutting. The cut plants which sprouted on later dates matured earlier and were less affected by cutting than early sprouting plants. The effect may be also due to immediate regeneration of late sprouting plants which contrasted with early sprouting plants which ceased sprouting and after cutting required some time to resume this activity.

Body mass and time investments to reproduction may explain the observed differences between early and late

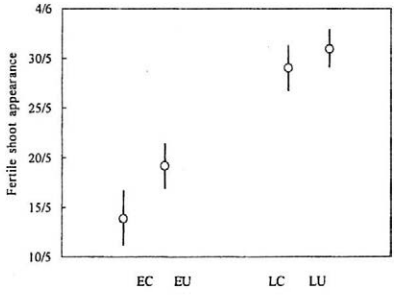

Figure 4. The average date (±*SE*) of fertile shoot appearance (in 1999) in plants that were cut or left intact in 1998; EC – early sprouting cut plants, EU – early sprouting control plants left intact, LC – late sprouting cut plants, LU – late sprouting control plants left intact

plants. The results revealed that the more time a plant has had to invest its resources into flowering, the less likely it was able to recover after cutting and the slower was the maturation once cut. Probably the plants that had a longer time between sprouting and cutting had committed more resources to the flowering shoot, had lost a greater proportion of their total biomass when cut, and therefore had the slowest and poorest regrowth. The most affected early plants postponed the flowering into the next vegetative season.

Delayed effects. The timing of fertile shoot appearance is typical for individual plants and the habit of early or late sprouting was maintained in successive years. However, cutting modified the intrinsic variation and accelerated the sprouting in the following year. Cutting may thus not only promote branching (Hughes et al. 1993; Pino et al. 1995) but also move its timing. Ecophysiological explanation of this effect remains to be studied.

Consequences. In general, the study indicated an important effect of seasonality on plant performance and response to cutting. This type of variation may affect the sensitivity of *R. obtusifolius* plants to agriculture practices. Earlier studies indicated some variability in response of *R. obtusifolius*. Some authors reported suppressive effects of cutting (Novák 1995; Hopkins et al. 1997), absence of negative effects (Nilsson and Halgren 1991; Fisher et al. 1993) or differences in response between populations (Aquilina and Clarke 1994). The early sprouting plants may be more affected by agriculture practices as cutting negatively affects their growth and seed production. On cut meadows we will thus expect the selection against early sprouting plants. The work revealed that seasonality is an important factor of *R. obtusifolius* biology.

Acknowledgements

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ABSTRAKT

Regenerace šťovíku tupolistého (*Rumex obtusifolius* L.) po posekání

Doba jarního rašení fertilních lodyh se u jednotlivých rostlin šťovíku tupolistého (*Rumex obtusifolius* L.) z populace v Praze-Ruzyni lišila o více než 30 dní. Zkoumali jsme vliv těchto rozdílů na obrůstání nových fertilních lodyh a zrání nažek po posekání nadzemních částí v červnu a v červenci. Počet rostlin, které regenerovaly, fertilní lodyhy a počet fertilních lodyh na rostlinu vzrůstal s původním (před posekáním) datem rašení. Nažky nesekaných rostlin zrály v první polovině srpna. Ačkoliv rozdíly v datu jarního rašení byly více než jeden měsíc, rozdíly v průměrném datu zrání byly pouze 12 dní. U rostlin posekaných v červnu a znovu obrostlých bylo zrání nažek zpožděno asi o jeden měsíc a rovněž zmizela pozitivní korelace mezi jarním datem rašení a datem zrání, zjištěná u rostlin neposekaných. Individuální rozdíly v sezonnosti rašení mezi rostlinami přetrvávaly v následujícím roce, avšak sekání v předchozím roce významně urychlovalo jarní rašení v následujícím roce. Individuální variabilita v datu rašení fertilních lodyh šťovíku tupolistého může ovlivnit tvorbu nažek a reprodukci v polních podmínkách.

