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Laser stimulation effect of seeds on quality of alfalfa

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A b s t r a c t. Alfalfa samples (sowing alfalfa – represented by cv. Legend, and hybrid alfalfa – represented by cv. Radius) were subjected to stimulation 1, 3 and 5 times with divergent beam of He-Ne laser. Alfalfa was harvested in the 3-cut system in years of full land use. Plant samples were used to determine the content of dry matter, total protein, specific protein, crude fibre, phosphorus, potassium, calcium, magnesium, copper, manganese, zinc, boron and molybdenum. Pre-sowing stimulation of seeds with laser light, at doses R6x5 and R6x3, caused a significant increase in the content of specific protein, phosphorus and molybdenum, while decreasing the content of crude fibre in the dry matter of the plants.

K e y w o r d s: alfalfa, seeds, cultivars, cuts, laser stimulation, quality

INTRODUCTION

Two alfalfa species are mainly grown in Poland: sowing alfalfa (*Medicago sativa* L.) and hybrid alfalfa (*Medicago x varia* T. Martyn). Sowing alfalfa is represented by foreign cultivars, while the hybrid alfalfa has been bred in Poland. In recent years, research was conducted on the yielding of different plants in conjunction with pre-sowing stimulation of seeds with laser light (Aladjadjiyan, 2007; Chen *et al*., 2005; Dong *et al*., 2007; Dziwulska *et al*., 2006; Ivanova, 1998). Laser stimulation of alfalfa seeds caused an increase in the number of shoots per 1 m^2 by 27%, increase in green matter yields by 7% and dry matter yields by 9%, and a decrease in dry weight of shoot by 12%, (Dziwulska *et al*., 2006). The higher yields of alfalfa stimulated with laser light probably result from increased efficiency of photosynthesis (Ćwintal and Olszewski, 2007; Chen *et al*., 2005, Pastore *et al*., 1996). Laser stimulation of seeds, cultivars, cuts, agrotechnical factors and weather conditions in particular years affected the ratio of leaf weight to stem weight, which suggests that it could have had an effect on the content of organic and mineral components in the dry matter of the plants (Ćwintal, 2000; Ćwintal and Wilczek, 2002; Thompson and Stout, 1996; Wilczek and Ćwintal, 2002). Varying ratio of weight of leaves to that of stems in treatments with laser light stimulation may have an effect on the quality of alfalfa, as most of the total and specific proteins, calcium, magnesium and phosphorus, and most microelements, are to be found in the leaves (Downs and Taylor, 1989; Falkowski *et al.*, 1990; Graham, 1991; Lanyon and Griffith, 1988; Wilczek and Ćwintal, 2000).

The aim of the study was to determine the effect of pre-sowing laser stimulation of alfalfa seeds, cultivars and harvest cuts on the organic component content and macro- and microelements in dry matter of alfalfa.

MATERIAL AND METHODS

Alfalfa samples for chemical analyses originated from the years 2003-2004, from a two-factor field experiment conducted with the random blocks method in four replications. The experiment was set up in the locality of Kolonia Spiczyn, Łęczna District, on a soil of the good wheat complex. The results concerning the yield structure and the yields of green and dry matter have been published in a paper by Dziwulska *et al.* (2006). This report presents the content of organic and mineral components in alfalfa against the background of the following experimental factors:

– two species of alfalfa (sowing and hybrid alfalfa represented by cv. Legend, and cv. Radius, respectively);
– seed irradiation with divergent beam of He-Ne laser, with power density of 0, 3 and 6 mW cm^{-2} (designated as R0, R3, R6).

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Seeds were subjected to laser stimulation once, three, and five times (designated as x1; x3; x5). Seed irradiation was performed using the device of Koper and Dygdała (1994). The time of exposure of seeds in free fall was 0.1 s, and the wavelength applied was 632.4 nm. Seed samples originated from years of alfalfa full land use, with harvesting performed in 3 cuts: the first and the third cuts in the budding phase, and the second cut at the beginning of blooming. From each experimental plot and each cut samples of plants were taken, with weight of 0.5 kg, on which the following determinations were made: dry matter content – with gravimetric method at 105ºC, total protein – with Kiejdahl method, specific protein – with Motehs and Engel method, crude fibre – with gravimetric method, phosphorus – with flow spectrophotometric method, potassium – with emission flame spectrophotometric method, calcium, magnesium, copper, manganese and zinc – with atom absorption spectrometry method (AAS), boron and molybdenum – with the method of electrothermal atom absorption spectrometry (cuvette). The analyses were made at the Central Laboratory of Chemical Analyses, Institute of Cultivation, Fertilization and Soil Science, National Research Institute, Puławy. The weather data were obtained from the Meteorological Station of the University of Life Sciences in Lublin. The obtained results were processed statistically using analysis of variance and $LSD_{0.05}$ according to the Tukey test.

