



CONDITIONING OF NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.) SOWING MATERIAL

*Hanna Szajsner*¹, *Anna Koszelnik-Leszek*², *Karolina Błasiak*³

¹ ORCID: 0000-0003-3429-8004

² ORCID: 0000-0002-5929-9372

^{1,3} Department of Genetics, Plant Breeding and Seeds Production

² Department of Botany and Plant Ecology

Wrocław University of Environmental and Life Sciences, Poland

Key words: semiconductor laser, Norway spruce, seeds, seedlings, bio-stimulation.

Abstract

Norway spruce (*Picea abies* (L.) Karst.) is a relatively demanding species (e.g. it does not tolerate drought, is very sensitive to environmental pollution, diseases and pests) compared to other conifers. It is therefore important to provide it with optimal growth conditions in the early stages of development. The laboratory and pot experiments were carried out on seed material of 10 different genotypes of Norway spruce bio-stimulated with semiconductor laser light. The following variants were used: control without irradiation (C) and multiplied dose variants ($0.25 \cdot 10^{-2} \text{ J/cm}^2$), three-fold (D3), six-fold (D6), and nine-fold (D9). Morphological features, fresh and dry weight of seedlings and the content of photosynthetically active pigments were assessed. Under the influence of the applied radiation, morphological features of seedlings were stimulated, and dose D6 turned out to be the most effective. It caused the stimulation of the tested features both in the experiment conducted in laboratory conditions and in a tunnel. The application of doses D3 and D9 caused an increase in chlorophyll content.

Introduction

Norway spruce (*Picea abies* (L.) Karst.) is an evergreen, fast-growing, erect tree species with many forms and varieties (MATĚJKA et al. 2014). It naturally grows in European forests from Scandinavia to Greece. In Central and southern Europe, the species naturally occurs mostly only in the mountains. Norway spruce moderately adapts to various climatic conditions. A growing season of at least 60 days and a minimum of 120 days of winter rest with freezing temperatures is necessary for it to develop properly (SVYSTUNA et al. 2021).

It has moderate soil requirements; it grows well on brown, fresh, or
Address: Anna Koszelnik-Leszek, Wrocław University of Environmental and Life Sciences, Wrocław, Poland, e-mail: anna.koszelnik-leszek@upwr.edu.pl

loam soils which are deep, moderately rich, not too acidic, and have an average depth of groundwater level (MURAT 2002, JAWORSKI 2011, MATĚJKA et al. 2014).

In Polish forests, the share of stands with a predominance of spruce is 5.6%. Spruce acts as an ecological admixture or acts as a second floor in stands in various habitats. Unfavorable changes occur in forests as a result of negative abiotic and biotic factors. In Europe, these changes include disturbances in the physiological processes of trees, such as flowering, fertility and quality of seeds obtained from forest tree seeds. The occurring changes in the genetic material result in a heritable mutation of some genotypes in individuals. Thus, a reduction in the quality of seeds and seedlings may be the effect of these changes (KORCZYK et al. 2010).

It seems that not only environmental contamination, but also long-term storage of seed material, e.g. in gene banks, also causes a quality drop associated with changes in their condition – aging – and therefore the seeds are subjected to various forms of refinement (COPELAND and MC DONALD 2001, KUBALA et al. 2013). In pre-sowing conditioning of seed materials, stratification is used (GRZESIK et al. 2012). Chemical methods are also used, e.g. treating dry, hard seeds with concentrated sulfuric acid (WOJCIECHOWSKA 1983).

In recent years, due to the global desire to reduce the excessive use of chemical compounds in plant production and the increasing acreage of plants cultivated using organic methods, in the improvement of seeds, other factors are used that have a beneficial effect on physiological processes (GRZESIK et al. 2012, HERNANDEZ-AGUILAR et al. 2016).

One of these factors is radiation, for example caused by a semiconductor laser (RYBIŃSKI AND GORCZYŃSKI 2004, KLIMONT 2006, SOLIMAN and HARITH 2010, GRZESIK et al. 2012). It is mainly used to stimulate cultivated plant growth (KLIMONT 2006). Seeds conditioning with laser rays positively influences germination processes, plant growth and development, as well as the size and quality of the obtained crop (DOBROWOLSKI and RÓZANOWSKI 1998, HERNANDEZ-AGUILAR et al. 2016, SZAJSNER et al. 2017, KOSZELNIK-LESZEK et al. 2019, SZAJSNER et al. 2019).

Due to the lack of reports on the effects of laser radiation on seeds of forest trees (e.g. germination parameters and morphological characteristics of seedlings), an attempt was made to study the response of seed material of ten different genotypes of Norway spruce using semiconductor laser light under laboratory conditions and in a pot experiment.