Klíčová slova: *Rumex obtusifolius*; šťovík tupolistý; sezonnost; sekání; obrůstání; velikost rostlin; reprodukce; zrání nažek

Corresponding author:

Ing. Zdenka Martinková, CSc., Výzkumný ústav rostlinné výroby, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika, tel.: + 420 2 33 02 22 88, fax: + 420 2 33 31 06 36, e-mail: martinkova@vurv.cz

SHORT COMMUNICATION

Identification of weed species using dimensionless analysis of leaves

P. Axman[1], J. Křen[1,2]

[1]*Mendel University of Agriculture and Forestry in Brno, Czech Republic*
[2]*Agricultural Research Institute Kroměříž, Ltd., Czech Republic*

ABSTRACT

To identify weed species in digital images, a dimensionless analysis of leaves was tested at two locations in the Czech Republic with different soil and climatic conditions (Žabčice and Břevenec) in 1999 and 2000. Differences were determined among the following six weed species: *Agropyron repens* (AGRRE), *Chenopodium album* (CHEAL), *Galinsoga parviflora* (GALPA), *Amaranthus retroflexus* (AMARE), *Malva neglecta* (MALNE), and *Convolvulus arvensis* (CONAR). Discrimination was carried out according to the following leaf features: compactness, roundness, elongation, roughness and aspect (ratio of ellipse length and width at the equivalent area). It was possible to distinguish significantly the presented weed species from each other. There was the only case (location Žabčice in 2000) when significance of differences between the species GALPA and CONAR could not be assessed.

Keywords: field weeds; identification; image analysis

Standard methods for assessment of weed infestation in field crops are based on either subjective with visual (scoring) evaluation or are very laborious (count and weight methods). Progress in new technologies provides possibilities of reducing laboriousness and achieving more accurate quantification. One of them is dimensionless analysis of leaf area.

The above-mentioned method enables us to determine some shape features of leaf area, i.e. its elongation, length/width ratio, a number and frequency of indents on blade margin, roundness, etc. The most frequently used features for distinguishing leaves reported in literature are compactness, roundness, elongation, roughness, aspect, and central invariant moment. These features allow us to evaluate characteristics genetically dependent on the species, and thus they are suitable to identify it.

The simplest and least intensive computational method is the use of the outline, or the so-called borders of the leaf (Jain 1989; Gonzales and Woods 1992). To describe leaf area, Kincaid and Schneider (1983) used outlines of individual leaves with normalized Fourier's coefficient, leaf complexity. Woebbecke et al. (1995) identified weeds using the following leaf shape features: roundness, aspect, leaf perimeter length, elongation, and central invariant moment. They report the ability to discriminate between monocotyledons and dicotyledons from 60 to 90% when the most suitable distinguishing features were aspect and central invariant moment. Yonekawa et al. (1996) tested a possibility of discrimination of idealized leaf blades using the shape features compactness, roundness, elongation, lobation, and roughness. They concluded that up to 1280 types of leaf blades can be theoretically distinguished. Guyer et al. (1986) were able to distinguish eight weed species at the error of 10% on the basis of leaf area analysis using the features elongation and compactness. Petry and Kühbauch (1989) distinguished six weed species at different growth stages using up to nine features at the error of about 18%. McDonald and Chen (1990) demonstrated utilization of morphological analysis to distinguish leaves of African violet from *Glechoma* sp., i.e. in species that are very similar in leaves.

Results in the cited studies were obtained under well-prepared model conditions when the plants were carefully displayed in the image. Under these conditions, they confirmed that dimensionless analysis of leaves was able to distinguish standardized blades in selected weed species. Our work was focused on utilization of this method for selected weed species occurring in field crops.

MATERIAL AND METHODS

The evaluation was carried out in 1999 and 2000 on six weed species with different character of leaf area as follows: *Agropyron repens* (AGRRE), *Chenopodium album* (CHEAL), *Galinsoga parviflora* (GALPA), *Amaranthus retroflexus* (AMARE), *Malva neglecta* (MALNE), and *Convolvulus arvensis* (CONAR). The weeds are designated with five-place codes according to the nomenclature of the EWRC – European Weed Research Society (Kohout 1996). The growth stages were assessed according to the decimal BBCH scale (Meier 1997). Selection of the weed species depended on its occurrence at both locations:

– Žabčice, maize-growing region, about 20 km south of Brno, gley fluvisols;
– Břevenec, cereal-growing region, about 30 km north of Olomouc, typical cambisols, saturated and unsaturated (acid) with different skeleton.

At anthesis (BBCH 59-71), leaves without stalks were cut from weed plants and images were immediately taken in dispersed sunlight on white background using a digital camera Olympus 1400. Thirty leaf samples (evaluated as replicates) were taken per weed species from the top part of plants, maximum two leaves per plant.

