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Original Research

Effects of Organic Fertilization and Mulching under Micro-Sprinkler Irrigation on Growth and Mycorrhizal Colonization of European Larch Seedlings, and Occurrence of Soil Mites

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Abstract

Our investigation determined the influence of organic fertilization and mulching (edaphon inoculation) on the vitality of European larch (*Larix decidua* Mill.) seedlings and their mycorrhizal structure, as well as the occurrence of soil mites (Acari). Nursery trials were conducted in 2005-07. It was confirmed that the investigated factors affected the growth of seedlings and mycorrhizal colonization, as well as soil properties and the occurrence of soil mites.

Keywords: edaphon inoculation, European larch, mycorrhiza, soil mites

Introduction

European larch (*Larix decidua* Mill.) has a very limited natural range but it is planted throughout the forests of Poland [1]. One-year production cycles of European larch seedlings are usually carried out in forest nurseries [2]. On the other hand, production of older and larger seedlings is – in some cases – also justified. Such seedlings are aimed at afforestation under difficult conditions [2-5].

The establishment and performance of outplanted tree seedlings may be significantly influenced by ectomycorrhizal fungi [6, 7]. Because the number and composition of mycorrhiza has decreased in many Polish bare-root nurseries [8], an improvement of nursery soil by inoculation with edaphon derived from forest soil (mulching with forest ectohumus) seems to be a good solution [9, 10].

Suitable application of organic fertilizers – e.g. composts – can secure the high productivity of soils in forest-tree nurseries [2, 9-11]. In this experiment, compost produced from sewage sludge was applied. Sewage sludge is being used for reclamation of devastated areas and for fertilization of arable soils [12]. The natural use of sewage sludge is justified from an ecological point of view [11, 13].

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The aim of our paper was to determine the influence of organic fertilization and mulching (edaphon inoculation) on the vitality of European larch (*Larix decidua* Mill.) seedlings and their mycorrhizal structure, as well as the occurrence of soil mites (Acari).

In our trials, which were carried out previously [9, 10], we investigated the effect of micro-irrigation (microjet sprinkling and drip irrigation) as well as organic fertilization (80% of treated sewage sludge and 20% of highmoor peat) on the seedling vitality of Scots pine (*Pinus sylvestris* L.) and white birch (*Betula pendula* Roth), as well as on the occurrence of soil mites (Acari) after edaphon inoculation.

It should be also noted that similar experiments on Scots pine (*Pinus sylvestris* L.) seedlings were conducted simultaneously under the same soil-climatic conditions [14].

Materials and Methods

Two nursery trials were conducted in 2005-07 at the Forest Nursery in Białe Błota, Forest District in Bydgoszcz. The first two-year experiment was carried out in 2005-06 (2 cycles of one-year-old seedling production), and the second in 2006-07 (2 cycles of two-year-old seedling production). These experiments were run in a split-plot system with four replications. Two different factors were compared. The first row factor – organic fertilization, was used in the two following treatments (main plots): F_1 – treated sewage sludge (⅔) + bark (⅓), and F_2 – treated sewage sludge (⅔) + sawdust (⅓). The second row factor – mulching, was used in two variants (subplots): C – without mulching (control) and M – mulching with litter. Seed origin and seeding density were similar to standard nursery practices [2]. European larch seeds were obtained from the seed stand of the Forest district in Bydgoszcz. The plot area was 2 m^2 and contained 4 rows (2 m length) of European larch seedlings. Total number of plots in each experiment was 16 (2×2×4).

Organic fertilizer was produced on a base of treated sewage sludge (⅔) and Scots pine bark (⅓) or sawdust (⅓). This fertilizer was applied at doses of 100 $t \cdot ha^{-1}$ in spring and mixed with the topsoil (10 cm deep) before European larch seed time.

Mulching with litter (ectohumus) obtained from fresh coniferous forest was done with a dose of 100 $m^3 \cdot ha^{-1}$. Introduction of edaphon consisted of mixing topsoil (2 cm deep) with organic matter obtained from the surface of fresh coniferous forests designed for tree felling. This substrate contained the living soil mesofauna, which was abundant. This measure was conducted after the emergence of European larch seedlings in the years 2005 and 2006.

