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YIELD PARAMETERS OF OLD AND YOUNG LUCERNE PLANTS UPON PRE-SOWING ELECTROMAGNETIC SEED STIMULATION

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A b s t r a c t. Lucerne is a fodder perennial plant from the *Fabaceae* family grown under field conditions as feed for various groups of animals. This paper is concerned with the effect of electromagnetic stimulation of lucerne seed on the yield, yield parameters, and content of photosynthetic pigments in 1-2- and 5-6- year old lucerne plants. In 2012-2013 a field experiment was conducted with 5-6-year old lucerne, which was initiated in 2008. Another experiment was started in 2012 and conducted on 1-2-year old lucerne. The research material were seeds of hybrid lucerne (*Medicago* x *varia* T. Martyn) variety Radius and sowing lucerne (*Medicago sativa* L.) variety Ulstar. Before sowing the seeds were stimulated with the following combinations of physical factors: C – control (untreated sample), L – laser light with a surface power density of 6 mW cm^{-2} during 3-fold free fall, F – alternating magnetic field with an induction of 30 mT and exposure time of 30 s; L + F – laser light and magnetic field in the above doses. Electromagnetic stimulation contributed significantly to an increase in the number of shoots per 1 m^2 as compared to the control. No significant difference in the weight of a single shoot was observed between the experimental combinations. 5-6-year old lucerne was characterised by a statistically greater shoot mass as compared with 1-2-year old plants. Statistical analysis showed that the highest yield increase was observed for the samples where seeds were stimulated with laser light and both laser light and magnetic field, relative to the control. The best results concerning concentrations of chlorophyll *a* and *b* and carotenoids were observed for alternating magnetic field, where the increase amounted to 9, 11 and 13.0%, respectively, as compared to the control.

K e y w o r d s : lucerne, crop, photosynthetic pigments, electromagnetic stimulation, field experiment

INTRODUCTION

Lucerne is one of most important perennial forage crops in the world (Schnurr *et al.* 2007). In Poland it is cultivated on arable lands as a pure crop as well as a fodder mixture with perennial grass. Its plantations can be used as long as 5-7 years (Gaweł 2012, Gaweł and Grzelak 2013). It can be used as a green crop, hay, and as dried material. Recently, increasingly popular is its usage in the form of protein-xantophyll concentrate (PX) or as a leaf extract (EFL) for human diet supplements, allowed by WE 258/97 European Parliament decree and by European Council (Mattera *el al.* 2013, OJEU 2009).

Lucerne is a source of valuable nutrients, it is rich in protein, minerals, vitamins and carotenoids (Gaweł 2012, Gaweł and Grzelak 2013).

Green lucerne is also used in human nutrition in the greater part of Russia, in China, America, as well as in South Africa as a spinach substitute (Mielmann 2013).

In order to improve the cultivation of lucerne and, most of all, for the improvement of the quality of seeds and to provide the optimal crop structure, physical methods of pre-sowing seed improvement such as laser light or magnetic field treatments are applied (Aladjadjiyan 2010, Ćwintal *et al.* 2010, Ćwintal and Dziwulska-Hunek 2013, Dziwulska *et al.* 2006, Hernandez *et al.* 2010, Pietruszewski *et al.* 2007).

Studies on the effect of treatment of the seeds of crop plants with electromagnetic factors showed its positive impact on seedling emergence, yielding, as well as on plant quality characteristics (Ćwintal *et al.* 2010, Ćwintal and Dziwulska-Hunek 2013, Hernandez *et al.* 2010). The experiments conducted previously were concerned mainly with annual plants (Vasilevski 2003) or perennial plants in the initial vegetation period, and were focused on the evaluation of variability of crop and crop structure and on the crop yield (Ćwintal and Dziwulska-Hunek 2013). In the available literature only few reports on the effect of electromagnetic stimulation on the photosynthetic pigment content exist (Atak *et al.* 2007, Prośba-Białczyk *et al.* 2013, Sujak *et al.* 2013). Chlorophyll content is one of the plant photosynthetic activity indices. The highest concentration of chlorophylls was observed at the beginning of plant flowering phase. In most of the reports the ratio of chlorophylls *a*/*b* amounts to 3:1. Carotenoids are the supporting photosynthetic pigments incorporated into the photosynthetic antennas and photosynthetic reaction centres (Bojović and Stojanović 2005). Surveys by other authors indicate c.a. 35 000 ppm of chlorophylls and c.a. 1 050 ppm of carotenes in fresh juice pressed of lucerne (Gaweł and Grzelak 2013). However, photosynthetic pigment concentrations undergo major changes as the years of plantation use go by.

