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Influence of laser light on mycelial growth of *Hebeloma mesophaeum* and ectomycorrhizal development on Scots pine

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Abstract The effects of exposure to helium-neon (He-Ne) or Argon (Ar) laser light ($\lambda = 632.8$ nm and 514 nm, respectively) on the growth of *Hebeloma mesophaeum* mycelium in pure culture were studied. Growth rates were highest after exposure to the He-Ne laser (1×60 s) + Ar laser (2×60 s) and to the He-Ne laser for 3×30. Container-grown *Pinus sylvestris* (pine) seedlings were inoculated with a water suspension of *H. mesophaeum* mycelium previously exposed to different kinds of laser light. After 3 months, the percentage of mycorrhizal associations on pine roots was 34.3% higher after He-Ne laser treatment and 47.1% higher after Ar laser treatment than in the controls with untreated fungus. However, seedlings infected with treated fungus were smaller than the control. Overall, laser light stimulated growth of *H. mesophaeum* mycelia in pure culture and enhanced mycorrhizal development on Scots pine seedlings.

Key words *Hebeloma mesophaeum* · Laser light · Mycelial growth · Mycorrhiza · *Pinus sylvestris*

Introduction

In Poland, the production of seedlings with abundant ectomycorrhiza is particularly important in the afforestation of post-agricultural land, soils degraded by air pollution and post-fire areas. In such conditions, the growth of Scots pine (*Pinus sylvestris* L.*)* is inhibited even to the point where seedlings do not survive after outplanting. Many studies have concerned themselves with the artificial production of mycorrhizal seedlings for afforestation and contaminated soils. One fungus being investigated by the authors for such mass seedling mycorrhization is *Hebeloma mesophaeum* (Pers.) Quel., a species characteristic of the juvenile stage in pines which develops best in the slightly alkaline soils typical of ex-agricultural land.

The dynamics of cell bioenergetic processes is known to increase after exposure of biological material (e.g. bacteria, yeast, algae, seeds, some plants grown in *in vitro* culture, plant bulbs and tubers) to laser light of specific wavelength (Dobrowolski et al. 1995, 1997). The energy from laser light has also been found to stimulate the intensity of the transmembrane electrochemical proton gradient in mitochondria and cell proliferation and to produce morphological changes in cells and organisms in connection with the use of ATP reserves (Lehninger 1978; Friedmann et al. 1991). Inyushin *et al.* (1983) reported an increase in pine seed germination after short (0.58 s) exposures to laser radiation. On the other hand, large doses of laser light can evoke damage in cell structures and morphological deformations associated with enzymatic changes (Karu 1988; Dobrowolski et al. 1997).

The main goal of the present study was to determine the influence of laser light of different kinds and exposure times on *in vitro* growth dynamics and mycorrhizal development in *H. mesophaeum*. The hypothesis tested was that properly chosen irradiation of *H. mesophaeum* mycelium could cause bio-stimulation reactions and thereby positively influence fungal growth rate and increase ectomycorrhizal development with Scots pine.

Materials and methods

Pure cultures of *H. mesophaeum,* obtained from one sporocarp collected in 1992 from a 60-year-old Scots pine forest in the Krynki Forest District, were provided by Professor R. Pachlewski (FRI). Isolate No. 3037 was grown in Petri dishes on Ingestad (1960) agar medium as modified by Pachlewski and Pachlewska (1974). Inocula of about 7-diameter were taken from the margin of the culture for laser irradiation. This took place in closed dishes on the fifth day after inoculation, in seven separate replicates for each experimental treatment.

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The following lasers were used (Dobrowolski et al. 1997): (i) an argon (Ar) laser of wavelength $\lambda = 514$ nm and density of irradiation of 5 W/m^2 (C. Zeiss Jena), and (ii) a helium-neon (He-Ne) laser of wavelength $\lambda = 632.8$ nm and density of irradiation of 2 W/m^2 (C. Zeiss Jena).

The experimental treatments were: (1) He-Ne laser, exposure time 3×30 s, (2) He-Ne laser, exposure time 3×60 s, (3) He-Ne laser, exposure time 3×120 s, (4) Ar laser, exposure time 3×60 s, (5) He-Ne laser, exposure time 1×60 s and then Ar laser, exposure time 2×60 s, (C) the control without irradiation.