Irrigation was done with the use of micro-sprinklers (q=80-100 $dm^3 \cdot h^{-1}$) [15-20]. Terms of irrigation and water rates were established according to directives for irrigation of forest nurseries on bare areas [16]. It should be noted that the vicinity of Bydgoszcz is characterized by relatively high irrigation requirements [21].

Soil analysis

The experiment was conducted on a Cambic Arenosol [22] formed on fluvioglacial sand characterized by the following soil pedon: Ap-Abv-Bv-BvC-C. Soil samples were taken from the 0-15 cm surface layer of all experimental plots. Soils were air-dried at room temperature and passed through a 1 mm sieve.

The following physico-chemical and chemical properties were determined [23]:

- pH in water and in 1 M KCl (1:2.5) was measured using a pH meter (model CPC-551 Elmetron)
- organic carbon (C_{org}) was determined by oxidizing the soil sample with a mixture of potassium dichromate and sulfuric acid, diluting the suspension with water and back titrating the excess dichromate with standardized ferrous sulfate solution (Tiurin method)
- total nitrogen (N_{total}) was determined by Kjeldahl method
- available potassium and phosphorus was determined by the Egner-Riehm method, by extraction of soil samples in solution of calcium lactate; P was determined by colorimetric method, with the use of UV-VIS spectrometer model Marcel Media, and K was determined by emission spectrometry with the use of a PU-9100X spectrometer (Philips)
- available magnesium was determined by Schachtschabel method, by extraction of samples in solution of 0.0125 M $CaCl_2$; measurement of Mg content was determined by atomic absorption spectrometry, with the use of a PU-9100 X spectrometer
- available microelements (zinc, copper, manganese) were determined by atomic absorption spectrometry, with the use of a PU-9100 X spectrometer, after their previous extraction from the soil samples in 1 M HCl
- granulometric analysis was carried out according to the method of Bouyoucos in modification of Casagrande and Prószyński

Growth and Mycorrhizal Analysis

Plant growth was evaluated in late autumn (October). The height of seedlings (cm) and shoot diameter (cm) were measured.

The root systems of the seedlings were studied using a stereomicroscope (magnification 10-50x). To standardize the samples, lateral roots were taken from three different levels of root system of each (top, middle, and bottom), cut into small pieces (ca. 1cm) and mixed in a Petri dish containing water. The percentage of mycorrhizal short roots for each plant was assessed by counting at least 200 short roots from the mixture of segmented roots under the stereomicroscope. Following the guidelines of Agerer and Rambold [24], and Ingleby et al. [25], morphological data were recorded on overall morphology and color.

Sampling and Observation of Soil Mites

To investigate the occurrence of mites, soil samples were taken twice a year (in June and October) in successive years. Samples of 17 cm^2 and 3 cm deep were taken from all plots in 3 replications (3 samples per plot). Mites were extracted during 7 days in high gradient Tullgren funnels. A total of 1,496 mites (Acari) were identified to order, according to Hammen's systematics [26].

The data were statistically processed by analysis of variance [27]. The Fisher-Snedecor test was used to determine the significance of experimental factors, and Tukey test was used to define significant differences between combinations. The data of mites were ln-transformed (x+1) prior to analysis [28].

Results and Discussion

Weather Conditions, Water Needs and Irrigation

Air temperature during the vegetation period (April-September) – on average in the years 2005-07 – was 14.7ºC, ranging from 14.2ºC to 15.1ºC in 2005 and 2006, respectively (Fig. 1). Mean monthly values of air temperature in the years of the study varied from 7.7ºC in April to 19.9ºC in July. (More details are given in the paper on Scots pine (*Pinus sylvestris* L.) seedlings grown simultaneously under the same soil-climatic conditions of the vicinity of Bydgoszcz [14].) Żarski et al. [29] showed that in the vicinity of Bydgoszcz during the period 1949-2008 the mean air temperature of May and August was characterized by an increase with time. In spite of this, a statement that the climate in the vicinity of Bydgoszcz is getting warmer should be threated with caution due to – among other things – different trends of linear equations that are dominated by insignificant equations.