The characteristics and origin of particular Norway spruce genotypes

Norway spruce seeds were obtained from Forest Gene Bank in Kostrzyca (Forest Reproductive Material 50°81'70"N 15°77'71"E) and were used for both the foil tunnel experiment (pot experiment) and the laboratory experiment. 10 spruce genotypes were selected (including A-11/ZP/12; B-106/ZP00; C-251/ZP04; D-251/ZP/04; E-262/ZP/07; F-470/ZP/04; G-514/ZP/03; H-567/ZP/03; J-986/ZP/06; K-1514ZP/00). Batches of seeds were collected during multiple years (genotypes: A – 2011, B – 2000, C – 2004, D – 2006, E – 1992, F – 2009, G – 2003, H – 2003, J – 2006, K – 2000).

The selected genotypes came from the following sites:

- Forest District Borki (A, D), 54°08'97"N – 21°91'22"E;
- Forest District Rokita (B, C), 53°76'76"N – 14°83'76"E;
- Forest District Nowy Targ (F, J), (49°69'95"N – 20°06'24"E);
- Forest District Śnieżka in Kowary (E), 50°79'06"N – 15°83'81"E;
- Forest District Wałbrzych (G), 50°73'57"N – 16°21'25"E;
- Forest District Ujsoły (K, H), 49°47'35"N – 19°11'78"E.

The propagating material used in the experiment belongs to the selected or qualified category (1999/105EC EU directive seed categories) and was of indigenous origin. The purity of the seed material used in the experiment varies as follows: 96% – H, 96.2% – J, 97% B, 97.2% – G, 97.4% – C, 98% – D, 98.3% – E, 99% – K, 99.9% – A, 100% – G; the average moisture content of all seeds was 5,3% (KORCZYK et al. 2010).

Laboratory experiment. The laboratory experiment was carried out in a SANYO environmental chamber, type MLR-351H. Norway spruce seeds were treated with semiconductor laser irradiation, type CTL 1106MX (with a power of 200 mW and a wavelength of 670 nm), working with a CTL-1202 S scanner. Three-(D3), six-(D6) and nine-fold (D9) doses were applied, with the basic dosage of $0.25 \cdot 10^{-2} \text{ J/cm}^2$. The duration of individual exposure was 4.1 min. The control group C(D0) consisted of seeds without any dosages.

Control and treated seeds laser light were placed in cuvettes lined with filter paper soaked in water distilled. The experiment was set up in three repetitions, 30 seeds per repetition. Energy and germination capacity was tested respectively 7 and 14 days after sowing (accordance with the methodology proposed by ISTA (2008). Moreover, the length of the radicle, hypocotyl and aboveground part of seedlings (stem) were measured. The content of fresh and dry weight of seedlings was also assessed.

Pot experiment. The experiment with sowing material of Norway spruce treated with laser radiation (see laboratory experiment) was also conducted also in pots grown under a cover (foil tunnel). The substrate in pots was peatmoss with NPK fertilizer. After four weeks, the following seedling parameters were assessed: the number and length of roots, the height of seedlings and the content of fresh weight of roots and above-ground parts of plants. Moreover, photosynthetically active pigments were tested in plant material obtained after eight weeks – chlorophyll a, b, and total. Chlorophyll was tested using spectrophotometry by measuring the absorbance of the prepared filtrates at a wavelength of 663 (chlorophyll A), 645 (chlorophyll B), 652 (chlorophyll A + B) and 765 for polyphenols (LICHTENTHALER 1987).

Statistics. The results of the research were statistically evaluated using variance analysis for a two-factor experiment, with the use STATISTICA 13.1 by Stat Soft Polska. The significance of differences was calculated at the level of $\alpha = 0.05$ using the Duncan test.

Results and Discussion

Laboratory experiment

The statistical analysis of data on energy and germination capacity for all tested genotypes showed no significant influence of laser radiation on these features.

This is most likely due to the fact that the seed material used in the experiment was of high quality – they belonged to the selected or qualified categories (KALINIEWICZ et al. 2011).

The studied morphological features of the seedlings: the length of radicle and hypocotyl, the length of the stem and the fresh weight of the seedlings showed a significant stimulating effect of the applied semiconductor laser radiation. The length of the embryonic root increased by 14.79% in relation to the roots of control plants after six- and nine-fold seed irradiation (Table 1). The length of hypocotyl showed stimulation under the influence of any applied doses of laser radiation – by 7.8% for dose D6; respectively 5.4% for D3 and 5% for nine-fold exposure (Table 1). In the case of stem length, the value of this feature was increased only through dose D6 – over 7.5%. The fresh weight of the seedlings grown from the irradiated seeds, in relation to the control seedlings, was higher by 16.5% and by 15%, respectively, after the application of the D6 and D9 doses. For the dry matter of seedlings, no effect of pre-sowing treatment of seeds with laser radiation was found (Table 1).

