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Morphological changes and yield of selected species of leguminous plants under the influence of seed treatment with laser light

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A b s t r a c t. Field and pot experiments were carried out between 1998 and 2000. The factors of the 1st order were faba bean and white lupine varieties, and the factors of the 2nd order were doses of laser irradiation. Seed irradiation was carried out using an apparatus equipped with a helium-neon laser. The seed treatment with laser light favourably influenced both the sprouting and shooting stages of the white lupine and the faba bean and modified the course of the individual phases of the plants' development. The result of these changes was accelerated flowering and plant maturity in the pot experiments, and accelerated drying out of those plants in the field experiments. Seed irradiation caused an increase of leaf surface area – the largest occurring in the period of intensive plant growth from the 2-3 leaf phase to full flowering. Irradiation of the sowing material influenced seed yield positively in the leguminous plants studied. An increase in yield was the effect of the increased pod complement of the plants in the field experiments and also of a decrease in plant losses during vegetation. The 1000 seed weight and the number of seeds per pod did not undergo any significant changes. The effectiveness of the treatment applied in the different individual study years relates the effects of irradiation to weather conditions.

K e y w o r d s: laser beam, leguminous plants, emergence, yield

INTRODUCTION

The development of modern agriculture and the propagation of rules for the rational use of natural resources related thereto demand a search for a safe method of increasing the size and improving the quality of cultivated plants. The appropriate preparation of the sowing material is an important yield generating factor which aims to improve seed sprouting ability and the vigour of the seedlings grown from them (Górecki and Grzesiuk, 1994). Young and more vigorous plants develop better, and are better suited to endure unfavourable habitats; they are also less susceptible to disease and require less intensive chemical protection. These initial growth stages decide then, to a higher degree, the further development of plants and their ultimate yield. Many chemical, physical and physiological methods for improving sowing material are well known at present. The best recognised and the most often used methods in actual practice are chemical methods of seed dressing with various substances (seed dressings, growth regulators, *etc*.). The substances used, even though highly efficient, pose a danger to the environment since many active substances can penetrate the seed and modify its chemical composition and also pollute the soil environment. Hence, in recent years, more attention has been paid to some physical factors which favourably influence the sowing material of cultivated plants (Drozd, 1994; Olchowiak and Dziamba, 1994; Phirke *et al*., 1996; Pietruszewski, 1993). The prevailing opinion is that physical methods for the processing of pre-sowing seed stimulate only the physiological and biochemical changes in the seeds (Anisimov *et al*., 1997; Galova, 1996; Grzesiuk and Kulka, 1986; Podleśny, 2000a; Smith, 1991) and hence, are safe for the environment. No physical method could be a substitute for highly efficient chemical methods, but they could adequately supplement them. One of the physical methods which can be applied to the improvement of the sowing material is seed treatment with laser light (Inyushin *et al*., 1981; Ivanova, 1998: Koper, 1994; Podleśny, 2002).

The aim of the present study was to determine the influence of the treatment of pre-sowing seed with laser light on the initial growth stages of the plant and the changes to some morphological properties and the yield of white lupine and the faba bean.

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METHODS

The present studies were carried out in the Institute of Soil Science and Plant Cultivation in Puławy in the period of 1998-2000. Pot experiments were carried out in the vegetation hall in Mitscherlich's pots with a mixture of 5 kg of garden soil and 2 kg of sand. The object of the study was white lupine and the faba bean species of leguminous plants characterised by their long vegetation periods and their relatively low seed yield which varies annually.

Ten white lupine and faba bean seeds were sown in each pot. Immediately after shooting, the plants were thinned leaving only 5 plants per pot. NPK fertilisation was used in the form of 0.30 g - NH_4NO_3 and 4.4 g - KH_2PO_4 which was added to each pot. An automatic apparatus for precise watering of the plants was used, coupled with a fertiliser dose-feeder. The plants were watered with distilled water up to 60% of the field water capacity of the soil. The factor of the 1st order was the two varieties of white lupine: Bardo - a traditional form, and Katon – a determinate form; and faba bean: Nadwiślański – a traditional form, and Tim – a determinate form; the factor of the 2nd order was the doses of laser irradiation: D0 – no irradiation, D3 – triple irradiation, D5 – fivefold irradiation of the seeds. A single exposure dose was 4 10^{-3} J $cm^{-2}s^{-1}$. Since the first emergence appeared, they were counted every day in order to estimate the dynamics of plant emergence (D_e). The D_e coefficient was counted by using the following formula:

$$D_e\,(\%) = N_e\, N_s^{-1}\, 100\%,$$

where: N_e – % of emerging plants, N_s – number of seeds sown.