The study assumed that electromagnetic stimulation of seeds can produce variable effects on yield and quality parameters in hybrid and sowing lucerne as well as in young or old plants, positively influencing their yield.

This experiment was conducted in order to compare the effects of pre-sowing electromagnetic stimulation of seeds on lucerne plants at different maturity stages (different years of use). The aim was to determine the effects of the electromagnetic factors on yield levels, yield structure and content of photosynthetic pigments in hybrid and sowing lucerne plants at the beginning (1-2 years of use after stimulation), and at the end of its use as fodder (5-6 years).

MATERIAL AND METHODS

The field experiment with 5-6-year old lucerne was started in 2008 (Ćwintal and Dziwulska-Hunek 2013), while the other equivalent experiment was initiated in 2012. Lucerne sown in 2008 during the experiments in the years 2012 and 2013 was a 5-6-year old plantation, while lucerne sown in 2012 was a 1-2-year old plantation.

The field experiments were conducted at the experimental farm in Felin (51°13′21.9″N, 22°37′55.85″E), on a soil classified in the good wheat complex (class IIIa), with the method of random blocks in four replications, on micro-plots with harvest area of 1 m^2.

The experimental material consisted of two species of lucerne: hybrid (*Medicago x varia* T. Martyn) – *cv.* Radius, and sowing (*Medicago sativa* L.) – *cv.* Ulstar.

Prior to sowing the seeds were pre-treated with physical factors such as radiation with laser light (L), with surface power density of 6 mW cm^{-2} during 3-fold free fall, 30 s radiation with 30 mT and 50 Hz alternating magnetic field (F), or with the combination of laser light and magnetic field (L+F). Untreated group was taken as the control.

Laser stimulation was performed using the device designed by Koper and Dygdała (patent; 1993), the basic element of which is a He-Ne laser with wavelength of 632.8 nm, and the magnetic field was generated by means of the Pietruszewski electromagnet (utility pattern; 2003). Seeds were irradiated with laser light during free fall. The time of a single exposure to laser light was 0.1 s. The stimulation of seeds was performed a day before sowing.

Lucerne seeds, calculated as 100% of germination capacity, were sown in the first decade of May 2008 and 2012, in the amount of 800 seeds m^{-2}, into rows spaced at 20 cm, to the depth of ca. 1 cm. Mineral fertilisation was applied pre-sowing and also before the start of vegetation, at the doses of 35 P and 100 K kg ha^{-1} $year^{-1}$. In each year the lucerne parameters from the 1^{st} and 2^{nd} cut were estimated on plants collected during the early phase of blossoming. After emergence, the density of plants per 1 m^2, mean mass of single shoot, dry matter yield from 1 m^2, percentage of leaves in dry matter yield, as well as photosynthetic pigment content in leaves were determined.

The yield structure elements and ratios of leaves and stems in lucerne plants were estimated on the basis of 1 kg samples collected during harvesting. The samples were also used for the determination of dry matter with the gravimetric method, after drying at the temperature of 105°C for 24 hours.

In random chosen samples of lucerne leaves the concentrations of chlorophylls (*a* and *b*) were determined using the spectrophotometric method. The leaves were collected during the early blossoming phase (61, according to BBCH scale).

Chlorophylls and carotenoids were isolated from leaves in darkness, with acetone containing 0.01% w/v BHT (butylated hydroxytoluene), in order to avoid pigment oxidation. UV-Vis spectra were measured by means of a double beam Carry Bio 300 spectrophotometer, and the pigment concentrations were calculated according to the previously published procedure of Lichtenthaler and Buschmann (2001).

The weather conditions were elaborated on the basis of data from the Meteorological Station at Felin.