Table 1

Laboratory experiment: Average values and homogenous groups for tested features of Norway spruce

Dose	Length of the radicle [mm]	Length of the hypocotyl [mm]	Length of the stem [mm]	Fresh weight [g]
C(D0)	25.15 ^b	29.76 ^b	39.23 ^b	0.206 ^b
D3	26.46 ^b	31.38 ^a	40.90 ^{a,b}	0.229 ^{a,b}
D6	28.87 ^a	32.08 ^a	42.19 ^a	0.240 ^a
D9	28.87 ^a	31.26 ^a	40.69 ^{a,b}	0.237 ^a

The performed statistical analysis showed an interaction of the studied genotypes with the applied doses of laser radiation for all examined morphological features and for both the fresh and dry weight of seedlings. The length of the embryonic root increased after all doses in genotypes D, J and K. Doses D3 and D9 induced stimulation in genotypes C. Six-fold seed exposure stimulated the root length in four out of ten tested genotypes: A, E, F and G.

The reduction in length was observed twice: in B under the influence of doses D3 and D6, and in H for doses D3 and D9. Very diverse reactions to pre-sowing laser irradiation were observed for the length of hypocotyl. Under the influence of all applied doses, genotype F showed stimulation, while genotype B showed a reduction in the value of this feature. Stimulation was also observed in form A (dose D6), form C (doses D3 and D9), and form E – D9. In genotype H there was a reduction after application of doses D3 and D9.

Four of the studied genotypes (D, G, J and K) showed no significant response to the treatment of seeds with semiconductor laser radiation. The interaction obtained for the length of the stem with the needles showed a significant stimulation in genotype F under the influence of all applied doses, in genotypes C and G under the influence of three-fold irradiation, in genotypes E – dose D9, K – dose D6. Three of the tested forms (genotype A, D, J) did not respond to pre-sowing seed irradiation. Stem length reduction occurred in form B after all doses, and in form H after doses D3 and D9 (Table 2). The results obtained in the studies conducted by SZAJŠNER et al. (2017) on sugar beet (*Beta vulgaris*) showed that a magnetic field and laser radiation modify the process of cluster germination. Moreover, they extend the germinal root and stimulate pigment condensation in seedlings

For fresh weight of seedlings, a response to irradiation was observed in six out of ten tested genotypes. Three of them – form C, F and G – were characterized by an increase in the content of fresh weight of seedlings

after applying three irradiation dosages, in relation to the control plants. Genotype E reacted to dose D9; genotype K to dose D6. No reaction was observed in forms A, B, D and J. Reduction in fresh weight of seedlings occurred only in genotype H under the influence of three-fold irradiation. The dry matter content of seedlings obtained from irradiated seeds was reduced only in the F genotype under the influence of D3 and D9 doses. The remaining forms did not show any reaction to the applied laser radiation.

Table 2
Laboratory experiment: Average values and homogenous groups for tested features of Norway spruce – interaction between genotype and does

Genotype	Dose	Length of the radicle [mm]	Length of the hypocotyl [mm]	Length of the stem [mm]	Fresh weight [g]	Dry weight [g]
1	2	3	4	5	6	7
A	C(D0)	25,53 ^b	34.30 ^b	47.20 ^a	0.25 ^a	0.037 ^a
	D3	25.70 ^b	38.17 ^{a,b}	51.2 ^a	0.22 ^a	0.041 ^a
	D6	31.97 ^a	41.60 ^a	52.93 ^a	0.28 ^a	0.041 ^a
	D9	25.87 ^b	38.70 ^{a,b}	49.37 ^a	0.25 ^a	0.043 ^a
B	C(D0)	28.40 ^a	32.53 ^a	42.97 ^a	0.23 ^a	0.040 ^a
	D3	21.13 ^b	21.2 ^b	28.20 ^b	0.19 ^a	0.050 ^a
	D6	21.37 ^b	25.17 ^b	34.17 ^b	0.21 ^a	0.040 ^a
	D9	24.30 ^{a,b}	23.47 ^b	33.80 ^b	0.22 ^a	0.050 ^a
C	C(D0)	23.27 ^c	29.20 ^b	33.57 ^b	0.19 ^b	0.040 ^{a, b}
	D3	27.93 ^b	37.77 ^a	41.77 ^a	0.28 ^a	0.050 ^a
	D6	25.73 ^{b,c}	31.20 ^b	33.57 ^b	0.24 ^b	0.030 ^b
	D9	34.90 ^a	38.00 ^a	39.50 ^{a, b}	0.26 ^{a,b}	0.030 ^b
D	C(D0)	19.03 ^b	30.93 ^a	41.93 ^a	0.20 ^a	0.030 ^a
	D3	25.47 ^a	33.10 ^a	44.10 ^a	0.21 ^a	0.030 ^a
	D6	24.0 ^a	32.63 ^a	44.70 ^a	0.21 ^a	0.040 ^a
	D9	25.77 ^a	33.37 ^a	44.43 ^a	0.22 ^a	0.040 ^a
E	C(D0)	26.63 ^c	26.27 ^b	36.23 ^b	0.17 ^b	0.040 ^a
	D3	24.23 ^c	27.57 ^{b,a}	35.63 ^b	0.20 ^{a,b}	0.040 ^a
	D6	31.73 ^b	29.3 ^{b,a}	40.93 ^{a, b}	0.19 ^{a,b}	0.040 ^a
	D9	36.37 ^a	31.63 ^a	43.43 ^a	0.22 ^a	0.040 ^a
F	C(D0)	21.50 ^b	17.83 ^c	24.07 ^c	0.16 ^b	0.050 ^a
	D3	28.63 ^b	30.47 ^a	41.77 ^a	0.23 ^a	0.030 ^b
	D6	33.13 ^a	32.2 ^a	42.47 ^a	0.14 ^b	0.040 ^a
	D9	24.53 ^b	25.27 ^b	33.27 ^b	0.20 ^{a, b}	0.030 ^b