In the pot experiments, the D_e coefficient was referred to the number of seeds in pot, and in the field experiments - to the number of seeds sowed within an area of 1 m^2.

During the whole vegetation period, detailed observations of the plants' growth and development were carried out. During flowering, biometric measurements were carried out to determine plant height, leaf surface area and the number of leaves, while at full maturity parameters such as the distance from the first to the last pod, the number of pods, the number and weight of the seeds and seed humidity were also determined. The results of the above experiments were the mean values for the three pots.

The field experiments were carried out in the Agricultural Experimental Station in Puławy-Kępa in the period of 1998-2000, using a layout of split-blocks on the soil from a good, IIIa class, wheat complex. In each experimental year, the pre-yields were cereals. Mineral fertilisation was as follows: P_2O_5 - 60 and K_2O - 80 kg ha^{-1}; fertilisation with nitrogen was not applied. The surface area of the plots for yield collection was 12 m^2. The factor of the 1st order was the two varieties of white lupine: Bardo – a traditional form, and Katon – a determinate form; and the two varieties of faba bean: Nadwiślański - a traditional form, and Tim – a determinate form; whereas the factor of the 2nd order was the three doses of laser irradiation, the same as in the pot experiments. The lupine seeds were sown to a depth of about 3-4 cm, at a density of 80 plants per 1 m^2, and the faba bean seeds at a depth of 10-12 cm and at a density of 40 plants per 1 m^2. Weeds were controlled using the 'Afalon' preparation at a dose of 1.2 kg ha^{-1} in the soil after sowing; in the later period of the lupine development, weeds were destroyed mechanically. During vegetation, detailed observations and measurements of the plants and their infections by pathogens were carried out. Plants per 1m^2 were counted several times during the shooting stage and immediately before harvest. The dates of the occurrence of the consecutive phases of the plants' development, *ie*: full emergence, the beginning and end of flowering, browning of about 5 and 80% of the pods, were noted. Before harvest, 10 plants were randomly selected from each plot and the following parameters were determined: height from the first to the last – or top – pod and to the plant top, the number of pods per plant, and the number of seeds per plant. After harvest, the following parameters were determined: plot yield, humidity and weight of 1000 seeds. Seed yield was calculated for a 14% humidity level. Seed irradiation applied in both pot and field experiments was carried out at the Chair of Physics of the Agricultural University of Lublin using an apparatus for the treatment of pre-sowing seed with laser irradiation, equipped with a helium-neon laser (Koper, 1996). The results of the experiment were statistically calculated using variance analysis. Tukey's interval of confidence at a level of significance of $\alpha = 0.05$ was applied to the statistical analysis.

RESULTS

Initial plant growth in the pot experiments was uniform and occurred earlier than that in the field experiments. Initial white lupine and faba bean growth in the vegetation hall was observed, respectively, after 8 and 14 days (Fig. 1), whereas in the field experiments, after 11 and 18 days from sowing (Fig. 2). The pattern of weather conditions had a high influence on the dynamics of initial plant growth. Due to heavy precipitation levels during sowing in 1998 and 1999, initial plant growth was more uniform and occurred 5-6 days earlier than in 2000 which was characterised by a deficiency of precipitation in this period. Pre-sowing seed irradiation had a favourable influence on the date and dynamics of the initial plant growth stages of the species studied. It was observed that the treatment applied resulted in a higher number of emerging plants and a 2-3 day acceleration of the dates where initial growth became evident. The noticeable influence of the treatment of seed irradiation on the initial growth period was observed in both the pot experiments and