The results obtained were processed statistically using the analysis of variance ANOVA (STATISTICA 6.0). Intervals of confidence were determined with the Fisher test (LSD) at the level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The course of weather condition during particular vegetation periods plays an important role in the field studies. Table 1 shows the basic characteristics of the course of air temperature and the distribution of rainfall during the vegetation period of lucerne. The average air temperature in each month was compared with the average monthly temperature in a multi-year period. Analysis of temperatures shows that in 2012 (1st experimental year) the warmest month was July. In 2013 (2nd experimental year) it was relatively cold in March, when the average daily air temperature was below zero. April 2013 was also colder as compared to the long term average. Such a thermal pattern during the spring of 2013 resulted in later start of the lucerne growing season. In other months, there were no wide discrepancies in average air temperatures.

In 2012, except June, in the remaining months of lucerne growing rainfall was lower than the average of several years. The largest differences occurred in May and August. In 2013 the situation was reversed. In all months except August higher rainfall was recorded as compared to the multi-year. Especially a lot of rain fell in May, June and July.

Table 1. Air temperature and distribution of rainfall during the vegetation period

Month	2012	2013	Long-term average (1958-2008)
	Mean temperature (°C)		
III	4.1	–2.4	2.0
IV	9.2	8.1	8.5
V	14.7	15.3	14.3
VI	16.8	18.5	17.2
VII	20.8	19.2	19.4
VIII	18.5	19.2	18.2
IX	14.4	11.8	12.8
Mean	14.1	12.8	13.2
	Total rainfall (mm)		
III	27.4	60.8	27.7
IV	31.3	51.1	36.0
V	34.0	101.6	53.3
VI	68.1	105.9	56.2
VII	58.0	126.1	69.1
VIII	44.5	17.8	68.1
IX	37.7	64.6	49.7
Sum	301.0	528.3	360.1

The yield structure of the lucerne grown for feed consists of two major components: the number of shoots and the weight of a single shoot. Table 2 shows the number of shoots per 1m^2.

Table 2. Number of shoots per 1 m^2

Specification		C	L	F	L+P	Mean
Year of use	Young	326^{aA}	389^{aA}	373^{aA}	388^{aA}	369^{A}
	Old	357^{aA}	405^{aA}	397^{aA}	423^{aA}	396^{A}
Variety	Radius	356^{aA}	416^{aA}	391^{aA}	416^{aA}	395^{A}
	Ulstar	327^{aA}	383^{aA}	379^{aA}	395^{aA}	371^{A}
Cut	1	343^{aA}	408^{aA}	392^{aA}	411^{aA}	389^{A}
	2	340^{aA}	391^{aA}	378^{aA}	401^{aA}	378^{A}
Year	2012	282^{aB}	337^{aB}	318^{aB}	336^{aB}	318^{B}
	2013	402^{bA}	463^{abA}	452^{abA}	475^{aA}	448^{A}
Mean		342^{b}	400^{a}	385^{a}	406^{a}	–

Y – young plant, 2012 and 2013 – 1st and 2nd year of experiment (1st and 2nd year of full land use), O – old plant, 1 and 2nd year of experiment (5th and 6th year of full land use); see details in Materials and Methods, C– control, untreated seeds, L– seeds subjected to laser stimulation, F– seeds stimulated with alternating magnetic field, L+F– seeds subjected to both laser and magnetic field stimulation. Data were analysed with Statistica 6.0, ANOVA analysis, LSD test, at the level of $\alpha = 0.05$: a → c– different small letters indicate significant differences between control and electromagnetic methods for comparison between objects and means. A → B –various capital letters denote significant differences between objects and interactions : objects x electromagnetic factor

Average numbers of shoots in the particular cuts were statistically different concerning the experimental year and the electromagnetic stimulation method.

Statistical analysis shows that more lucerne shoots per 1 m^2 were developed in 2013, which was probably dependent on favourable weather conditions during the growing season. In all the experiments with stimulation of seeds the number of shoots per 1 m^2 was statistically significantly higher in comparison with the control. The largest mean plant density was observed for plants grown from seeds stimulated both with laser light and alternating magnetic field, although no statistically significant differences were found as compared with other experimental combinations. The increase of the density of shoots per 1 m^2 was statistically dependent on interactions between the years of full land use and on the seed stimulation factors applied. Statistically significantly higher number of shoots was observed in 2013 for the experimental combinations where seeds were stimulated both with laser light and alternating magnetic field (L+F), as compared to the control.