cont. Table 2

1	2	3	4	5	6	7
G	C(D0)	27.07 ^b	36.47 ^{a,b}	47.50 ^b	0.28 ^b	0.040 ^a
	D3	30.77 ^{a,b}	40.20 ^a	55.73 ^a	0.33 ^a	0.040 ^a
	D6	32.5 ^a	35.23 ^b	47.00 ^b	0.28 ^b	0.040 ^a
	D9	26.93 ^b	33.10 ^b	44.83 ^b	0.28 ^b	0.050 ^a
H	C(D0)	28.43 ^a	30.43 ^a	38.77 ^a	0.28 ^a	0.040 ^a
	D3	18.83 ^c	20.93 ^b	26.67 ^c	0.18 ^b	0.050 ^a
	D6	26.00 ^{a,b}	28.97 ^a	39.50 ^a	0.26 ^a	0.040 ^a
	D9	22.83 ^{b,c}	23.23 ^b	32.17 ^b	0.24 ^a	0.050 ^a
J	C(D0)	22.13 ^c	29.97 ^a	40.60 ^{a, b}	0.22 ^a	0.050 ^a
	D3	27.47 ^b	30.80 ^a		0.21 ^a	0.040 ^a
	D6	28.17 ^{a, b}	30.70 ^a	41.13 ^{a, b}	0.25 ^a	0.040
	D9	32.60 ^a	34.40 ^a	45.77 ^a	0.24 ^a	0.050 ^a
K	C(D0)	29.53 ^b	29.70 ^a	39.41 ^b	0.22 ^b	0.050 ^a
	D3	34.47 ^a	33.53 ^a	44.10 ^{a, b}	0.25 ^{a, b}	0.050 ^a
	D6	34.10 ^a	33.70 ^a	45.37 ^a	0.27 ^a	0.050 ^a
	D9	34.63 ^a	31.43 ^a	40.33 ^{a, b}	0.26 ^{a, b}	0.050 ^a

Pot experiment

In the experiment conducted in foil tunnels, like in the laboratory experiment, the D6 dose turned out to be the most effective. It stimulated four out of five examined morphological features of spruce seedlings. The length of the root increased by 15.9%, the height of the seedling by 29.3%, the weight of the above-ground part of the seedling by 26.7%, and the weight of the roots by 28.6%. The stimulation of seedling height was caused by the D9 dose and the increase in seedling weight by the D3 dose. The only feature reduced under the influence of laser radiation was the number of roots – on average from 3.84 PCs to 2.54 PCs (Table 3).

Table 3

Pot experiment: Average values and homogenous groups for tested features of Norway spruce

Dose	Height of the seedling [mm]	Root numbers [pcs]	Root length [mm]	Weight of the above-ground part of the seedlings [g]	Root weight [g]
C(D0)	21.26 ^b	3.84 ^a	74.02 ^b	0.15 ^b	0.05 ^b
D3	21.18 ^{a, b}	2.78 ^{b, c}	71.36 ^b	0.19 ^a	0.04 ^b
D6	27.48 ^a	3.12 ^b	85.8 ^a	0.19 ^a	0.07 ^a
D9	26.12 ^a	2.54 ^c	72.64 ^b	0.15 ^b	0.04 ^b

The performed statistical analysis showed a diversified reaction of the studied spruce genotypes to the applied doses of laser radiation (Table 4). Only dose D6 stimulated the height of the seedling in 4 out of 10 tested spruce genotypes. They were as follows: genotype C, F, H and K (by 38.90%; 53.79%; 115.38% and 53.33%, respectively). Genotype H deserves special attention – its elongation was over 115%. In genotype G, a reduction in the value of this feature was observed after the use of any of the three doses of laser radiation, and in genotype B under the influence of the strongest dose – nine-fold irradiation. The remaining genotypes (A, D, J) did not show any reaction to the applied doses of radiation.