The sum of rainfall from 1 April to 30 September, on average for 2005-07, amounted to 295.4 mm, ranging from 203.0 mm to 366.6 mm, in 2005 and 2007, respectively. Among the months of the vegetation period, May, July, and August were characterized by the highest rainfall amount (more than 60 mm), and April by the lowest (25.6 mm). Rainfall amounts of particular months differentiated in the period of study, e.g. the amounts of rainfall ranged widely from 21.8 mm (2006) to 103.4 mm (2007), or from 30.4 mm (2006) to 111.3 mm (2007), as well as from 20.9 mm (2005) to 114.5 mm (2006) in June, July, and August, respectively. There were three rainfall-free decades in the vegetation period 2005 (1/VII, 3/VIII, and 1/IX), five in 2006 (1/IV, 2/IV, 3/VI, 1/VII, and 2/IX), and only one (2/IV) in 2007 [14].

Water requirements of European larch seedling nursery during the vegetation period (1 April-30 September), determined as the water use – according to a method given by Pierzgalski et al. [16], amounted to 508 mm, ranging from 494 mm to 533 mm, in 2005 and 2006, respectively. The mean monthly amount of water requirements varied from 51 mm in April to 130 mm in July (Fig. 2).

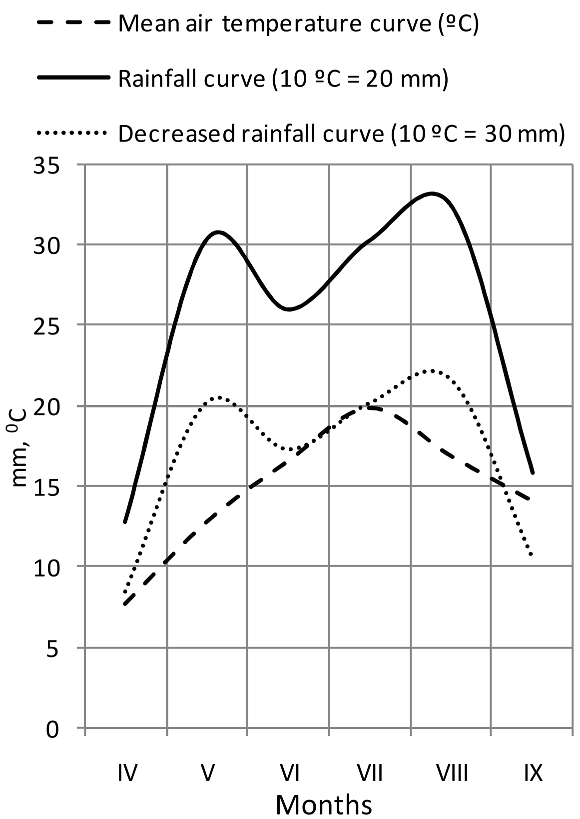

Fig. 1. Course of air temperature and rainfall during the vegetation period in 2005-07.