Similar effects of increase in the number of shoots upon laser stimulation and the stimulation with a combination of laser light and magnetic field were reported previously by Ćwintal and Dziwulska-Hunek (2013). The pre-sowing stimulation with magnetic field brought in 50% a positive effect, equally distributed between the varieties in the case of young plants, and higher in the case of mature plants from cv. Ulstar.

The increase of shoot density of lucerne per 1m^2 following the seed stimulation treatment can be explained in terms of an increase of the number of seeds germinating normally and a decrease of the number of hard seeds (Wilczek *et al.* 2005a, 2005b). Following that, an increase of the field emergence capacity of lucerne is observed, which translates into higher plant density and stems per unit area (Ćwintal and Sowa 2006, Dziwulska *et al.* 2006a).

Table 3. Mass of single shoot (g)

Specification		C	L	F	L+P	Mean
Year of use	Young	1.13^{aB}	1.08^{aB}	1.07^{aB}	1.10^{aB}	1.10^{B}
	Old	1.66^{aA}	1.65^{aA}	1.61^{aA}	1.63^{aA}	1.64^{A}
Variety	Radius	1.45^{aA}	1.41^{aA}	1.40^{aA}	1.43^{aA}	1.42^{A}
	Ulstar	1.33^{aA}	1.32^{aA}	1.28^{aA}	1.29^{aA}	1.31^{A}
Cut	1	1.41^{aA}	1.40^{aA}	1.35^{aA}	1.37^{aA}	1.38^{A}
	2	1.37^{aA}	1.33^{aA}	1.34^{aA}	1.36^{aA}	1.35^{A}
Year	2012	1.31^{aA}	1.27^{aA}	1.27^{aA}	1.30^{aA}	1.29^{A}
	2013	1.47^{aA}	1.46^{aA}	1.42^{aA}	1.42^{aA}	1.44^{A}
Mean		1.39^{a}	1.37^{a}	1.34^{a}	1.36^{a}	–

Explanations as in Table 2

Table 3 shows the mean mass of single lucerne shoot during two cuts and two experimental years, respectively, for young and mature lucerne. This parameter was not statistically different with relation to the stimulation method, although a tendency of a decrease of this parameter was observed. On the other hand, a significant variation of the mean mass of a single shoot was observed concerning 1-2-year old and 5-6-year old plants. Statistically greater mass of single shoot was observed for older lucerne plants.

Dry matter yield of lucerne was significantly dependent on the electromagnetic stimulation of seeds, the year of research, and the age of the plantation (Tab. 4).

Table 4. Mean dry matter yield (kg m^{-2})

Specification		C	L	F	L+P	Mean
Year of use	Young	0.39^{aA}	0.45^{aB}	0.43^{aA}	0.45^{aA}	0.43^{B}
	Old	0.59^{aB}	0.67^{aA}	0.64^{aA}	0.68^{aA}	0.65^{A}
Variety	Radius	0.53^{aA}	0.60^{aA}	0.57^{aA}	0.61^{aA}	0.58^{A}
	Ulstar	0.4^{aA}	0.52^{aA}	0.51^{aA}	0.53^{aA}	0.50^{A}
Cut	1	0.52^{aA}	0.61^{aA}	0.56^{aA}	0.60^{aA}	0.57^{A}
	2	0.46^{aA}	0.52^{aA}	0.50^{aA}	0.53^{aA}	0.50^{A}
Year	2012	0.39^{bA}	0.72^{aA}	0.43^{cbA}	0.47^{bA}	0.50^{B}
	2013	0.58^{aA}	0.67^{aA}	0.63^{aA}	0.66^{aA}	0.64^{A}
Mean		0.49^{b}	0.59^{a}	0.53^{b}	0.57^{a}	–

Explanations as in Table 2

The highest dry matter yield was obtained from seeds subjected to pre-sowing laser stimulation, as compared to the control and to other electromagnetic stimulation methods (Table 4). No statistically significant differences were found between the untreated sample (C), alternating magnetic field (F) and the combination of both laser light and magnetic field (L+F).

Statistically significantly higher dry matter yield from cuts was obtained in 2103 year as compared to 2012. Concerning the experimental years, attention should be paid to the differences in the effects caused by the various stimulation methods in 2012. The highest dry matter yield was obtained from the laser stimulated group (L), where statistical differences were found, as compared to the control and to other stimulation methods.