Data from a memory card of the camera Olympus 1400 were imported to the computer hard disk using Flash Path. The analysis of images was carried out on Pentium 350 MHz computer at the memory size of 128 MB using the Image Pro Plus 3.1 software. If necessary, the images for individual assessments were corrected by thresholding and pseudocolours.

We assessed the features compactness, roundness, elongation, and roughness as reported by Yonekawa et al. (1996), and aspect (Woebbecke et al. 1995). Particular features (Figure 1) were defined as follows:

Compactness (*C*) indicates roundness or compactness in dependence on leaf perimeter and shape. It is defined as:

$$C = \frac{4\pi A}{P^2}$$

C = compactness, A = leaf area, P = leaf perimeter

Roundness (*R*) indicates roundness or compactness relating leaf perimeter and is defined as:

$$R = \frac{4A}{\pi . L^2}$$

A = leaf area, L = maximum leaf length, R = leaf roundness

Elongation (*E*) defines elongation in dependence on leaf length/width ratio:

$$E = \frac{L}{W}$$

E = leaf elongation, L = maximum leaf length, W = maximum leaf width

Aspect (*A*) is defined as a ratio of maximum length and maximum width of ellipse (leaf) with the equivalent area:

$$A = \frac{L_e}{W_e}$$

A = aspect, L_e = maximum ellipse length, W_e = maximum ellipse width

Roughness (*G*) reflects leaf margins in dependence on their irregularities (the crenate, dentate, and others), and is defined as:

$$G = \frac{H}{W}$$

H = leaf perimeter obtained by joining two edge points (convex hull), W = maximum leaf width

Based on assessed numerical values measured by Image Pro Plus 3.1, values of individual features were processed using Microsoft Excel software. Their variability was assessed by coefficients of variation. Then a single factor analysis of variance for statistical evaluation was used where the number of leaf samplings within one species (a number of replicates) was 30. Differences among weeds were calculated by Tukey's test at the significance level of 95%. Unistat 4.53 software was used for all statistical assessments.

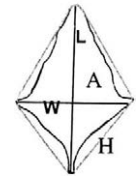

Figure 1. Scheme of measured leaf dimensions

RESULTS

An assumption for distinguishing selected weed species was a significant difference at least in one feature.

Coefficients of variation for the features are given in Table 1 and 3, and mean values in Table 2 and 4. Significance of differences is illustrated using confidence intervals in Figures 2, 4, 6, and 8. The differences are insignificant if a join of the points (in Figures 3, 5, 7, and 9), which characterize individual weed species, converges in one or more common points.

Location Žabčice

Coefficients of variation for measured features were rather low in both years. They exceeded a value of 15% in some cases only (Table 1). AGRRE was the only species that showed high values in leaf elongation in 1999, and particularly in compactness and roundness in 2000. The high variation was caused by a different leaf length in the measured set (up to double in some cases).

Table 2 shows rather good balance of evaluated features in individual weeds except for AGRRE that considerably differs from the other species.

Figures 2 and 3 show that all weeds differed at least in one feature, which confirms applicability of this method for distinguishing evaluated weed species.

Significant differences among individual weed species were assessed in all evaluated shape features also in 2000 (Figure 4). Only the differences between GALPA and CONAR were insignificant, which means that they cannot be distinguished from each other. It was probably due to larger variance in roundness, particularly in CONAR where a coefficient of variation was higher than in the other cases.

Location Břevenec

Out of all locations and years the highest coefficients of variation were assessed for AGRRE in 1999 (Table 3). Variation in leaf length was highest. As the analysis shows (Figures 6 and 7), it did not negatively affect weed discrimination.