The other applied doses of laser radiation did not produce any effect. Genotype G again turned out to be a form sensitive to any dose of laser radiation used in the experiment. This time, the number of roots was stimulated. The greatest effect was obtained with the dose D9 (from 2.0 to 4.0). The number of roots also increased in genotype A after irradiation with the D6 dose (from 3.6 to 7.0); while in genotype F, under the influence of six- and nine-fold irradiation, from 1.8 to 3.6 for D6 and 2.6 for D9. Genotype B responded by reducing the number of roots to irradiation with any dose. The lowest dose (D3) reduced the number of roots in forms E and F while the highest dose (D9) – in forms A, D and K. The remaining genotypes – C and J – did not show any reaction to pre-sowing irradiation.

The increase in the fresh weight of the above-ground part of the seedlings was achieved in the C form – by 35.30%, the F form – by 42.9%. and the H form – by 50%; in all cases after the D6 dose. The G and K genotypes responded with a reduction in seedling fresh weight to the application of any of the three doses of laser light. The other genotypes showed no significant effect of seed irradiation. An increase in root weight by 57% was obtained with the D6 dose only in genotype A. Genotypes B, D and K showed a reduction in root mass. while genotypes C, E, G, H and J did not respond to pre-sowing irradiation.

Table 4

Pot experiment: Average values and homogenous groups for tested features of Norway spruce – interactions

Genotype	Dose	Height of the seedling [mm]	Root numbers [pcs]	Weight of the above-ground part of the seedlings [g]	Root weight [g]
1	2	3	4	5	6
A	C(D0)	24.0 ^a	3.6 ^b	0.20 ^{a,b}	0.07 ^b
	D3	16.0 ^a	2.8 ^b	0.11 ^b	0.05 ^{b,c}
	D6	23.0 ^a	7.0 ^a	0.21 ^a	0.11 ^a
	D9	21.0 ^a	3.0 ^b	0.15 ^b	0.03 ^c

cont. Table 4

1	2	3	4	5	6
B	C(D0)	23.4 ^a	7.4 ^a	0.20 ^a	0.12 ^a
	D3	21.0 ^{a,b}	3.8 ^b	0.15 ^{a,b}	0.04 ^b
	D6	28.0 ^a	2.8 ^{b,c}	0.20 ^a	0.04 ^b
	D9	13.0 ^b	1.6 ^c	0.10 ^b	0.03 ^b
C	C(D0)	26.2 ^b	3.0 ^a	0.17 ^b	0.04 ^a
	D3	26.8 ^b	2.2 ^a	0.16 ^b	0.04 ^a
	D6	36.4 ^a	2.2 ^a	0.23 ^a	0.04 ^a
	D9	20.6 ^b	2.6 ^a	0.12 ^b	0.03 ^a
D	C(D0)	20.0 ^a	3.8 ^a	0.13 ^a	0.10 ^a
	D3	19.0 ^a	4.2 ^a	0.16 ^a	0.04 ^b
	D6	19.0 ^a	3.4 ^a	0.13 ^a	0.05 ^b
	D9	25.2 ^a	1.4 ^b	0.13 ^a	0.03 ^b
E	C(D0)	18.6 ^a	2.8 ^a	0.14 ^a	0.04 ^a
	D3	24.6 ^a	1.6 ^b	0.15 ^a	0.04 ^a
	D6	23.0 ^a	2.8 ^a	0.14 ^a	0.03 ^a
	D9	21.8 ^a	3.6 ^a	0.14 ^a	0.04 ^a
F	C(D0)	26.4 ^b	1.8 ^b	0.14 ^b	0.05 ^a
	D3	17.6 ^b	2.0 ^b	0.18 ^{a,b}	0.02 ^b
	D6	40.6 ^a	3.6 ^a	0.20 ^a	0.03 ^{a,b}
	D9	20.8 ^b	2.6 ^a	0.16 ^{a,b}	0.03 ^{a,b}
G	C(D0)	44.8 ^a	2.0 ^b	0.311 ^a	0.07 ^a
	D3	30.0	3.2 ^a	0.20 ^b	0.05 ^a
	D6	22.2 ^b	3.0 ^a	0.20 ^b	0.07 ^a
	D9	25.0 ^b	4.0 ^a	0.19 ^b	0.06 ^a
H	C(D0)	15.6 ^b	3.2 ^{a,b}	0.16 ^b	0.05 ^a
	D3	22.0 ^b	3.8 ^a	0.17 ^b	0.06 ^a
	D6	33.6 ^a	2.6 ^{a,b}	0.24 ^a	0.05 ^a
	D9	24.8 ^{a,b}	2.0 ^b	0.17 ^b	0.04 ^a
J	C(D0)	30.0 ^a	2.8 ^a	0.18 ^a	0.05 ^a
	D3	20.8 ^a	2.6 ^a	0.15 ^a	0.03 ^a
	D6	28.0 ^a	3.4 ^a	0.19 ^a	0.03 ^a
	D9	24.4 ^a	3.2 ^a	0.18 ^a	0.06 ^a
K	C(D0)	21.0 ^b	3.0 ^a	0.25 ^a	0.07 ^a
	D3	14.0 ^b	1.6 ^{a,b}	0.13 ^{b,c}	0.04 ^b
	D6	32.2 ^a	2.4 ^a	0.17 ^b	0.03 ^b
	D9	16.0 ^b	1.4 ^b	0.11 ^c	0.02 ^b