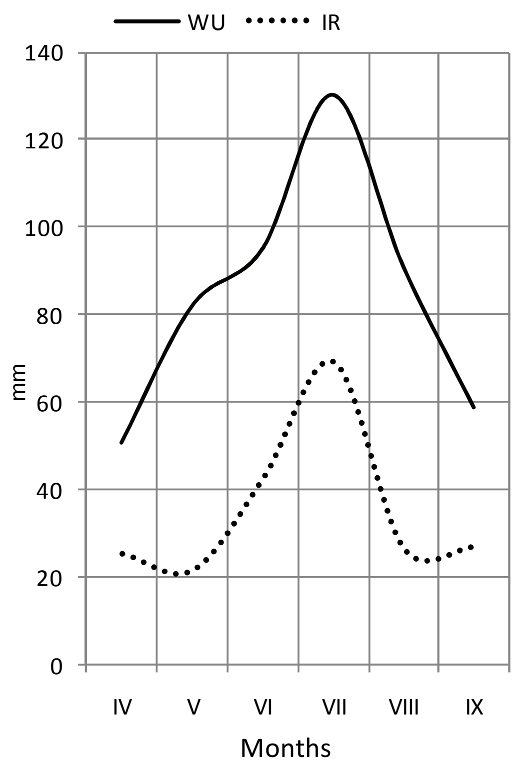

Fig. 2. Water use from the soil layer of controlled moisture (WU) and irrigation requirements (IR) at forest nursery Białe Błota near Bydgoszcz, acc. to Pierzgalski et al. [16], mean for 2005-07 (mm).

Table 1. Characteristics of some physico-chemical properties of the soils.

Treatment	Soil characteristics									
	pH H_2O	pH KCl	$C_{org.}$ %	N_{total} %	K_2O	P_2O_5	Mg	Zn	Cu	Mn
					mg·100 g^{-1}			mg·kg^{-1}		
CF_1	6.84	6.59	1.29	0.10	2.45	3.15	2.11	0.69	0.35	15.67
CF_2	6.91	6.63	1.13	0.09	1.96	3.22	2.45	0.88	0.24	18.90
MF_1	6.50	6.34	1.86	0.14	2.84	5.14	2.20	1.29	0.68	24.55
MF_2	6.39	6.21	1.77	0.18	2.60	5.94	1.56	2.14	0.51	22.22
Mean	6.66	6.44	1.51	0.13	2.46	4.36	2.08	1.25	0.44	20.33
Organic fertilization										
F_1	6.67	6.46	1.57	0.12	2.64	4.14	2.15	0.99	0.51	20.11
F_2	6.65	6.42	1.45	0.13	2.28	4.58	2.00	1.51	0.37	20.56
Mulching										
C	6.87	6.61	1.21	0.09	2.20	3.18	2.28	0.78	0.29	17.28
M	6.44	6.27	1.81	0.16	2.72	5.54	1.88	1.71	0.59	23.38

F_1 – treated sewage sludge (⅔) + bark (⅓); F_2 – treated sewage sludge (⅔) + sawdust (⅓);
C – without mulching (control); M – mulching with litter (ectohumus).

Irrigation requirements during the vegetation period (April-September), determined as water deficits – on average for 2005-07 – amounted to 212.6 mm, ranging from 130.4 mm to 291 mm, in 2007 and 2005, respectively. Of all months, July was characterized by the highest irrigation needs – about 70 mm. On the other hand, these requirements were very differentiated (due to rainfall amounts) in particular years of the study. For example, they amounted to 0.7 mm and 122.6 mm in 2007 and 2005, respectively.

Total amounts of irrigation water were higher in the case of one-year-old seedling production cycle (2005-06) than those for the two-year-old seedlings (2006-07), and amounted to 197 mm and 76 mm, respectively. It resulted – to a great extent – from the different irrigation needs of the seedlings during their first or second growing seasons [15, 16, 30]. Prudent irrigation and fertilization management can diminish costs of forest seedling production and minimize groundwater contamination [31].

In trials on Scots pine seedlings that were carried out simultaneously at the same forest nursery, the seasonal irrigation rates were similar to those for European larch [14]. But Höhnel maintained – according to Møller – that larch consumes 5-10 times more water than other conifers, while Schubert stated – according to Kiellander – that water uptake and evaporation is twice as high for larch than for Norway spruce, and four times higher than for Scots pine [32]. Unfortunately, there are no experimental data to verify these claims [32]. On the other hand, it is known that the covered (mulched) fields are characterized by lower water consumption as compared to the uncovered plots. It is mainly due to the reduction in surface evaporation from the topsoils [33].

Soil Characteristics

Our nursery experiments on European larch were conducted on a Cambic Arenosol formed on fluvioglacial sand [22]. According to Vidakovic [34], as well as Larsson-Stern [32], European larch grows most strongly on moderately rich soils, often giving very good results on light loams. On the other hand, healthy seedlings must be well supplied with all nutrients in proper proportions [35].