RESULTS

The weather in the years 2003-2004 was similar in terms of precipitation totals during the period of alfalfa vegetation (Table 1), but air temperature was higher by an average of 2.7°C in 2003. The greatest differences were recorded during the vegetation of the third regrowth, when air temperature in 2003 was higher by 3.9°C than the corresponding value in 2004.

Among the organic components, laser irradiation caused significant differentiation only in the concentration of specific protein and crude fibre in the dry matter of alfalfa (Table 2). The highest content of the former of those components was recorded in treatment R6x5, while of the latter – in the control treatment and in treatment R3x5. The cuts of alfalfa significantly differentiated the content of all organic components. The levels of dry matter, total and specific proteins increased from regrowth 1 to 3, while the content of crude fibre was the highest in alfalfa from the 1st cut and the lowest in that from the 3rd. More favourable weather in 2003 caused significantly higher concentration of dry matter, total protein and specific protein as compared to 2004. The content of crude fibre, on the other hand, showed an opposite trend, with higher levels recorded in 2004. Out of the two alfalfa cultivars under study, cv. Legend was characterized by significantly higher content of specific protein.

The levels of phosphorus, calcium and magnesium were affected to the greatest extent by the cuts and by the weather conditions in the years of full land use (Table 3). The highest content of P and K was recorded in alfalfa from the 1st cut, and the lowest in that from the 3rd. Calcium, in turn, increased its content in the plants in the order from the 1st to the 3rd cuts. Significantly higher contents of phosphorus and calcium were observed in the plants from 2004, when the vegetation period was longer by 24 days, while the content of potassium was significantly higher in plants from 2003. Harvest cuts and years did not significantly differentiate the concentration of Mg in alfalfa dry matter. Laser light differentiated significantly only the content of P in alfalfa. The highest content of that component was recorded in treatments R6x5, R6x3 and R6x1. Concentration of K and Ca in the plants was also affected by the interaction of cuts and years. The cultivars did not have a significantly differentiating effect on the content of the macroelements studied.

Factors that had a significant effect on the content of microelements in alfalfa, such as B, Cu, Mo and Zn, were the harvest cuts, years, and cultivars (Table 4). The content of Cu, Mo and Zn was the highest in plants from the 1st cut, and the lowest in those from the 3rd, while the level of boron and

T a b l e 1. Meteorological profile for years of full performance

Specification	Year	Cut			$\Sigma / \bar{x}$
		I	II	III	
Dates	2003	15.04-27.05	28.05-07.07	08.07-30.08	-
	2004	02.04-31.05	01.06-15.07	16.07-10.09	-
Duration of vegetation (day)	2003	43	41	54	138
	2004	60	45	57	162
Daily mean air temperature (°C)	2003	12.1	17.5	18.9	16.2
	2004	9.9	15.7	15.0	13.5
Rainfall sum (mm)	2003	91.6	60.2	102.9	254.7
	2004	71.6	77.1	120.7	269.4
Number of days with rainfalls	2003	12	14	13	39
	2004	10	12	15	37

manganese was the highest in the third regrowth. The weather in 2003 caused significantly greater uptake of B, Cu, Mo and Zn by alfalfa in comparison with 2004. It should be noted that the years did not significantly differentiate the content of Mn. Alfalfa cv. Legend had a significantly higher content of B, Cu, Mn, Mo and Zn than cv. Radius. Laser stimulation of seeds caused a significant increase only in the content of molybdenum, for most of the applied doses of irradiation (R6x5, R3x5, R6x3, R3x3 and R6x1). Noteworthy is the fact that the content of Mo was affected by the interaction of cuts x years and laser stimulation x years, while that of Cu – by the interaction of cuts x years.

DISCUSSION

In recent years there has been a growing interest in research on the quality of alfalfa which is used as green fodder, hay, hay-silage, and dry fodder. The interest in the crop plant increases also due to its application in human feeding (germs, leaf extracts) and in the production of various fodder supplements for swine, horses and poultry (Dong *et al*., 2007; Downs and Taylor, 1989; Grela, 2008; Ueda and Ohshima, 1989). A notable achievement was the obtainment of protein-xanthophyll concentrate (PX) from alfalfa (Grela, 2008).