Table 1. Coefficients of variation (%) for measured features in weed species at Žabčice

Feature	AGRRE		CHEAL		GALPA		AMARE		MALNE		CONAR	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Aspect	17.6	24.1	19.3	15.2	12.8	12.3	8.9	9.1	4.9	5.3	7.1	12.1
Compactness	17.6	36.7	12.2	8.8	7.1	6.1	5.8	6.9	11.3	5.9	11.1	12.2
Roundness	17.5	35.3	18.0	15.1	12.9	13.7	9.9	10.0	5.0	5.1	9.8	14.2
Elongation	19.6	16.7	16.0	15.6	11.1	12.4	8.9	8.4	5.5	4.8	15.7	11.9
Roughness	2.8	1.7	4.9	5.2	3.8	4.8	2.9	3.1	2.1	2.4	5.6	8.7

Table 2. Means of evaluated features at Žabčice

Weed	Feature									
	aspect		compactness		roundness		elongation		roughness	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
AGRRE	16.64	20.29	0.13	0.11	0.05	0.04	0.06	0.05	2.00	2.00
CHEAL	1.87	1.82	0.52	0.63	0.39	0.45	0.58	0.62	2.38	2.45
GALPA	1.42	1.40	0.75	0.78	0.57	0.59	0.68	0.69	2.60	2.60
AMARE	1.77	1.61	0.74	0.74	0.46	0.52	0.55	0.62	2.40	2.51
MALNE	1.17	1.20	0.67	0.60	0.82	0.83	0.87	0.88	2.98	3.02
CONAR	1.79	1.53	0.52	0.77	0.45	0.61	0.64	0.65	2.74	2.70
LSD	0.52	0.85	0.02	0.02	0.02	0.03	0.03	0.03	0.04	0.05

In 2000, coefficients of variation for individual weed species in all features were mostly lower than 20%. The feature elongation in AGRRE was the only that reached 25%, which was caused by differences in leaf length again (Table 3). But this fact did not influence subsequent discrimination despite of a high coefficient of variation for AGRRE (Figures 6 and 7). All studied weeds could be significantly distinguished at the location Břevenec in both years. Discrimination ability was not influenced by a high coefficient of variation in AGRRE.

Table 3. Coefficients of variation (%) for measured features in weed species at Břevenec

Feature	AGRRE		CHEAL		GALPA		AMARE		MALNE		CONAR	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Aspect	42.7	19.4	42.7	12.5	11.0	8.0	6.8	12.6	12.8	7.5	13.0	12.4
Compactness	154.7	16.6	154.7	14.3	9.9	9.0	7.0	7.7	2.6	10.2	8.8	7.4
Roundness	153.6	16.9	153.6	16.4	13.2	12.5	7.8	12.5	4.2	7.6	13.5	11.9
Elongation	46.9	25.7	46.9	10.6	11.7	8.9	6.1	11.3	4.5	6.7	13.4	13.0
Roughness	1.3	0.5	1.3	4.5	5.1	5.5	2.1	3.6	3.1	3.1	6.1	7.7

Table 4. Means of evaluated features at Břevenec

Weed	Feature									
	aspect		compactness		roundness		elongation		roughness	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
AGRRE	19.67	18.74	0.08	0.11	0.03	0.04	0.06	0.05	2.01	2.01
CHEAL	1.64	1.62	0.59	0.66	0.48	0.50	0.66	0.66	2.50	2.51
GALPA	1.30	1.19	0.73	0.75	0.60	0.67	0.73	0.80	2.62	2.74
AMARE	1.56	1.65	0.72	0.76	0.51	0.51	0.63	0.61	2.49	2.48
MALNE	1.30	1.22	0.59	0.62	0.73	0.79	0.82	0.87	2.91	3.00
CONAR	1.78	1.72	0.72	0.74	0.53	0.54	0.55	0.56	2.57	2.58
LSD	1.46	0.63	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.05

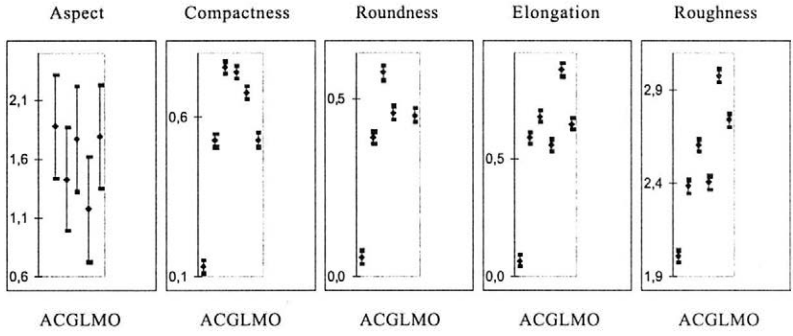

Figure 2. Confidence intervals for evaluated features in individual weed species, Žabčice 1999 (A – AGRRE, C – CHEAL, G – GALPA, L – AMARE, M – MALNE, O – CONAR)