Mycelium irradiation was carried out at the Faculty of Mining Geodesy and Environmental Engineering AGH in Cracow, in collaboration with Professor J.W. Dobrowolski. The Petri dishes with irradiated mycelium were incubated for 3 weeks at 25 °C. Growth increments expressed as the diameter of each fungal colony were measured at 24-h intervals and colour and structure recorded (Molina and Palmer 1982; Kottke et al. 1987). Mean diameter increments were determined for each experimental treatment after 7, 14, 21 and 28 days of culture. Data were analysed by one-way analysis of variance (ANOVA), least significance differences (LSD), multiple range test (MRT) and analysis of regression (REG) using Statgraphics v.5 (STSC Inc.).

Fungal cultures from treatments 1 and 4 showing regular colony growth and characteristic colour were used, along with control cultures, for inoculation of the roots of pine seedlings in a pot experiment. Sterile (30% H_2O_2, 30 min) pine seeds were sown in sterile plastic pots (5.5×11.5 cm) filled with an autoclaved mixture of peat and leca (burned clay keramsit) (2:1 v:v). The pots were put into a plastic dish and watered gravimetrically to 70% field capacity with sterile (nutrient-free) water. Four weeks after seedling germination, the medium in the pots was inoculated with 42-day-old irradiated or non-irradiated cultures of *H. mesophaeum*. The mycelium was removed from the surface of the agar medium aseptically, homogenized in sterile distilled water and applied as 15 ml of mycelial slurry per pot. The pots were covered with aluminium foil with a hole left for the seedling stem. The experiment, involving treatments 1, 4 and the control with 6 replicates each, was run for 3 months in a greenhouse with a controlled environment: 20–25 °C, >50% relative humidity, sunlight. At the end of the experiment seedling growth (length of shoot and root) was measured and the number of mycorrhizal root tips per seedling counted and compared to the control.

Results

Influence of laser irradiation on mycelial growth

On the second day after laser irradiation of *H. mesophaeum* cultures, the highest increase in the diameter of mycelial cultures occurred in the control (mean 0.6 mm, Fig. 1). On the third day, growth increases