Earlier studies have shown that weather conditions, harvest cuts, fertilization and cultivars have a differentiating effect on the content of organic and mineral components (Ćwintal, 2000; Downs and Taylor, 1989; Lanyon and Griffith, 1988; Wilczek *et al*., 1996). However, there is no information on the effect of laser stimulation of seeds on the quality of alfalfa. As it has been mentioned, laser light stimulation caused a significant increase in the number of stems per 1 m^2, with simultaneous decrease in their unit weight (Dziwulska *et al*., 2006). Decrease in stem weight caused a significant reduction in the content of crude fibre in plants from treatment R6x5. This finding is in agreement with the opinion of Ćwintal (2000) that the concentration of crude fibre increased with increase in the stem unit weight. Moreover, stems with lower mass were characterized by a higher

T a b l e 2. Content of organic elements in alfalfa and hybrid alfalfa (years of full land use)

Object	Dry matter (%)	Crude protein	True protein	Crude fibre
		(% d.m.)		
A. Radiation rates				
R0	24.8	15.8	10.4	30.9
R3x1	25.2	16.5	11.3	29.4
R3x3	25.5	16.3	11.0	29.0
R3x5	25.3	16.2	11.2	29.6
R6x1	25.4	16.0	11.1	29.3
R6x3	25.4	16.3	11.4	29.0
R6x5	25.2	16.5	11.8	28.3
$LSD_{0.05}$	n.s.	n.s.	1.0	2.5
B. Cut				
I	21.4	14.0	9.6	30.2
II	24.7	16.8	11.8	29.8
III	29.9	18.3	12.0	27.4
$LSD_{0.05}$	2.4	1.6	0.9	2.2
C. Years				
2003	26.6	17.6	11.8	28.0
2004	24.1	15.2	10.4	30.4
$LSD_{0.05}$	2.1	1.4	0.7	1.9
D. Cultivars				
Legend	25.2	16.9	11.8	29.0
Radius	25.5	16.0	10.5	29.5
$LSD_{0.05}$	n.s.	n.s.	0.7	n.s.
Interaction BxC	n.s.	4.1	2.4	n.s.

T a b l e 3. Content of P, K, Ca, Mg in alfalfa and hybrid alfalfa (years of full land use)

Object	P	K	Ca	Mg
	(% d.m.)			
A. Radiation rates				
R0	0.27	2.45	1.49	0.22
R3x1	0.28	2.38	1.53	0.21
R3x3	0.28	2.44	1.54	0.22
R3x5	0.29	2.46	1.53	0.21
R6x1	0.30	2.42	1.54	0.22
R6x3	0.30	2.46	1.53	0.23
R6x5	0.31	2.45	1.53	0.22
$LSD_{0.05}$	0.030	n.s.	n.s.	n.s.
B. Cut				
I	0.32	3.09	1.22	0.22
II	0.30	2.48	1.41	0.22
III	0.25	1.73	1.95	0.23
$LSD_{0.05}$	0.028	0.24	0.16	n.s.
C. Years				
2003	0.28	2.60	1.43	0.23
2004	0.31	2.27	1.64	0.22
$LSD_{0.05}$	0.026	0.21	0.13	n.s.
D. Cultivars				
Legend	0.28	2.37	1.58	0.22
Radius	0.30	2.49	1.48	0.23
$LSD_{0.05}$	n.s.	n.s.	n.s.	n.s.
Interaction BxC	n.s.	0.61	0.43	n.s.

T a b l e 4. Content of B, Cu, Mn, Mo, Zn in alfalfa and hybrid alfalfa (years of full land use)

Object	B	Cu	Mn	Mo	Zn
	(% d.m.)				
A. Radiation rates					
R0	21.3	6.39	48.7	0.45	24.1
R3x1	21.4	6.52	49.3	0.47	24.8
R3x3	20.7	6.38	49.7	0.49	24.4
R3x5	21.0	6.47	48.9	0.50	24.3
R6x1	21.1	6.40	48.1	0.48	24.6
R6x3	20.7	6.47	45.9	0.49	24.7
R6x5	21.0	6.51	59.8	0.51	24.4
$LSD_{0.05}$	n.s.	n.s.	n.s.	0.030	n.s.
B. Cut					
I	19.5	6.76	41.8	0.73	28.6
II	21.4	6.38	45.9	0.47	24.1
III	22.3	6.22	59.8	0.23	20.6
$LSD_{0.05}$	1.81	0.52	4.10	0.028	2.08
C. Years					
2003	22.9	7.39	49.5	0.64	27.1
2004	19.4	5.51	48.9	0.32	21.9
$LSD_{0.05}$	1.52	0.46	n.s.	0.025	1.87
D. Cultivars					
Legend	22.4	6.95	51.3	0.57	25.4
Radius	19.9	5.96	47.2	0.40	23.6
$LSD_{0.05}$	n.s.	0.93	n.s.	0.13	n.s.
Interaction BxC	n.s.	n.s.	n.s.	0.25	n.s.