On average, higher crop yield was harvested from 5-6-year mature lucerne as compared to 1-2-year young plants.

The application of laser light resulted in a significant increase in the density of shoots, but at the same time the mass of a single shoot decreased. However, the increase in the number of shoots per unit area was higher than the decrease in the mass of a single shoot, which resulted therefore in an increase of dry matter yield. Such a reaction of lucerne to stimulation with laser light was previously reported by other authors (Ćwintal and Dziwulska-Hunek 2013, Ćwintal and Sowa 2006, Dziwulska *et al.* 2006b).

In the light of literature (Stanisavljevic *et al.* 2012), the mean dry matter yield obtained from cuts in this study was comparable to those obtained in the year of sowing, but lower than those reported as harvested during the years of full land use.

The leaves are the most valuable component of the yield of lucerne, therefore the quality of feed is determined by the fraction of leaves in the lucerne yield. The leaves are more valuable nutritionally than the stems. Besides, they are the main source of carotenoids and chlorophylls (Gaweł 2012).

The dependence of the share of leaves in dry matter on the variety (hybrid lucerne variety Radius and sowing lucerne variety Ulstar) and on the stimulation method is shown in Fig. 1. It follows that in both years of survey a ca. 2% higher leaf fraction was observed for cv. Ulstar. Besides, as compared to the control, a higher leaf fraction was observed in treatments where seeds were subjected to electromagnetic stimulation.

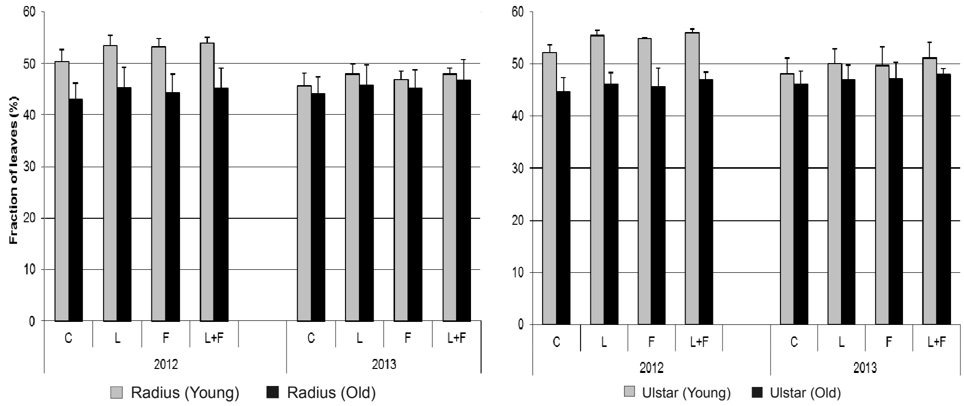

Fig. 1. Fraction of leaves versus variety

A greater effect of stimulation of seeds on the increase in the fraction of leaves was observed in 2012. In 2013 leaves constituted a smaller fraction in the lucerne plant and stimulation had also a smaller effect on this parameter. Comparing young and old lucerne plants in terms of the share of leaves in the yield, the greatest diversity of this feature was observed in 2012 (Fig. 1). More leaves in dry matter were observed at young plants, for which this was the year of sowing. Bigger also was the influence of stimulation on the increase of the share of leaves as compared with older plants.

In 2013 a similar trend occurred, but with much less differentiation between 1-2 and 5-6-year old lucerne plants.

The highest diversity in the leaf fraction of young and old lucerne plants was found for the 1st experimental year (Fig. 1). Generally, more leaves in dry matter were observed in young lucerne for which the particular year was the year of sowing. A higher influence of electromagnetic stimulation of seeds on the fraction of leaves was also observed for younger plants. A similar tendency took place in the second experimental year.

The fact that the weather has a decisive influence on the yield of lucerne is commonly observed (Ćwintal and Dziwulska-Hunek 2013, Stanisavljević *et al.* 2012, Testa *et al.* 2011). In this study the average density of lucerne stems per 1 m^2 in the cuts was lower than reported by other authors (Stanisavljevic *et al.* 2012). This should be explained by less favourable weather conditions for the growth of young plants in the first experimental year.