Mulched plots (treatments M) were characterized by lower pH values in comparison to those on control plots (without mulching) (Table 1). The same tendency was noted in the experiment on Scots pine seedlings that was conducted simultaneously in the same forest nursery [14].

The plots mulched by litter also had higher content of C_{org} and N_{total} as compared to those on control treatments. In trials on Scots pine seedlings carried out simultaneously at the same forest nursery [14], the contents of organic carbon (C_{org}), as well as that of N_{total}, were also higher in the treatments with mulching.

The measure of mulching also increased the content of available K and P. These results corroborate those of Klimek et al. [14] concerning Scots pine. No major differences of the Mg content (except for MF_2) were noted in the combinations of the experiment.

The mulched plots were also characterized by increased concentrations of Zn, Cu, and Mn in comparison to those on control plots (without mulching). The same tendency was noted in the above-mentioned experiment on Scots pine [14]. It can be added that availability of the most readily released manganese lessens with depth of the soil profile from a pine-covered area [36].

Table 2. Effects of organic fertilization and mulching on European larch seedling height and diameter.

Organic fertilization	Mulching	One-year-old seedling*		Two-year-old seedling**	
		height (cm)	diameter (cm)	height (cm)	diameter (cm)
F_1	C	18.35	0.41	85.50	1.11
	M	24.76	0.46	93.61	1.26
F_2	C	18.67	0.43	79.35	1.20
	M	25.32	0.48	97.16	1.34
Influence of fertilization (I)					
F_1	-	21.55[a]	0.43[a]	89.55[a]	1.18[a]
F_2	-	21.99[a]	0.45[a]	88.25[a]	1.27[a]
Influence of mulching (II)					
-	C	18.51[a]	0.42[a]	82.42[a]	1.15[a]
-	M	25.04[b]	0.47[b]	95.38[b]	1.30[b]

F_1, F_2, C, M – see Table 1; *, **– mean for 2005-06 and 2006-07, respectively.
Means in a column followed by the same letter do not differ at 5% level of significance (Tukey test).

Growth of Seedlings

Growth of European larch seedlings was characterized by height ranging from 18.35 cm to 25.32 cm, and from 79.35 cm to 97.16 cm in the case of one-year-old seedlings and two-year-old seedlings, respectively (Table 2). For comparison, in similar trials on Scots pine seedlings conducted simultaneously under the same soil-climatic conditions [14], the height of seedlings ranged from 10.35 to 11.87 cm and from 34.12 to 36.42 cm for one-year-old seedlings and two-year-old seedlings, accordingly. Such results indicated that the growth response is highly species-specific.

The type of organic fertilizer (sewage sludge + bark or sawdust) did not significantly affect seedling growth, both in the first and second growing seasons.

Seedling diameter ranged from 0.41 to 0.48 cm and from 1.11 to 1.34 cm, for one-year-old and two-year-old seedlings, respectively. There were no significant differences in diameter between seedlings fertilized with treated sewage sludge and bark (F_1), and treated sewage sludge and sawdust (F_2). The same tendency was also noted in the above-mentioned experiment on Scots pine seedlings [14].

The influence of mulching on both the seedling growth parameters was significant in the case of one-year-old seedlings, as well as two-year-old seedlings. Seedlings grown on mulched plots were characterized by higher height and diameter as compared to those cultivated on control plots (without mulching). Measure of mulching with ectohumus (forest edaphon inoculation) significantly increased the height of seedlings from 18.51 to 25.04 cm and from 82.42 to 95.38 cm, for one-year-old and two-year-old seedlings, respectively.

These data are partly in agreement with results obtained in simultaneous experiments on Scots pine conducted in 2005-07 at Białe Błota forest nursery [14]. In the mentioned trial there were no significant differences in diameter between Scots pine seedlings cultivated on control plots (C) and those on mulched plots (treatments M), although seedlings cultivated on mulched plots were characterized by slightly increased diameter as compared to those of seedlings grown on control plots.