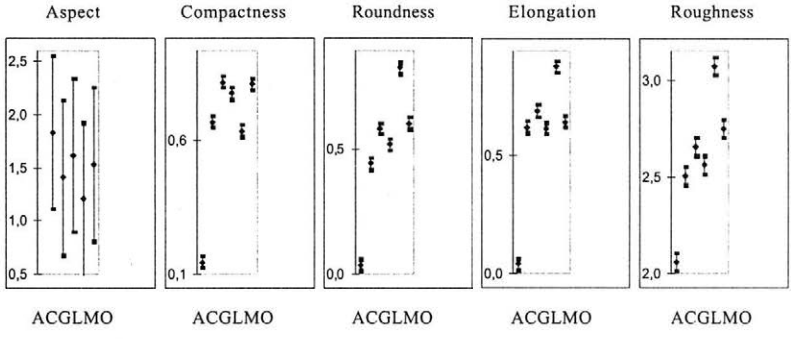

Figure 4. Confidence intervals for evaluated features in individual weed species, Žabčice 2000 (A – AGRRE, C – CHEAL, G – GALPA, L – AMARE, M – MALNE, O – CONAR)

DISCUSSION

Our measurements showed that combination of several selected leaf features enables in most cases to detect differences among individual weed species. This method is also suitable due to relatively simple measurements and modest requirements for equipment. To evaluate selected dimensionless features it is also necessary to choose appropriate software. Here we used one of the most common software for image analysis, Image Pro Plus 3.1, which proved to meet our goals.

However, it still remains to test applicability of the method at different development stages of weeds since morphological changes are observed during ontogenesis of a number of species, which can modify examined features. Thus it is necessary to carry out similar measurements at different growth and development stages of weeds. These problems have been solved in part by Woebbecke et al. (1995) who studied changes of the parameters roundness, aspect, perimeter/thickness, elongation and first invariant central moment in 10 important weeds till the 45th day after emergence. The least varia-

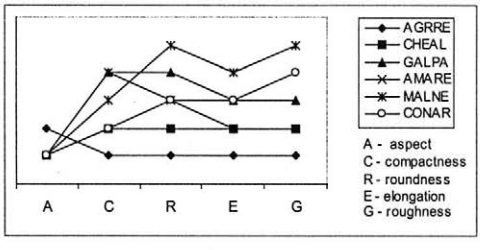

Figure 3. Significance of differences among individual weed species in evaluated features using Tukey's test, Žabčice 1999

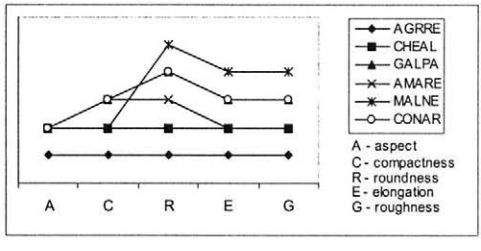

Figure 5. Significance of differences among individual weed species in evaluated features using Tukey's test, Žabčice 2000

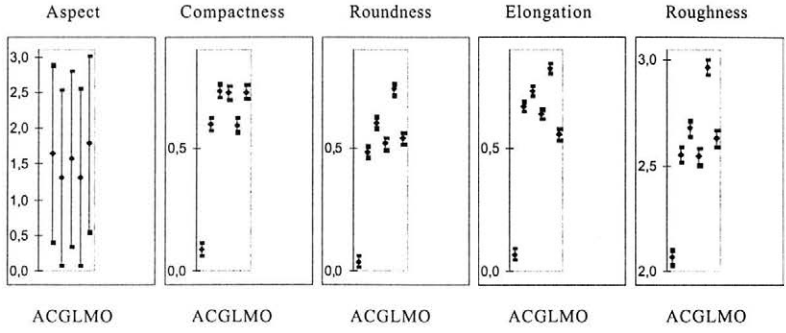

Figure 6. Confidence intervals for evaluated features in individual weed species, Břevenec 1999 (A – AGRRE, C – CHEAL, G – GALPA, L – AMARE, M – MALNE, O – CONAR)