This work also involved studying the effects of electromagnetic stimulation of lucerne seeds on the content of photosynthetic pigments. Table 5 presents the effect of electromagnetic stimulation of the seeds on chlorophyll *a* content in lucerne leaves (in $\mu g \cdot g^{-1}$). There was a statistically significant increase in the concentration of this pigment depending on the factors of electromagnetic stimulation against the control. The concentration of chlorophyll *a* was significantly higher in variety Radius, in the year of the study 2012, as well as in young lucerne plants (1-2-year old). No differences were found between the cuts. From the interaction between the examined factors it follows that stimulation with alternating magnetic field caused a statistically significant increase in the concentration of chlorophyll in the leaves in variety Ulstar in 2013 and in the 5-6-year old lucerne.

Table 5. Chlorophyll *a* content in lucerne leaves ($\mu g\ g^{-1}$)

Specification		C	L	F	L+P	Mean
Year of utilization	Young	2104.65^{aA}	2224.68^{aA}	2148.43^{aA}	2143.24^{aA}	2155.25^{A}
	Old	1763.88^{bB}	1866.47^{bB}	2124.93^{aA}	1935.10^{bA}	1923.85^{B}
Variety	Radius	2052.93^{aA}	2199.68^{aA}	2117.92^{aA}	2172.39^{aA}	2135.73^{A}
	Ulstar	1815.60^{bA}	1891.48^{bB}	2155.44^{aA}	1905.96^{bA}	1942.12^{B}
Cut	1	1964.83^{aA}	2028.71^{aA}	2107.38^{aA}	2094.66^{aA}	2048.90^{A}
	2	1903.69^{aA}	2062.45^{aA}	2165.98^{aA}	1983.67^{aA}	2028.95^{A}
Year	2012	2104.65^{aA}	2224.68^{aA}	2148.43^{aA}	2143.24^{aA}	2155.25^{A}
	2013	1763.88^{bB}	1866.47^{bB}	2124.93^{aA}	1935.10^{bA}	1923.85^{B}
Mean		612.39^{c}	1934.26^{b}	2047.61^{a}	2110.98^{a}	2036.32^{a}

Explanations as in Table 2

Studies on the effect of laser stimulation of lucerne seeds on the intensity of photosynthesis, carried out under strictly controlled conditions of the greenhouse, showed an increase in the average rate of photosynthesis from 17.2 to 18.9 μmol CO_2 m^{-2} s^{-1} for variety Radius and from 16.9 to 18.8 μmol CO_2 m^{-2} s^{-1} for the variety Legend (Ćwintal and Olszewski 2007).

Table 6. Chlorophyll *b* content in lucerne leaves (μg g^{-1})

Specification		C	L	F	L+P	Mean
Year of use	Young	652.40^{aA}	665.70^{aA}	654.33^{aA}	651.93^{aA}	656.09^{A}
	Old	572.39^{aA}	605.28^{aA}	704.76^{aA}	626.43^{aA}	627.22^{A}
Variety	Radius	660.71^{aA}	695.37^{aA}	668.28^{aA}	692.33^{aA}	679.17^{A}
	Ulstar	564.08^{aA}	575.61^{aB}	690.81^{aA}	586.06^{aA}	604.14^{B}
Cut	1	597.49^{aA}	600.19^{aA}	631.21^{aA}	627.10^{aA}	614.00^{A}
	2	627.30^{aA}	670.78^{aA}	727.88^{aA}	651.24^{aA}	669.30^{A}
Year	2012	762.65^{aA}	758.18^{aA}	831.61^{aA}	792.33^{aA}	786.19^{A}
	2013	462.13^{bB}	512.79^{bB}	527.48^{aB}	486.02^{bB}	497.11^{B}
Mean		612.39^{c}	635.49^{cb}	679.55^{a}	639.18^{ac}	–

Explanations as in Table 2

The concentration of chlorophyll b varied between the variants of stimulation, varieties and years of research, and depended on the interaction between these factors (Table 6). The highest chlorophyll b content was found in lucerne leaves grown from seeds stimulated with an alternating magnetic field. This result differed significantly from the control and also from the variant where seeds were stimulated with laser light, but did not differ from the experimental variant L + F. From the analysis of interactions it follows that upon laser stimulation variety Ulstar had significantly less chlorophyll b than variety Radius. On the other hand, in 2013 significantly more chlorophyll b was found in lucerne stimulated with an alternating magnetic field.