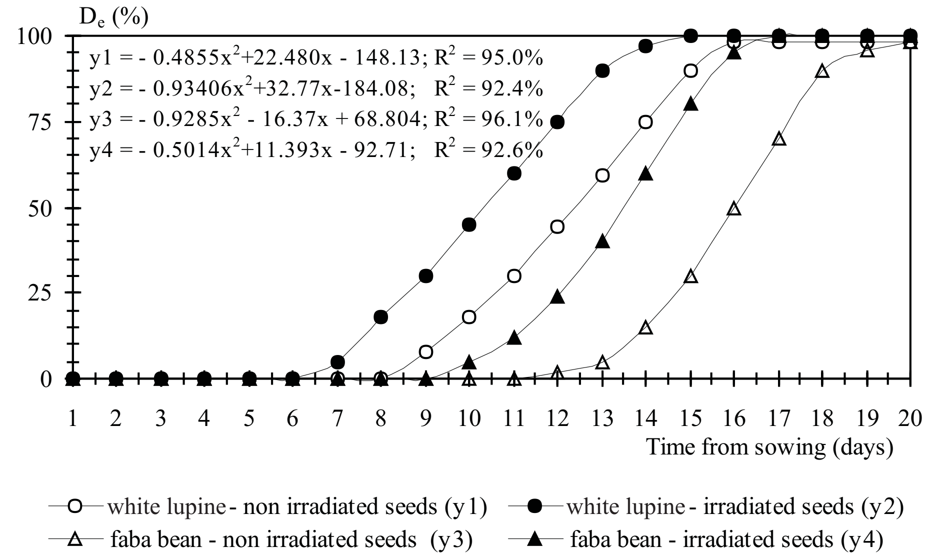

Fig. 1. Dynamics of white lupine and faba bean emergence in pot experiments.

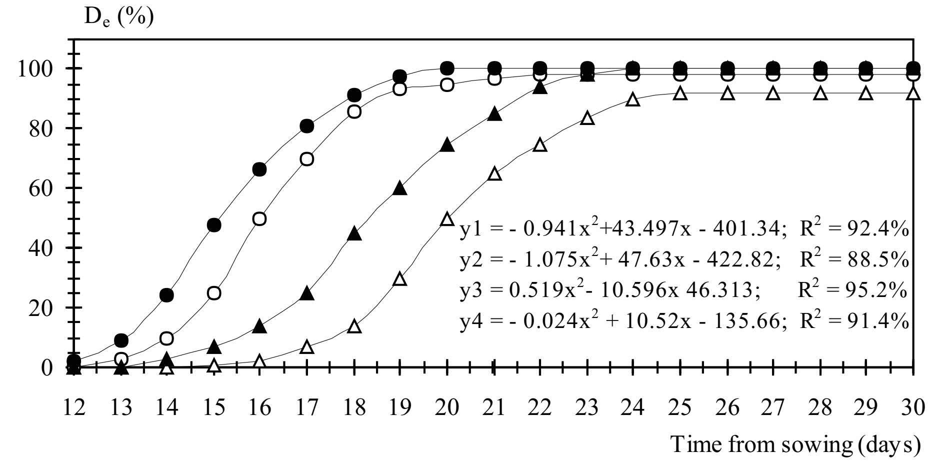

Fig. 2. Dynamics of white lupine and faba bean emergence at field experiments. Explanations as in Fig. 1.

the field experiments, but to a somewhat lesser extent in the latter.

Clear differences in the sprouting of the individual varieties of white lupine and faba bean were also evident. These resulted first of all from the different sprouting capacity of the seeds. Laser light similarly stimulated both the sprouting and the initial growth phases of both the self-ending and traditional varieties of the leguminous plant species studied. As a result of the accelerated seed sprouting, seedlings obtained from them were higher and better formed than the seedlings grown from the control seeds.

Detailed biometric measurements of individual plant organs showed that the irradiation of seeds modified their morphological properties. In plants grown from irradiated seeds, a significantly increased leaf surface area in the period from emergence to flowering was found. On the other hand, no significant differences in the leaf surface areas of the lupine varieties studied were observed; hence the graph illustrating the course of the changes described was presented jointly for the two varieties (Fig. 3). During flowering, the plants of the faba bean and the lupine grown from irradiated seeds formed a leaf surface area larger by 34 and 42%, respectively, than that formed in the control plants.