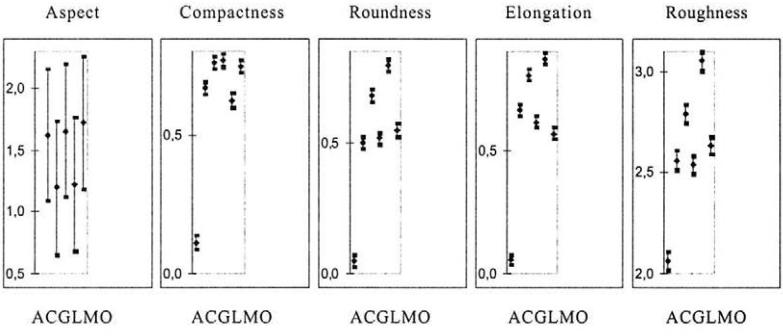

Figure 8. Confidence intervals for evaluated features in individual weed species, Břevenec 2000 (A – AGRRE, C – CHEAL, G – GALPA, L – AMARE, M – MALNE, O – CONAR)

tion in leaves and the highest discrimination ability were found on the 7th to 23rd day after emergence.

The values we have assessed should be rather stable in a longer period since the samples (leaves) were taken at the flowering stage when the growth habit of plants is finished and there are hardly any morphological changes on leaves. Among the examined shape features roundness and roughness exhibited the best discrimination ability. They enabled to distinguish the highest number of weeds and were followed by the features elongation and compactness. The lowest number of weeds could be distinguished using aspect. Just aspect together with central invariant moment are reported by Woebbecke et al. (1995) as the most suitable features to discriminate between monocotyledons and dicotyledons. However, it was measured at earlier growth and development stages (up to 30 to 50 days after emergence). But Katayama et al. (1998), in accordance with our results, found roundness and central invariant moment as the best distinguishing features in field flowers till the 55th day after emergence.

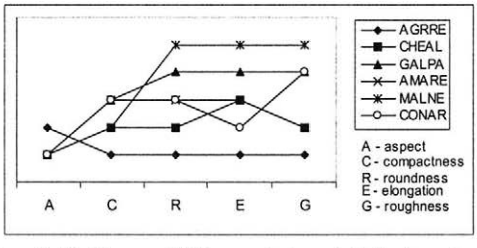

Figure 7. Significance of differences between individual weed species in evaluated features, Břevenec 1999

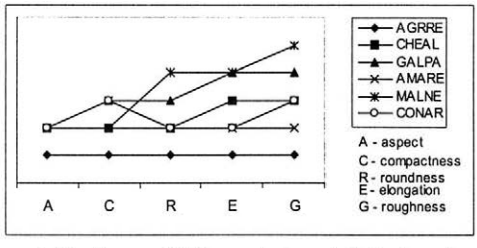

Figure 9. Significance of differences between individual weed species in evaluated features, Břevenec 2000

Furthermore, it is necessary to test sensitivity of the method in distinguishing weed species that are more similar to each other, and particularly possibilities of application of the dimensionless analysis of leaf area to discriminate individual species of monocotyledons. Based on a high coefficient of variation for assessed features in AGRRE, we can suspect some problems that could emerge, for example, in distinguishing among *Agropyron repens*, *Echinochloa-cruss gali*, *Apera spica venti*, etc. Use of this method is likely to be accompanied by some troubles. They could be avoided by a higher number of evaluated features, for instance, those that are reported to be able to catch even smaller differences among individual weed species. Yonekava et al. (1996) report that combination of a greater number of studied features allows us to distinguish a larger amount of weeds, theoretically up to 1280 species on the basis of leaf blades standardized according to Makino (1969). Our results suggest that even though this number will be markedly lower in practice, it can encompass the most important weeds common under our soil and climatic conditions.

A rather wide interval of accuracy in weed identification (40–90%) using dimensionless analysis reported by some authors (Woebbecke et al. 1995; Yonekawa et al. 1996; Guyer et al. 1986; Petry and Kühbauch 1989) can be explained by different weeds examined and different used features and their numbers. However, identifying weeds for on-line application of herbicides in practice it would be sufficient to detect a certain group of weeds only because broad-spectra herbicides can be applied.

In comparison with standard methods for assessment of weed infestation (count and weight methods) use of dimensionless analysis is of similar accuracy but less laborious, i.e. it is more comfortable due to visual and computational assessments of the digital image.