Following the comparison between the examined electromagnetic factors, stimulation with alternating magnetic field resulted in a statistically significant increase in chlorophyll *a* concentration in leaves of cv. Ulstar in the second experimental year as well as in mature plants. Chlorophyll *b* concentration varied between the stimulation variants, varieties and years of the experiment (Table 6). The highest chlorophyll *b* concentration was observed in lucerne plants whose seeds were subjected to pre-sowing stimulation with magnetic field.

The electromagnetic methods used in this study resulted in a change in the concentration of chlorophyll *a* and *b* in most cases. Under the laser stimulation, *cv.* Ulstar had less chlorophyll *b* than *cv.* Radius. On the other hand, in the 2^{nd} experimental year statistically more chlorophyll *b* was found in the plants from seeds stimulated with alternating electromagnetic field. Such a result was found previously in the preliminary studies conducted by Sujak *et al.* (2013) on the effect of electromagnetic stimulation factors on pigment content in lucerne (variety Sitel and Legend).

Iqbal *et al.* (2012) conducted research on the effect of alternating magnetic field on the content of chlorophylls *a* and *b* in pea and obtained highly varied effects as compared to the control sample. The highest increase in the pigment concentration was recorded following the seed treatment with a magnetic field of 60 mT and 120 mT and exposure time of 5 minutes. The alternating magnetic field of 180 mT applied for 5, 10 and 15 minutes caused an increase of chlorophyll *a* and *b* with relation to the control sample. In contrast, the concentration of chlorophyll *b* increased only after seed treatment with the magnetic field of 60 mT for 10 and 15 minutes.

Prośba-Białczyk *et al.* (2013) conducted experiments on the effect of pre-sowing application of semi-conductor laser light with power of 200 mW and wavelength of 670 nm on seeds of sugar beet. In that experiment, concentrations of the sum of chlorophylls and carotenoids were determined. The best results of the increase of concentrations of those pigments with respect to control were observed at five times the basic dose.

Experiments on concentration of chlorophyll *a* and *b* and carotenoids were also conducted on plants grown from seeds of lentils, irrigated with magnetised water (Abdul Qados and Hozayn 2010). The results indicate that the magnetised water contributed to increasing the content of carotenoids and chlorophylls *a* and *b*, as compared to tap water, by 14, 44 and 3%.

Atak *et al.* (2007) stimulated soybeans with magnetic field of 2.9-4.6 mT and exposure times of 2.2 and 19.8 s. Their study shows that the magnetic field applied for 2.2 s resulted in increase of the concentration of chlorophyll *a* and *b* by 21 and 13%, respectively, compared to control treatments.

Concentration of carotenoids in lucerne seeds varied considerably depending on the stimulation method, cut, and on the year of experiment (Table 7). The highest concentration of these pigments was found in the leaves of lucerne previously stimulated with magnetic field (F). The results obtained were statistically different from the control as well as from the laser stimulated variants. Statistical differences in carotenoids concentration were also found between the control (C) and the laser stimulated samples (L), as well as between the control and the combination of laser light and magnetic field (L+F). Besides, richer in this pigments were Radius variety plants from the 1st cut and young lucerne plants. On the other hand, lower concentration of carotenoids was observed in Ulstar variety pre-treated with laser light, and an increase in the magnetic field-treated plants. Lower carotenoid concentration was determined at variety Ulstar in experimental variant with laser stimulation. Analysis of interactions shows that lower carotenoid concentration was found in Ulstar variety stimulated with laser light while higher concentrations of these pigments were found in experimental variants with stimulation with magnetic field (F). In conclusion it should be emphasised that the highest contents of chlorophyll a and b and carotenoids were observed in lucerne stimulated with alternating magnetic field, where the effect of an increase of concentrations of photosynthetic pigments amounted to 9, 11, and 13%, respectively, as compared to the control.