ratio of leaf weight to stem weight (Ćwintal, 2000; Dziwulska *et al*., 2006). In such circumstances, laser light caused a significant increase in the content of specific protein in treatments R6x5 and R6x3. Thus, the principle known with relation to alfalfa, that with increasing content of protein there is a decrease in the content of fibre and the other way round, had found confirmation (Ćwintal and Wilczek, 2002; Downs and Taylor, 1989; Wilczek and Ćwintal, 1996). The warm weather with sufficient rainfall in 2003 was favourable for alfalfa yielding as well as for the uptake of potassium, boron, copper, molybdenum and zinc by the plants. With colder weather (2004) and a good distribution of precipitations an extension of the vegetation of alfalfa plants was observed, with a significant increase in crude fibre content in the plants. These results find confirmation in literature (Ćwintal and Wilczek, 2002; Khaiti and Lemaire, 1992). Harvest cuts were a factor that notably differentiated the chemical composition of alfalfa. A significantly higher content of dry matter, total and specific protein, calcium, boron and manganese was found in plants from the 3rd cut, while the first cut of alfalfa was characterized by the highest content of fibre, phosphorus, potassium, copper, molybdenum and zinc. These results are in line with literature data (Ćwintal, 2000; Lanyon and Griffith, 1988; Wilczek and Ćwintal, 2002). The relation between the contents of K and Ca in the cuts followed the known regularity that when the content of K in the cuts increases, that of Ca decreases, and the other way round. In our experiment the highest content of K was recorded in plants from the 1st cut, and the lowest in those from the 3rd. In the case of Ca the arrangement of results was the opposite – the highest content in plants from the 3rd cut, and the lowest in those from the 1st. These relations were probably determined by the antagonism in K^{+} and Ca^{++} uptake by alfalfa (Falkowski *et al.*, 1990; Graham, 1991; Lanyon and Griffith, 1988), and by the higher ratio of leaf to stem weight ratio in plants from the 3rd regrowth. It should be kept in mind that most Ca is to be found in the leaves, and K in the stems (Ćwintal, 2000; Wilczek and Ćwintal, 2002; Wilczek *et al*., 1996).

From the two compared cultivars, the multi-leaf cv. Legend was characterized by better quality, with its significantly higher content of specific protein, B, Cu, Mn, Mo and Zn compared to those of cv. Radius. These results are related with the greater share of leaves in the dry matter yield of cv. Legend (Ćwintal and Wilczek, 2002; Hill and Jung, 1974; Wilczek and Ćwintal, 2002).

The characterized contents of organic and mineral components in the dry matter of the sowing and hybrid alfalfa cultivars, against the background of literature (Ćwintal, 2000; Falkowski *et al*., 1990; Graham, 1991; Hill and Jung, 1974; Wilczek and Ćwintal, 2000, 2002; Wilczek *et al*., 1996), permit to evaluate the alfalfa cultivars studied as valuable fodder material. The slight deficit of molybdenum can be compensated for with suitable foliar feeding of the plants.

CONCLUSIONS

1.The quality of the sowing and hybrid alfalfa cultivars was affected to the greatest extent by the weather conditions and the harvest cuts in the years of the study. Those factors caused significant differences in the content of dry matter, total and specific protein, crude fibre, phosphorus, potassium, calcium, boron, copper, manganese, molybdenum and zinc. Alfalfa from the 3rd cut was characterized by the highest nutritive value.

2. Alfalfa cv. Legend was significantly superior to cv. Radius in terms of its content of specific protein, boron, copper, manganese, molybdenum and zinc.

3. Pre-sowing stimulation of seeds with laser light at doses of R6x5 and R6x3 caused a significant increase in the content of specific protein, phosphorus and molybdenum in dry matter of the plants, and a decrease in the content of crude fibre.

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