A great disadvantage of the method (at current state of elaboration) is sampling leaves by hand and taking digital images. Taking images of individual leaves directly in the canopy would enable to work with larger sets of data (individual samples) and thus to make measurements more accurate. The biggest problem at work in the canopy are differences in the position of leaves towards a digital camera (scanning component), which necessitates mathematical adjustments of scanned areas and makes the assessments rather difficult.

CONCLUSIONS

The used factors compactness, roundness, elongation, roughness and aspect showed to be suitable to distinguish the species *Agropyron repens* (AGRRE), *Chenopodium album* (CHEAL), *Galinsoga parviflora* (GALPA), *Amaranthus retroflexus* (AMARE), *Malva neglecta* (MALNE), and *Convolvulus arvensis* (CONAR). The equipment allowed us to obtain respective accuracy. Neither markedly different length of leaves in AGRRE had negative effects on distinguishing individual species. There was the only case when two weeds, GALPA and CONAR, were impossible to distinguish from each other (Žabčice, 2000). It could be explained by a higher variation in leaves of CONAR. Based on visual evaluation, some leaves were slightly deformed, which resulted in deviations from mean values. Therefore, it is desirable to take into account even potential adverse (stress) effects at the location that influence leaf shape. If such plants are present at a low number, it is suitable to eliminate them because they can considerably confuse the results.

Identifying weeds at the flowering stage when the growth habit of plants is finished and most leaves attained a final shape, roundness and roughness exhibited the best discrimination ability. They enabled to distinguish the highest number of weeds. They were followed by the features elongation and compactness. The lowest number of weeds could be distinguished using aspect. However, the results achieved by other authors given in the discussion suggest that discrimination ability of individual features depend on ontogenesis (growth and development stage of plants).

At the current stage of elaboration this method is suitable particularly for experimental measurements in small-plot trials. The advantages and disadvantages mentioned in the discussion are mostly of technical character. Their elimination on the basis of further research and tests will be crucial for their application in non-destructive assessment of both quantity and quality of weed infestation of field crops. Possibilities of practical use are, for instance, in evaluation of herbicide efficacy in field trials or in precise farming at on-line herbicide application.

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ABSTRAKT

Rozlišování plevelných druhů pomocí bezdimenzionální analýzy listů

Za účelem identifikace jednotlivých druhů plevelů na digitálních snímcích byla ve dvou letech (1999 a 2000) a na dvou lokalitách v ČR s odlišnými půdně-klimatickými podmínkami (Žabčice a Břevenec) ověřována bezdimenzionální analýza listů. Byly zjišťovány rozdíly mezi šesti plevelnými druhy: *Agropyron repens* (AGRRE), *Chenopodium album* (CHEAL), *Galinsoga parviflora* (GALPA), *Amaranthus retroflexus* (AMARE), *Malva neglecta* (MALNE) a *Convolvulus arvensis* (CONAR), a to na základě sledování těchto vlastností (znaků) listů: kompaktnost (compactness), kulatost (roundness), podlouhlost (elongation), drsnost okraje (roughness) a poměr délky a šířky elipsy s ekvivalentní plochou (aspect). Uvedené druhy plevelů bylo možné statisticky průkazně vzájemně rozlišit. Pouze v jednom případě (na stanovišti Žabčice v roce 2000) nebylo možné stanovit průkaznost rozdílů mezi druhy GALPA a CONAR.

Klíčová slova: polní plevele; identifikace; analýza obrazu

Corresponding author:

Doc. Ing. Jan Křen, CSc., Mendelova zemědělská a lesnická univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika, tel.: + 420 5 45 13 31 06, fax: + 420 5 45 13 31 07, e-mail: kren@mendelu.cz

RECENZE

Regulačné technológie v produkčnom procese plodín

M. Demo, P. Bielek a kol.

SPU Nitra, VÚPOP Bratislava, 2000. 648 s., 94 tab., 175 obr.