Chlorophyll A and chlorophyll B are the main photosynthetic pigments. Both absorb light, but chlorophyll A plays a unique and key role in the conversion of light energy into chemical energy (BICZAK et al. 2016). When examining the effect of the applied doses of laser radiation on spruce seeds, a significant stimulating effect on the content of chlorophyll in the obtained seedlings was found. Both three- and nine-fold irradiation increased the content of chlorophyll A, B and total (dose D3 by 26.7%, 13.33% and 22.80%, respectively; and dose D9 by 24.47%, 15.86% and 21.94%, respectively). Six-fold irradiation caused an increase only in the content of chlorophyll A (by 10.13%) see Table 5. Statistical analysis of the obtained results showed the interaction of genotype x applied laser light dose for chlorophyll A, B, and total. Under the influence of pre-sowing irradiation, five of the studied spruce genotypes produced seedlings with significantly increased chlorophyll content and under the influence of any of the three applied doses. These were genotypes B, C, G, H and K. Forms B and C showed the highest content under the influence of dose D9 (by 40.5% and 44.80%, respectively), while the lowest dose (D3) increased the content of chlorophyll A in genotypes G (by 33.40%), H (by 20.60%), and K (by 45%).

Table 5

Pot experiment: Contents of chlorophyll A, chlorophyll B and total chlorophyll in Norway spruce seedlings [mg g^{-1} F.W.]

Dose	Chlorophyll A	Chlorophyll B	Total chlorophyll
C(D0)	1.046 ^c	0.435 ^b	1.481 ^b
D3	1.326 ^a	0.493 ^a	1.819 ^a
D6	1.152 ^b	0.440 ^b	1.592 ^b
D9	1.302 ^a	0.504 ^a	1.806 ^a

Genotypes A, F reacted to doses D3 and D9 with stimulation, while dose D6 caused a reduction in chlorophyll A content in three tested genotypes – A, D, and E. The content of chlorophyll B was stimulated in genotypes A, C, H after three- and nine-fold irradiation. dose D3 caused an increase in the content of this pigment in forms D and K, while D9 – in F, G and J. Genotype B turned out to be the most sensitive and reacted to all applied doses of laser light with a significant stimulation of chlorophyll B content. The reduction was observed in genotype E (doses D6 and D9). For total chlorophyll, the best effects were achieved in genotypes B, C, G, H – stimulation after all applied doses. D3 increased the content of this pigment in forms A, D, E and K, while dose D9 in forms A, F and J. The reduction occurred only in two cases – in forms A and E under the influence of dose D6 (Table 6). In studies conducted on other species, it was found that short-term exposure to pre-sowing electromagnetic radiation of

amaranth seeds affects the germination energy, but not the content of photosynthetic pigment (DZIWULSKA-HUNEK et al. 2013). Higher chlorophyll content and leaf surface area under the influence of a magnetic field were observed by EŞİTKEN and TURAN (2004), whereas SZAJSNER et al. (2017). in their research on sugar beet pre-sowing stimulation, found that a magnetic field and laser radiation modify the sprouting process and stimulate pigment condensation in seedlings. SACALA et al. (2012) also observed a significant stimulating effect of laser radiation on the content of chlorophyll and carotenoids in the study on sugar beet as a result of five- and seven-fold irradiation of the clusters with semiconductor laser rays.

Table 6
Pot experiment: Contents of chlorophyll A, chlorophyll B and total chlorophyll in Norway spruce seedlings for interaction genotype x dose – average values and homogenous group