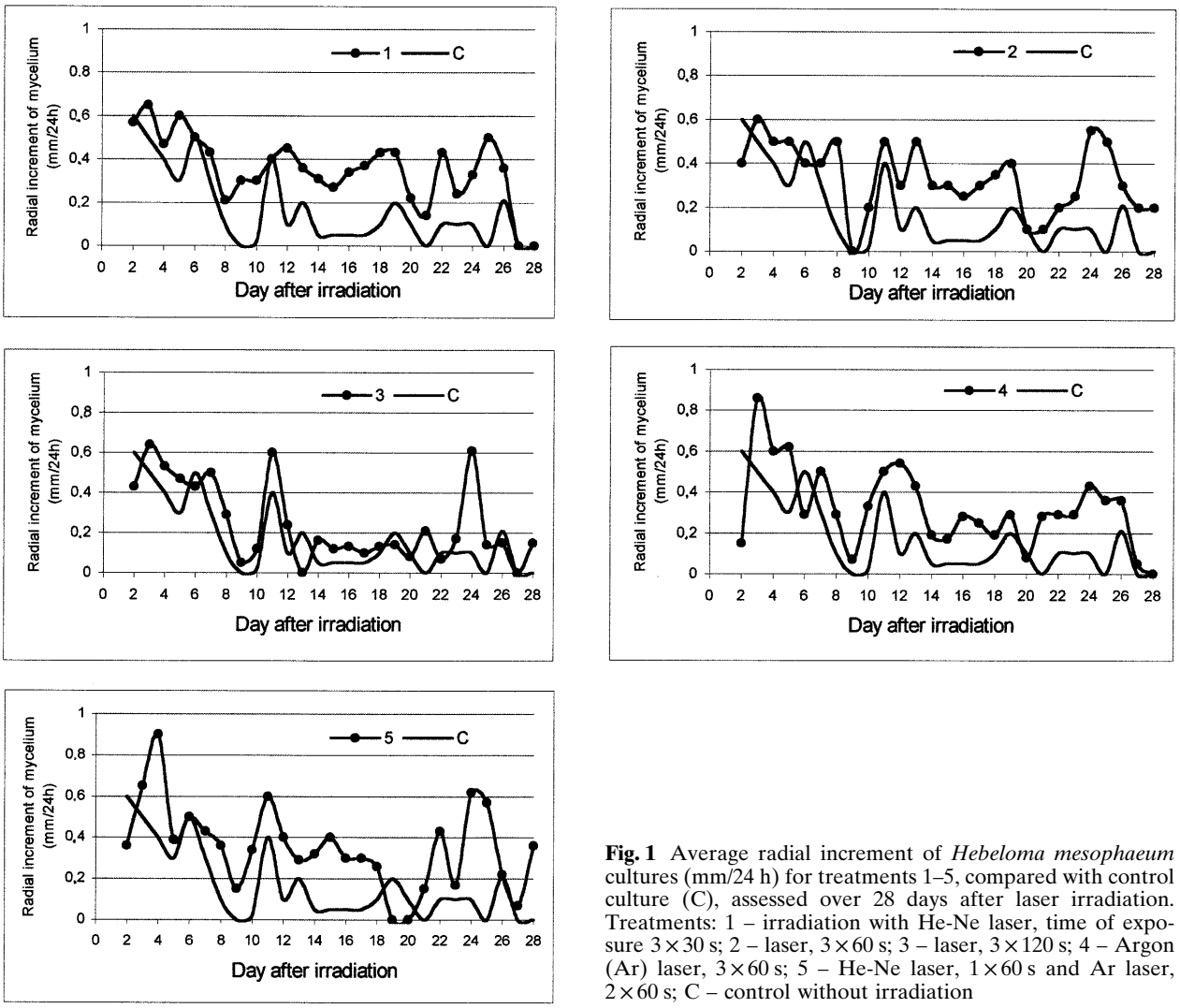

Fig. 1 Average radial increment of *Hebeloma mesophaeum* cultures (mm/24 h) for treatments 1–5, compared with control culture (C), assessed over 28 days after laser irradiation. Treatments: 1 – irradiation with He-Ne laser, time of exposure 3×30 s; 2 – laser, 3×60 s; 3 – laser, 3×120 s; 4 – Argon (Ar) laser, 3×60 s; 5 – He-Ne laser, 1×60 s and Ar laser, 2×60 s; C – control without irradiation

Table 1 Mean diameters of *Hebeloma mesophaeum* cultures 7, 14, 21 and 28 days after laser irradiation, standard deviation (σ), coefficient of variation (V%), significance level (ANOVA) and homogenous groups (MRT). Treatments: 1 – irradiation with Helium-Neon (He-Ne) laser, time of exposure 3×30 s; 2 – He-Ne laser, 3×60 s; 3 – He-Ne laser, 3×120 s; 4 – Argon (Ar) laser, 3×60 s; 5 – He-Ne laser, 1×60 s and Ar laser, 2×60 s; C – control without irradiation. The vertical alignment of Xs shows overlapping groups

Treatment	7 days			14 days			21 days			28 days		
	Diameter	σ	V%	Diameter	σ	V%	Diameter	σ	V%	Diameter	σ	V%
1	14.1	2.0	13.8	18.9	3.3	17.7	23.6	4.6	19.4	26.4	4.0	15.1
2	13.6	0.9	6.6	17.4	3.0	17.5	20.8	5.2	24.8	25.6	8.4	33.0
3	13.6	1.4	10.3	16.1	2.3	14.0	17.9	3.3	18.7	20.4	5.1	24.8
4	13.7	1.5	10.9	18.1	2.3	12.9	21.4	3.6	17.0	24.3	4.2	17.1
5	14.1	1.3	9.5	19.0	1.2	6.1	22.9	2.4	10.5	27.6	4.0	14.6
C	12.4	1.9	15.7	13.8	2.5	18.0	15.0	1.6	10.5	15.6	2.1	13.3
Significance level:		0.4712			0.0109*			0.0025*			0.0019*	
		C	X		C	X		C	X		C	X
		3	X		3	XX		3	XX		3	XX
Homogenous		2	X		2	XX		2	XX		2	XX
groups		4	X		4	XX		4	XX		4	XX
(MRT)		1	X		1	XX		2	X		1	X
		5	X		5	X		1	X		5	X

* $p<0.05$

were apparent in all treated cultures with the highest in treatments 4 and 5 (0.86 mm); the lowest diameter increment occurred in the control (mean 0.5 mm) (Fig. 1). With the control mycelium, the highest diameter increments were found during the first 5 days of the growth, whereas in the treated mycelia growth increments varied with time and were usually lowest on the days 9 and 20 (Fig. 1).

Table 1 shows changes in the diameter of the *H. mesophaeum* cultures in the different treatments during 28 days of growth. Cultures from treatments 1 and 5 had similar mycelial growth (linear regression: $y=0.693x+8.973$; $R^2=0.991$), different to treatment 3 ($y=0.415x+0.755$; $R^2=0.944$) and the control ($y=0.241x+9.989$; $R^2=0.890$). After 28 days growth, the highest diameters were recorded in cultures of treatments 5 (He-Ne + Ar), 1 (He-Ne3×30 s) and 4 (Ar, 3×120 s). The control cultures gave the lowest values. The differences between treatments and control were statistically significant after 14 (P0.0109), 21 (P0.0025) and 28 (P0.0019) days of growth. Multiple range tests revealed statistically significant differences between some treatments, e.g. at 28 days after irradiation, treatments 1 versus 3 and 5 versus 3. Three homogeneous treatment groups were distinguishable based on total growth of cultures: were (i) the control and treatment 3, (ii) treatments 2, 3, 4, and (iii) treatments 1, 2, 4, 5 (Table 1).