Prof. M. Demo je v současnosti zřejmě nejplodnějším autorem v oblasti zemědělských věd na Slovensku. Necelý rok po vyjití knihy *Trvalo udržateľný rozvoj* se nám dostala do rukou obsáhlá publikace, kterou sestavil za přispění dalších předních odborníků. Od doby vydání *Zemědělských soustav* akademika Kudrny nevyšlo v Čechách a na Slovensku tak souborné a přehledné dílo v tomto oboru. Publikace má sloužit především jako vysokoškolská učebnice, ale současně bude jistě vítána i odbornou zemědělskou veřejností. Je totiž přes vysokou vědeckou úroveň velmi přehledná, srozumitelná, vhodně spojuje staré pravdy s novými poznatky. Nutí čtenáře k přemýšlení, nabízí variantní řešení každému pro vlastní užití. Ve stěžejních kapitolách plynule přecházejí informace souhrnného obecného charakteru až k významným detailům v jednotlivostech a obráceně.

Dílo je členěno na pět základních částí a osm kapitol. V prvé části je v úvodní kapitole pojednáno o krajinném prostoru jako objektu produkčního procesu a v následné kapitole je charakterizován produkční proces v krajinném prostoru. Zvláštní pozornost je věnována půdě jako objektu produkčních procesů a vodě jako všeobecnému médiu transportu přeměny látek a energie v produkčních procesech. Regulací energetických procesů v zemědělské soustavě se zabývá druhá část publikace. Pro celou publikaci, ale pro tyto části zejména, je charakteristické systémové pojetí, které je pro zemědělského odborníka nezbytné.

Největší prostor je věnován soustavám regulace produkčních procesů v zemědělské krajině, zvláště pak biologickým a mechanickým regulačním technologiím. Z biologických regulačních technologií je pozornost soustředěna na výběr plodin, osivo a sadbu, osevní postupy a biologické metody regulace škodlivých činitelů. Z mechanických regulačních technologií je preferována problematika zpracování půdy (text je doprovázen velkým množstvím schémat a fotografií nářadí a strojů na zpracování půdy), méně pak úprava vodních poměrů melioracemi a nakonec i mechanická regulace škodlivých činitelů.

Chemické regulační technologie jsou rozděleny na biogenní a abiogenní a pojednány poněkud zjednodušeně. Zájemce o chemickou regulaci škodlivých činitelů, jakož i výživu rostlin a aplikaci regulačních látek bude muset informace získávat z jiných zdrojů. Regulaci plevelů je věnována samostatná kapitola. V celé publikaci převládají biologické a ekologické aspekty produkčních procesů. Preference metod podporujících trvale udržitelný rozvoj, celostní a systémové pojetí, racionální přístup a nabídka alternativních řešení patří mezi přednosti nové publikace.

Doc. Ing. Jan Moudrý, CSc.

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Výsledky – při jejich popisu se k vyjádření kvantitativních hodnot dává přednost grafům před tabulkami. V tabulkách je třeba shrnout statistické hodnocení naměřených hodnot. Tato část by neměla obsahovat teoretické závěry ani dedukce, ale pouze faktické nálezy.

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Manuscript layout: paper of standard size (210 × 297 mm), double-spaced typescript. A PC diskette should be provided with separate text and graphic files. Tables, figures and photos should be enclosed separately. The text must contain references to all these appendices.

If any abbreviation is used in the paper, it is necessary to mention its full form for the first time it is used, abbreviations should not be used in the title or in the summary of the paper.

The **title** of the paper should not exceed 85 characters. Subheadings are not allowed.

Abstract should contain the subject and conclusions of the paper, not a mere description of the paper. It must present all substantial information contained in the paper. It should not exceed 170 words. It should be written in full sentences and contain basic numerical data including statistical data. It must contain keywords. It should be submitted in English and, if possible, also in Czech.

Introduction has to present the main reasons why the study was conducted, and the circumstances of the studied problems should be described briefly.

Review of literature should be a short section, containing only references closely related to the main topic of the paper.

Only original **methods** should be described, in other cases cite the method used and any modifications. This section should also contain a description of experimental material.

In the **Results** section figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables. This section should not contain either theoretical conclusions or deductions, but only experimental data.

Discussion contains an evaluation of the study, potential shortcomings are discussed, and the results of the study are compared with previously published results (only those authors whose studies are closely related to the published paper should be cited). The section Results and Discussion may be presented as one section.

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The author should give his full name (and the names of other collaborators), academic, scientific and pedagogic titles, full address of his workplace and postal code, telephone and fax number or e-mail.

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OBSAH – CONTENTS

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Rozšiřuje Ústav zemědělských a potravinářských informací, referát odbytu, Slezská 7, 120 56 Praha 2