Table 7. Carotenoids content in lucerne leaves (μg g^{-1})

Specification		C	L	F	L+P	Mean
Year of use	Young	255.99^{aA}	275.57^{aA}	285.79^{aA}	274.33^{aA}	272.92^{A}
	Old	214.44^{aA}	228.67^{aA}	243.80^{aA}	238.36^{aA}	231.32^{B}
Variety	Radius	237.69^{aA}	278.00^{aA}	267.89^{aA}	278.50^{aA}	265.52^{A}
	Ulstar	232.74^{aA}	226.24^{aB}	261.70^{aA}	234.19^{aA}	238.72^{B}
Cut	1	281.81^{aA}	283.01^{aA}	317.60^{aA}	297.45^{aA}	294.97^{A}
	2	188.61^{aB}	221.22^{aB}	211.98^{aB}	215.21^{aB}	209.26^{B}
Year	2012	227.92^{aA}	239.33^{aA}	250.59^{aA}	263.19^{aA}	245.26^{A}
	2013	242.50^{bA}	264.90^{bA}	278.99^{aA}	249.50^{bA}	258.97^{A}
Mean		235.21^{c}	252.12^{bc}	264.79^{a}	256.34^{ab}	–

Explanations as in Table 2

CONCLUSIONS

1. Electromagnetic stimulation of lucerne seed resulted in a significant increase of the number of shoots per 1 m^2, dry matter yield, as well as in the content of chlorophyll a, b and carotenoids in the leaves

2. Under the influence of electromagnetic stimulation an increase in the share of leaves in the yield, from 43.8 to 55.0%, was observed.

3. Significantly higher mass of a single shoot and dry matter yield were observed at 5-6-year old lucerne plants and in 2013, while a higher fraction of leaves in 1-2-year old plants and in 2012.

4. Significantly more chlorophyll *a* and *b* was found in leaves of Radius variety as well as at lucerne plants in 2012. The content of chlorophyll *a*, *b* and carotenoids was higher in young lucerne plants.

5. The observed variability of yield components and the qualitative characteristics of lucerne under the influence of electromagnetic stimulation of seeds, persisting in different time of plant use, needs further study.

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STRUKTURA PLONU STAREJ I MŁODEJ LUCERNY Z NASION PODDANYCH PRZEDSIEWNEJ STYMULACJI ELEKTROMAGNETYCZNEJ

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S t r e s z c z e n i e. Lucerna należy do wieloletnich roślin pastewnych z rodziny bobowatych i uprawiana jest w warunkach polowych na paszę dla różnych grup zwierząt. Niniejsza praca dotyczy wpływu stymulacji elektromagnetycznej nasion lucerny na plon i elementy jego struktury oraz na zawartość barwników fotosyntetycznych w roślinach 1-2 oraz 5-6 letnich. W latach 2012-2013 przeprowadzono doświadczenie polowe z lucerną 5-6 letnią, które założono w 2008 roku i drugie równorzędne założone w 2012 roku z 1-2 letnią. Materiałem badawczym były nasiona lucerny mieszańcowej (*Medicago x varia* T. Martyn.) odmiany Radius i siewnej (*Medicago sativa* L.) odmiany Ulstar. Przed siewem nasiona stymulowano w następujących kombinacjach: C – kontrola (bez stymulacji), L – światło lasera o powierzchniowej gęstości mocy 6 $mW \cdot cm^{-2}$ stosowane 3-krotnie, F – zmienne pole magnetyczne o indukcji 30 mT i czasie ekspozycji 30s; L+F – światło lasera i pole magnetyczne w powyższych dawkach. Stymulacja elektromagnetyczna wpłynęła istotnie statystycznie na wzrost liczby pędów na 1 m^2 na tle kontroli. Z kolei nie było istotnych różnic statystycznych w masie pojedynczego pędu. Lucerna 5-6 letnia odznaczała się statystycznie większą

masą pędu w porównaniu z 1-2 letnią. Z analizy statystycznej wynika, iż największy wzrost plonu był w przypadku stymulacji światłem lasera oraz połączonej światłem lasera i polem magnetycznym w stosunku do kontroli. Najlepsze efekty stężenia chlorofili a i b oraz karotenoidów były dla zmiennego pola magnetycznego, gdzie wzrost wyniósł odpowiednio: 9, 11 i 13,0% na tle kontroli.
Słowa kluczowe: lucerna, plon, barwniki fotosyntetyczne, stymulacja elektromagnetyczna, eksperyment polowy