Slight differences related to plant irradiation were observed in plant height before harvest, but these concerned mainly plants grown from seeds irradiated five times, and only for this last dose were these differences statistically proven (Table 1). No significant differences in plant height were found in the varieties of white lupine studied. The number of leaves per plant changed only slightly. The only observation made was a tendency towards an increase in the number of leaves per plant grown from the irradiated sowing material. Traditional varieties of leguminous plants produced a higher number of leaves than did the determinate

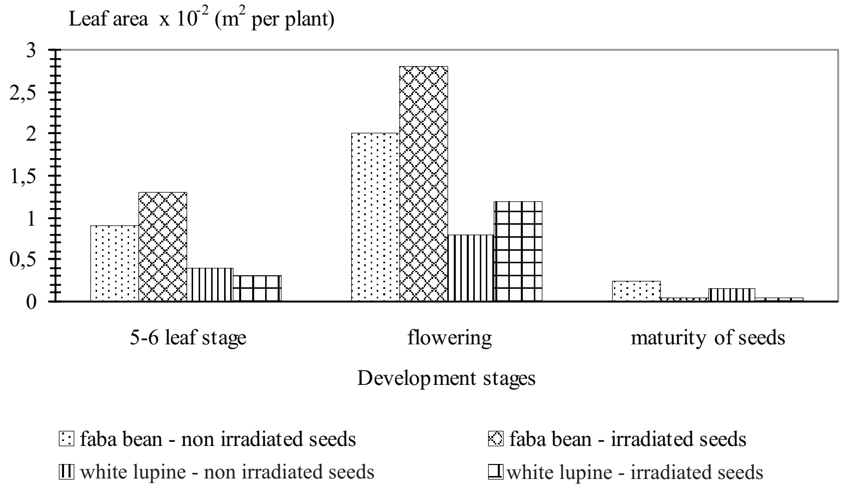

Fig. 3. Change of leaf area of white lupine and faba bean plants – pot experiments.

varieties, which resulted from the differences in the morphological constitution of these varieties.

Seed irradiation had a considerable impact on the shoot length with pods (Table 1). Both the three-fold and five-fold seed irradiation influenced favourably this morphological feature of the plants.

In the pot experiments, a clear influence of seed irradiation on the course of some phases in the plants' development was observed. Earlier flowering and maturity of both the white lupine and the faba bean were observed, whereas in the field experiments, no clear differences in the course of the development phases of the plants grown from the seeds treated and untreated with laser light were observed. Only the lower content of water in the seeds examined immediately after harvest proved the quicker maturity of the plants grown from irradiated seeds.

During the whole experimental period, the traditional varieties of faba bean and white lupine provided better yields than the self-ending varieties. Irradiation of the sowing material caused a significant increase in seed yield, with better effects observed in the white lupine than in the faba bean (Table 2).

Both irradiation doses applied in the experiment had a positive influence on the size of the seed yield obtained. On average, from the three study years and two varieties, the increase in the faba bean seed yield after the three-fold and the five-fold irradiation of the seeds as compared to those not irradiated was, respectively, 9.2 and 12.2%, whereas in the case of the white lupine the corresponding values were 17 and 14.9%, respectively.

Weather conditions during the vegetation period had a very great impact on the effectiveness of irradiation measured by the yield size of the faba bean seeds; hence the various values of the effect of the seed irradiation measured by the size of the faba bean seed yield were obtained in the individual study years. No significant differences in plant

T a b l e 1. Values of some morphological and utility features before harvest – pot experiments

Doses/varietes	Height of plant (m)		Length of stem with pods (m)		Number of seeds per pod	
	faba bean	white lupine	faba bean	white lupine	faba bean	white lupine
Doses of laser irradiation:						
D0	1.12a	0.457a*	0.32a	0.056a	6.1a	4.4a
D3	1.16a	0.463a	0.38b	0.088b	6.0a	4.0a
D5	1.19a	0.492b	0.36a	0.069b	6.2a	4.2a
Varieties						
Nadwiślański	1.27a	-	0.44a	-	6.7a	-
Tim	1.04b	-	0.26b	-	5.4b	-
Bardo	-	0.475a	-	0.075a	-	3.8a
Katon	-	0.467a	-	0.067b	-	4.3b

*values in columns marked with the same letters do not differ significantly.