The sizes and shapes of some *H. mesophaeum* cultures 40 days after laser light irradiation are shown in Fig. 2. Discoloration and changes in rates of growth of some parts of the fungal colony after 3×30 s with the He-Ne (treatment 1) and 3×60 s with Ar (treatment 4) are apparent.

Influence of irradiated mycelia on seedling growth parameters and mycorrhiza synthesis

There were not significant (at $P>0.05$) differences in seedling growth micorrhized with *H. mesophaeum* and controled (Table 2). The lengths of shoots and roots were comparable when mycelium was irradiated with either the Ar laser or the He-Ne laser.

Roots of Scots pine seedlings with *H. mesophaeum* which had been irradiated with He-Ne or Ar before inoculation had the highest number of mycorrhizal roots compared with the control (Table 2). Compared with non-irradiated mycelia, irradiation with the lasers appeared to cause an increase in the number of mycorrhizal associations on Scots pine roots of about 34.3% (He-Ne) and 47.1% (Ar). However, a statistically significant difference was only found between Ar irradiation and the control.

Discussion

Inyushin (1976) reparted that a dose of high energy He-Ne laser light with a short exposure time had a positive effect on cell activity. Using an He-Ne laser of energy equivalent 1.45×10^{-3} J for 0.58, Inyushin et al. (1983) increased the germination of pine seeds by 30%. Dobrowolski et al. (1997) found a stimulating effect of laser irradiation of radish seeds on root development, although a negative effect of overdosage (after 25 irradiations) was also noted.

In our experiment, the irradiation dose (2 W/m^2) and exposure times (were relatively low 30, 60 and 120). However, the treatments appeared to stimulate