Genotype	Dose	Chlorophyll A	Chlorophyll B	Total chlorophyll
1	2	3	4	5
A	C(D0)	0.879 ^c	0.324 ^b	1.203 ^b
	D3	1.015 ^b	0.406 ^a	1.421 ^a
	D6	0.721 ^d	0.284 ^b	1.005 ^c
	D9	1.046 ^a	0.407 ^a	1.453 ^a
B	C(D0)	0.945 ^d	0.351 ^c	1.296 ^c
	D3	1.094 ^c	0.455 ^{a,b}	1.549 ^b
	D6	1.144 ^b	0.436 ^b	1.580 ^b
	D9	1.328 ^a	0.501 ^a	1.829 ^a
C	C(D0)	0.872 ^c	0.327 ^c	1.199 ^c
	D3	1.117 ^b	0.416 ^b	1.533 ^b
	D6	1.043 ^b	0.384 ^{b,c}	1.427 ^b
	D9	1.263 ^a	0.503 ^a	1.766 ^a
D	C(D0)	1.365 ^{a,b}	0.491 ^b	1.856 ^b
	D3	1.427 ^a	0.581 ^a	2.008 ^a
	D6	1.254 ^c	0.508 ^b	1.762 ^b
	D9	1.344 ^b	0.526 ^{a,b}	1.870 ^b
E	C(D0)	1.246 ^b	0.494 ^a	1.740 ^b
	D3	1.363 ^a	0.532 ^a	1.895 ^a
	D6	1.098 ^c	0.407 ^b	1.505 ^c
	D9	1.212 ^b	0.462 ^b	1.674 ^b
F	C(D0)	1.122 ^c	0.434 ^b	1.556 ^b
	D3	1.207 ^b	0.447 ^{a,b}	1.654 ^b
	D6	1.145 ^{b,c}	0.432 ^b	1.577 ^b
	D9	1.316 ^a	0.502 ^a	1.818 ^a

cont. Table 6

1	2	3	4	5
G	C(D0)	1.112 ^d	0.441 ^b	1.553 ^c
	D3	1.483 ^a	0.441 ^b	1.924 ^a
	D6	1.225 ^c	0.497 ^a	1.722 ^b
	D9	1.370 ^b	0.529 ^a	1.899 ^a
H	C(D0)	1.114 ^c	0.456 ^b	1.570 ^c
	D3	1.344 ^a	0.519 ^a	1.863 ^a
	D6	1.213 ^b	0.493 ^{a,b}	1.706 ^b
	D9	1.313 ^a	0.494 ^a	1.807 ^{a,b}
J	C(D0)	1.331 ^b	0.504 ^b	1.835 ^b
	D3	1.373 ^b	0.531 ^{a,b}	1.904 ^b
	D6	1.320 ^b	0.538 ^{a,b}	1.858 ^b
	D9	1.477 ^a	0.581 ^a	2.058 ^a
K	C(D0)	1.267 ^c	0.531 ^b	1.798 ^b
	D3	1.837 ^a	0.603 ^a	2.440 ^a
	D6	1.354 ^b	0.421 ^c	1.775 ^b
	D9	1.353 ^b	0.530 ^b	1.883 ^b

Conclusions

1. The response of the studied genotypes of Norway spruce to pre-sowing seed irradiation was varied and depended on both the genotype and the applied doses of laser radiation.

2. In laboratory conditions, all examined morphological features of the seedlings showed a positive effect of stimulation.

3. The genotypes C, E and F turned out to be the most susceptible to laser irradiation in laboratory conditions and showed stimulation of all examined characteristics.

4. In the pot experiment, the examined features such as seedling height, root length or seedling and root weight showed a significant stimulation effect of laser radiation. Only the number of roots was reduced.

5. The D6 dose turned out to be the most effective, inducing the stimulation of the examined features both in the laboratory and in a pot experiment.

6. The application of doses D3 and D9 of laser radiation for pre-sowing spruce seed bio-stimulation increased the content of chlorophyll in seedlings, which may increase the intensity of photosynthesis and thus plant biomass.

Acknowledgements. The authors thank dr hab. inż. Przemysław Bąbiewski for help in the implementation of the pot experiment.