T a b l e 2. Yield of seeds (kg m^{-2}) and number of pods per faba bean and white lupine plants – field experiment

Doses/variates	Means from years							
	1998-1999				2000			
	white lupine		faba bean		white lupine		faba bean	
	yield of seeds	number of pods	yield of seeds	number of pods	yield of seeds	number of pods	yield of seeds	number of pods
Doses of laser irradiation:								
D0	0.18a*	20a	0.74a	11.4a	0.29a	25a	0.57a	11.2a
D3	0.22b	21b	0.81b	12.6b	0.33b	27b	0.62b	12.1b
D5	0.21b	22b	0.80b	12.6b	0.33b	26c	0.67b	12.8b
Varieties								
Nadwiślański	-	-	0.80a	12.3a	-	-	0.67a	12.0a
Tim	-	-	0.76b	12.1a	-	-	0.57b	12.3a
Bardo	0.24a	22a	-	-	0.34a	27a	-	-
Katon	0.18b	20b	-	-	0.26b	24b	-	-

*values in columns marked with the same letters do not differ significantly.

yields were observed, with the exception of those in 1998 and 1999; hence the value of the yield obtained was given as the mean value of those two years.

In the pot experiments, the increase of seed yield resulted from the increased pod complement per plant, and in the field experiments it was also a result of smaller plant losses during the vegetation period. During the three years of the present study, the mean value of plants dropping out of the canopy of the two varieties of faba bean from sowing to harvest was 4.6% of the plants grown from irradiated seeds, and 8.2% of the plants grown from non-irradiated seeds. For the two varieties of white lupine, the above values were, respectively, 6.8 and 14.2%. The white lupine grown from non-irradiated seeds and the seeds irradiated three and five times produced, respectively: 22.5; 24 and 22 pods. For the faba bean, the above values were, respectively, 11.3; 12.4 and 12.2 pods. The traditional varieties of white lupine and faba bean were characterised by larger pod complements per plant during harvesting than were the plants of the determinate varieties.

The seeds of the white lupine var. Katon and of the faba bean var. Nadwiślański were characterised by a higher weight of 1000 seeds than the seeds of the white lupine var. Bardo and the faba bean var. Tim. Seed irradiation did not exert any significant influence on the number of seeds per pod or the weight of 1000 seeds of the varieties of leguminous plants studied.

DISCUSSION

The present study showed a positive influence of pre-sowing seed irradiation on the increase of uniformity and emergence dates of the white lupine and faba bean grown both in a vegetation hall and in an experimental field. This is of special significance in the case of studies carried out on the species of leguminous plants studied, as they are characterised by a long vegetation period; seeds are collected often in late summer when weather conditions do not favour maturing or the drying out of the plants. Seeds obtained in such conditions are sowing material of poor quality, as their sprouting capacity is small and they develop unevenly. Seedlings grown from such seeds are weak and more susceptible to fungal infection than seedlings from well sprouting seeds. To date, very few studies have been undertaken on the possibility of increasing the sprouting capacity of leguminous plants by treating them with laser light and other physical factors. The influence of seed irradiation with laser light on the acceleration and uniformity of the initial growth phase of plants was especially visible in experiments carried out in controlled temperatures and soil humidity levels (Podleśny, 2000b). The study by Szyrmer and Klimont (1999) is one of the very few works on the impact of laser light on the sprouting of leguminous plants. This advances the fact that the number of dead and abnormally sprouting bean seeds significantly decreased as a result of their irradiation with helium-neon laser light. Further studies proved increased seed sprouting capacity and the acceleration of plant emergence dates for cereal plants and vegetables after the pre-sowing irradiation of seeds with laser light (Drozd and Szajsner, 1997; Drozd *et al*., 1996; Wilde *et al*., 1969; Zhidong and Shuzhen, 1990).