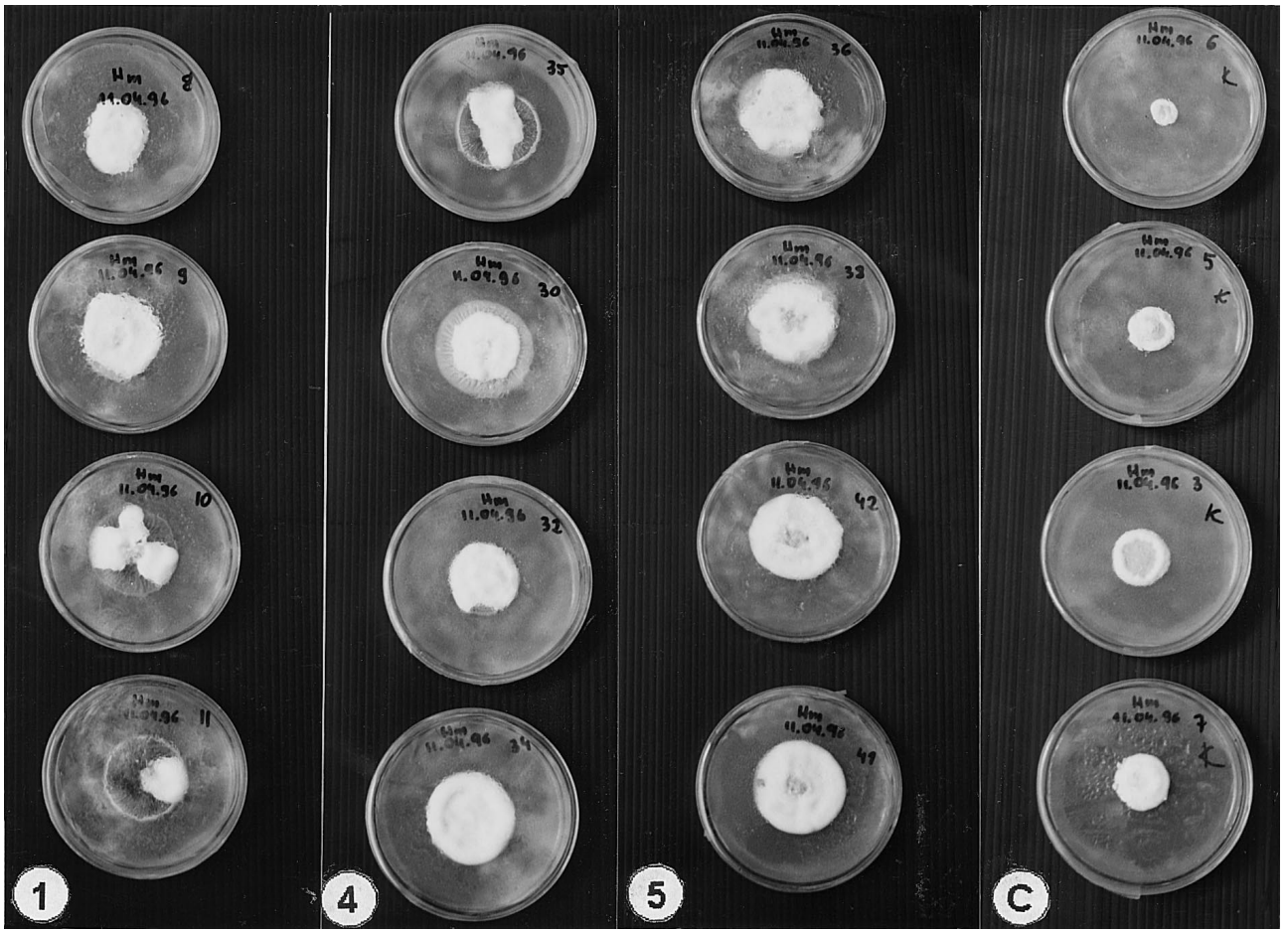

Fig. 2 Appearance of some control and treated cultures of *H. mesaphaeum* 40 days after laser irradiation. 1 – irradiation with He-Ne laser, 3 × 30 s; 4 – Ar laser, 3 × 60 s; 5 – He-Ne laser, time 1 × 60 s + Ar laser, 2 × 60 s, C – control

growth of *H. mesophaeum* hyphae compared with non-irradiated controls. Statistically significant differences between treatments appeared as early as 14 days after irradiation.

The duration and frequency of exposure were as important as the type and nature of the applied laser light. Dobrowolski et al. (1997) reported that one short (15 s) exposure to laser light increased the germination of *Lepidium sativum* L. seed by about 14%. These authors also found that some fungi exposed to Argon laser light for 180 s changed colour and underwent enzymatic changes limiting cell activity. Changes in colour of irradiated cultures of *H. mesophaeum* were also observed

Table 2 Growth parameters for pine seedlings, mycorrhiza formation per seedling, standard deviation (σ), coefficient of variation (V%) and significance. Groups: 1 – seedlings with non-irradiated mycelium (control), 2 – mycelium from treatment (1) irradiated with He-Ne laser, 3 – mycelium from treatment (4) irradiated with Ar laser

Growth pameter		Group			F	Significance level	Significant difference between groups (MRT)
		1	2	3			
Shoot length (cm)		8.2	7.4	7.3	1.165	0.3450	–
	σ	1.5	0.9	0.6			
	V%	18.1	12.1	7.7			
Root length (cm)		7.0	5.7	5.6	1.109	0.3650	–
	σ	2.7	0.6	0.4			
	V%	39.1	10.0	7.2			
Mean number of mycorrhizae		14.0	18.8	20.6	3.763	0.0539	1 versus 3
	σ	6.0	2.6	1.8			
	V%	43.2	13.8	8.7			
Percentage of group 1		100.0	134.3	147.1			

in our experiment. The discoloration and changes in growth rate of some parts of the fungal colonies resembled mutations affecting the enzyme synthesis, cell activity and pigment synthesis (Dobrowolski et al. 1997).

The use of mycelium irradiated with He-Ne and Ar laser light as mycorrhizal inocula showed that laser light also enhances synthesis of mycorrhizal association in vivo. Argon laser light with a wave-length of 514 nm, an exposure time of 3×60 s, and an energy of 5 W/m^2 was most adequate for stimulating mycorrhiza-forming activities.

The cause of the lower growth rates noted for pine seedlings inoculated with irradiated mycelium is unknown. Carbohydrates from the host plants may have been usurped for mycorrhiza synthesis (Pachlewski 1993). In a previous experiment on the influence of fertilization on growth and mycorrhization of Scots pine seedlings, Stenstrom et al. (1986) and Väre (1990) observed no growth enhancement in mycorrhizal seedlings over 3 years. Further research should be aimed at factors determining the stability of changes caused by laser light in roots and mycorrhizal fungi and their possible influence on, for example, the production of phenol and the auxin indole-3-acetic-acid.

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