Translated by T. JACEK JABŁOŃSKI

Accepted for print 9.03.2023

References

- BICZAK R., PAWŁOWSKA B., FEDER-KUBIS J. 2016. *Inhibicja wzrostu i stres oksydacyjny w roślinach pod wpływem chiralnej imidazoliowej cieczy jonowej z anionem tetrafluoroboranowym*. Chemistry Environment Biotechnology, 19: 35–45. [in Polish]
- COPELAND L.O., MC DONALD M.B. 2001. *Principles of Seed Science and Technology*. Boston. Kluwer Academic Publishers. 4 ed., pp. 181–220.
- DOBROWOLSKI J., RÓŻANOWSKI B. 1998. *The influence of laser light on accumulation of selected macro-, trace- and ultraelements by some plants*. In: Anke M. et al. (eds.) Mengen- und Spurenelemente. Schubert-Verlag, pp. 147–156.
- DZIWIŃSKA-HUNEK A., SUJAK A., KORNAZYŃSKI K. 2013. *Short-term exposure to Pre-sowing electromagnetic radiation of amaranth seeds affects germination energy but not photosynthetic pigment content*. Polish Journal of Environmental Studies, 22: 93–98.
- ESİTKEN A., TURAN M. 2004. *Alternating magnetic field effects on yield and plant nutrient element composition of strawberry (*Fragaria x ananassa* cv. *camarosa*)*. Acta Agriculturae Scandinavica. Section B, 54: 135–139.
- GRZESIK K., JANAS R., GÓRNIK K., ROMANOWSKA-DUDA M. 2012. *Biologiczne i fizyczne metody stosowane w produkcji i uszlachetnianiu nasion*. Journal of Research and Applications in Agricultural Engineering, 579(3): 47–152. [in Polish]
- HERNANDEZ-AGUILAR C., DOMINGUEZ-PACHECO A., CRUZ-OREA A., PODLEŚNA A., IVANOV R., CARBALO A.C., REYES M.C.P., HERNANDEZ G.S., BAUTISTA R.Z., LOPEZ-BONILLA J.L. 2016. *Laser biostimulation in seeds and plants*. Gayana Botanica, 73(1):132–149.
- ISTA. 2008. *International rules for seed testing*. Int. Seed Testing Association Press. Bassersdorf. CH, Switzerland.
- JAWORSKI A. 2011. *Hodowla lasu*. Tom III. *Charakterystyka hodowlana drzew i krzewów leśnych*. Warszawa. PWRiL, pp. 26–37. [in Polish]
- KALINIEWICZ Z., GRABOWSKI A., LISZEWSKI A., FURA S. 2011. *Analysis of correlations between selected physical attributes of Scots pine seeds*. Technical Sciences, 14 (1): 13–22.
- KLIMONT K. 2006. *Wpływ biostymulacji światłem lasera na wartość siewną nasion i plon wybranych roślin uprawnych*. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 242: 233–241. [in Polish]
- KORCZYK A., MATRAS J., BURZYŃSKI G., CZART J., FONDER W., PUCHNIARSKI T., TOMCZYK A., ZAŁĘSKA A. 2010. *Program zachowania leśnych zasobów genowych i hodowli selekcyjnej drzew leśnych w Polsce na lata 1991–2010*. Wydano na zlecenie Dyrekcji Generalnej Lasów Państwowych, Warszawa. [in Polish]
- KOSZELNIK-LESZEK A., SZAJSNER H., PODLASKA M. 2019. *The improving influence of laser stimulation on phytoremediation capabilities of selected *Silene vulgaris* ecotypes*. Archives of Environmental Protection, 45(3): 79–85.
- KUBALA S., WOJTYŁA Ł., GARNCZARSKA M. 2013. *fizjologiczne i biochemiczne podstawy kondycjonowania nasion*. Postępy Biologii Komórki, 40(2): 199–214. [in Polish]
- LICHTENTHALER H.K. 1987. *Chlorophylls and carotenoides pigments of photosynthetic biomembranes*. Methods in Enzymology, 148: 350–382.
- MATĚJKA K., LEUGNER J., KRPEŠ V. 2014. *Phenotype features in juvenile populations of *Picea abies* and their growth*. Journal of Forest Science, 60: 96–108.

- MURAT E. 2002. *Szczegółowa hodowla lasu*. Warszawa. Oficyna Edytorska Wydawnictwo Świat, 204. [in Polish]
- RYBIŃSKI W., GARCZYŃSKI S. 2004. *Influence of laser light on leaf area and parameters of photosynthetic activity in DH lines of spring barley (Hordeum vulgare L.)* International Agrophysics, 18: 261–267.
- SACAŁA E., DEMCZUK A., GRZYŚ E., PROŚBA-BIAŁCZYK U., SZAJSNER H. 2012. *Impact of presowing laser irradiation of seed on sugar beet properties*. International Agrophysics, 26: 295–300.
- SOLIMAN A.S., HARITH M.A 2010. *Effects of laser biostimulation on germination of Acacia farnesiana (L.) Willd.* Acta Horticulturae, 854: 41–50.
- SVYSTUNA T., LUNDSTRÖMERB J., BERLINC M., WESTIND J., JÖNSSONA A.M. 2021. *Model analysis of temperature impact on the Norway spruce provenance specific bud burst and associated risk of frost damage*. Forest Ecology and Management, 493: 119252, doi: 10.1016/j.foreco.2021.119252.
- SZAJSNER H., PROŚBA-BIAŁCZYK U., SACAŁA E., KOSZELNIK-LESZEK A., SZUBZDA B. 2017. *The effect of pre-sowing seed stimulation on the germination and pigment content in sugar beet (Beta vulgaris L.) seedlings leaves*. Polish Journal of Natural Sciences. 32(2): 207–222.
- SZAJSNER H., BĄBELEWSKI P., PYTLARZ-KOZICKA M., KOSZELNIK-LESZEK A. 2019. *Conditioning of purple coneflower seeds (Echinacea purpurea Moench) with laser beam: Osivo a Sadba: XIV. Národní odborný a vědecký seminář: Sborník referátů. Česká zemědělská univerzita*, pp. 225–230.
- WOJCIECHOWSKA B. 1983. *O twardości nasion*. Wiadomości Botaniczne, 3: 199–212. [in Polish]