Many researchers claim that the effects resulting from seed irradiation with laser light are especially visible in sprouting seeds and seedlings (Drozd and Szajsner, 1997; Szyrmer and Klimont, 1999; Toth *et al*., 1993; Wilde *et al*., 1969), which suggests that the reasons for the influence of irradiation should be researched using biochemical and physiological studies of seeds and young plants. The feasibility of such a line of thinking was proved by the studies by Durkova (1993), Galova (1996) and Podleśny (2000a) which showed the positive influence of laser light on a-amylase activity and the concentration of free radicals in the seeds of several winter wheat varieties as well as the faba bean and white lupine. In a very small number of studies, other changes in seeds subjected to irradiation with laser light were also found. For example, Chuvaeva *et al.* (1981) and Sebanek *et al.* (1989) found an increase in the activity of some phyto-hormones, mainly indolilo-3-acetic acid (IAA), in the irradiated seeds of sowing pea and maize. Grzesiuk and Kulka (1986) pointed to the versatile influence of laser light on seeds but emphasised the strongest influence on their enzymatic activity.

From the studies carried out so far, it follows that irradiation of the sowing material causes significant changes in the later phases of plant ontogenesis and the formation of yield-generating properties. Studies by other authors show a significant influence of seed irradiation on the course of plant ontogenesis, both during the period of vegetative growth and of generative development. A quicker rate of development of plants grown from irradiated seeds as compared to the control plants was observed. This caused earlier flowering and maturity. The above was also confirmed by studies on the course of changes in the dry mass content of plants grown from irradiated and non-irradiated seeds which showed that the biggest difference in the dry mass content of plants occurred during their maturing period. According to Grzesiuk and Kulka (1986), the influence of physical factors on seeds was often manifested in the later periods of ontogenesis, which then resulted in quicker plant maturing, and which is of special significance in the growing of species with a long vegetation period. The results obtained are especially valuable in relation to white lupine and faba bean which are plants with a long vegetation period (Gladstones *et al.*, 1998; Jasińska and Kotecki, 1993). It follows from the studies carried out that the rays of a helium-neon laser emitting a bundle of red light of high surface density (Popp, 1984) exert a similar influence on plant development and yields as some other physical factors (Grzesiuk and Kulka, 1986; Kurobaru *et al.*, 1979; Pittman *et al.*, 1979; Smith, 1991). The phenomenon of the reduction of the vegetation period caused by irradiation of the sowing material of lupine observed by the present authors was very distinct in the experiments carried out in the strictly controlled conditions of climatic chambers (Podleśny, 2000b). In field experiments, in which the influence of the habitat conditions on plants is greater, it is more difficult to observe the effect of the accelerated maturing process resulting from seed irradiation. The earlier maturing of plants grown in experimental fields was only observed on the basis of the water content in seeds after harvest (Podleśny, 2002), even though some researchers observed – during field experiments – (Cvetkovic *et al.*, 1996, Gieroba *et al.*, 1995; Shtanko, 1979) the earlier flowering of some plant species grown from irradiated seeds

Literature on the subject deals mainly with the final effect on the plants as measured by the size and quality of yield obtained in addition to the influence of seed irradiation on plant sprouting and emergence (Dziamba and Koper, 1992; Koper, 1994; Svetleva and Aladjadjian, 1996; Vasilevski *et al.*, 1996). The fact that the effects of seed irradiation are not manifested in all phases of the ontogenetic development of the plant is worth drawing attention to. This could be the reason for the divergence in the results obtained by the various researchers working on this problem. The above observations were confirmed by Grzesiuk and Kulka (1986) who are of the opinion that the effects resulting from the activity of some physical factors on seed can be visible in the ontogenesis of various plants.

The effect of the irradiation of the sowing material of lupine was an increase in seed yield. An analysis of the yield structure showed that an increase in white lupine and faba bean yield was a result of increased pod complement per plant which in leguminous plants is the property which most often changes due to the activity of other yield-generating factors; the number of seeds per pod is the least changeable property. Irradiation of the sowing material of the leguminous plant species studied did not exert any significant influence on the number of seeds per pod or the 1000 seed weight. Hence, in the case of the white lupine and the faba bean, an increase in yield was caused by a change in other yield-generating features than is the case in cereals – since according to Dziamba and Koper (1992) and some other authors (Inyushin, 1981; Rybiński *et al.*, 1993) – an increase of yield due to the irradiation of winter wheat seeds was caused by an increase of the weight of 1000 kernels and the number of kernels per spike.

Numerous studies on seed irradiation concentrate mainly on the size of yield obtained. The above studies, however, were not limited to the determination of the size of the harvest yield; they included a detailed analysis of the yield obtained through precise plant measurements. In the study presented herein, in addition to showing the influence of seed irradiation on leguminous plant development and yield, the authors have attempted to give reasons for the effects obtained. Among other considerations are the leaf surface area of those plants grown from laser radiation treated and untreated seeds and the positive influence of irradiation on the shaping of this important yield-generating factor. It is worth pointing to the fact that the differences in leaf surface area between those plants grown from the radiation treated and untreated seeds of both varieties were not identical in the individual phases of plant development. In plants grown from irradiated seeds, a significantly larger leaf surface area was observed during the period from emergence to flowering and pod formation; during maturing, a significantly smaller leaf surface area was noticed as compared to the control plants. The larger leaf surface area during the period of the vegetative growth of the plants from the irradiated seeds probably resulted from the quicker growth of these plants as compared to the control, whereas a decrease in the leaf surface area was a consequence of the earlier maturing of the former. An increase of leaf surface area can take place as a result of an increase of the surface area of one leaf or an increase in the number of leaves per plant. In both cases, this leads to an increase in the productivity of photosynthesis. In the studies of other authors, no significant differences in the number of leaves on the plants from the irradiated and non-irradiated seeds was observed. Hence, an increase in the total leaf surface area of a plant was due to an increase in the surface area of the individual leaves. There are only a few studies on the influence of laser light on the formation of the physiological indicators of plant productivity, hence it is difficult to compare the results obtained by the present authors with the results of other authors. Only Inyushin *et al.* (1981) stated that oat and cucumber plants grown from irradiated seeds produced larger leaf surface areas than plants grown from non-irradiated seeds; an increase of this surface took place as a result of an increase in both the number and size of the leaves.

Many researchers (Cepero *et al.*, 1997; Ivanova, 1998; Rybiński *et al.*, 1993; Svetleva and Aladjadjian, 1996) found that the effect of seed irradiation depended on the dose of laser light. Results of earlier studies (Podleśny, 2002) and of those presented in this work have not given any basis for the precise determination of the optimum irradiation dose for white lupine and faba bean seeds. Both irradiation doses applied had a favourable influence on the plants' growth and development. Studies by Rybiński *et al.* (1993) proved that it is necessary to determine a range of irradiation doses with a favourable influence and showed that going above these threshold doses of laser irradiation can lead to a decrease of yield and a worsening of its structure.

CONCLUSIONS

1. Seed irradiation positively influenced the sprouting and emergence of white lupine and faba bean and modified the course of individual phases in the development of these plants. The effect of the above changes was an acceleration of the emergence, the earlier flowering and maturing of plants in the pot experiments as well as the accelerated drying out of plants in the field experiments.

2. The pre-sowing laser treatment of seeds had a positive impact on seedling growth and development. Seedlings grown from irradiated seeds reached a significantly larger hypocotyl and root length compared to seedlings grown from non-irradiated seeds on the consecutive dates of measurements. A difference in plant height between those plants grown from irradiated seeds and those grown from the control seeds was also present in the later period of their growth and development.

3. A larger leaf surface was produced by those plants grown from the seeds treated with laser light than was produced by those plants grown from the control seeds.

4. Irradiation of the sowing material had a positive influence on the seed yield of both the varieties of white lupine and faba bean. The increase in yield was the result of an increased pod complement of the individual plants; in the field experiment there was also a decrease in plant losses during the vegetation period; the 1000 seed weight and the number of seeds per pod did not undergo any significant changes.

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