Mycorrhizal Colonization

Given the young age of larch seedlings, the number of mycobionts forming mycorrhizas on roots was quite high (Fig. 3). Leski et al. [37] observed that for one-year-old seedlings of larch, the mean number of mycorrhizal species was about 1.4. At the investigated treatments, species richness could result from soil enrichment with sawdust, pine bark, and sediment. The presence of *Suillus grevillei* (Klotzsch) Singer mycorrhizas on young seedlings of larch was observed by mentioned authors. Previously, it was thought mycorrhizas of this species are formed on roots of older trees [38]. The high percentage of mycorrhizas is a good indicator for better growth of seedlings after outplanting. *S. grevillei* is fungus that belongs to a group of specialist-fungi and has a narrow range of hosts. It is advantageous because the fungus may have greater physiological compatibility with the host. Higher physiological compatibility would enable a specialist fungus to obtain more carbohydrates from the plant host than generalist competitors do [39].

Dominance of *Thelephora terrestris* Ehrh. might result from abilities of this species to grow even in poor arable soils with high moisture, as well as high nitrogen concentration [40, 41]. A few authors are of the opinion that *T. terrestris* mycorrhizas are not the optimal type of mycorrhizas since they cannot promote seedling growth [42] as, for instance, other mycorrhizal fungi e.g. *Suillus bovinus* (L. ex Fr.) O.

Table 3. Abundance (103 individuals·m^{-2}) of mites under differentiated organic fertilization and mulching.

Group of mites	Year	Treatments				M	F
		CF_1	CF_2	MF_1	MF_2		
Actinedida	I	1.03^{a}	1.28^{a}	1.00^{a}	1.88^{a}	ns	ns
	II	4.94^{a}*	5.87^{a}*	1.51^{a}	1.73^{a}	0.033	ns
	Mean	2.98^{a}	3.57^{a}	1.25^{a}	1.81^{a}	ns	ns
Gamasida	I	0.03^{a}	0.38^{b}	0.05^{a}	0.28^{b}	ns	<0.001
	II	0.13^{a}	0.10^{a}*	0.25^{a}*	0.35^{a}	<0.001	ns
	Mean	0.08^{a}	0.24^{b}	0.15^{a}	0.31^{b}	ns	0.006
Oribatida	I	0.03^{a}	0.18^{a}	2.51^{b}	4.01^{b}	<0.001	ns
	II	0.45^{a}*	0.43^{a}	5.79^{b}*	3.11^{c}	<0.001	ns
	Mean	0.24^{a}	0.30^{a}	4.15^{b}	3.56^{b}	<0.001	ns
Tarsonemida	I	-	0.03^{a}	-	0.05^{a}	ns	ns
	II	-	-	0.08^{a}	0.08^{a}	ns	ns
	Mean	-	0.01^{a}	0.04^{a}	0.06^{a}	ns	ns
Acari total	I	1.08^{a}	1.86^{a}	3.56^{b}	6.22^{b}	<0.001	ns
	II	5.52^{a}*	6.40^{a}*	7.63^{bc}*	5.27^{ac}	0.001	ns
	Mean	3.30^{a}	4.13^{a}	5.59^{b}	5.74^{b}	<0.001	ns

[a] – the same letter means lack of significant differences, p<0.05;
* – significant between I and II year, p<0.05;
M – mulching effect (p); F – fertilization effect (p).

Kuntze [43]. Nevertheless, Lehto [44] in a short term experiment found that mycorrhias of *Thelephora terrestris* supported development and growth of *Picea abies* seedlings affected by drought. A positive role of *Thelephora terrestris* in the colonization of Scots pine roots following the planting of seedlings on former farmland was found by Hilszczanska et al. [41]. The second, most abundant mycorrhizas on roots of two-year-old seedlings were those created by *Wilcoxina mikolae* (Yang & Wilcox) and *Wilcoxina* sp.

The presence of ectendomycorrhizas belonging to *Wilcoxina* indicates a high concentration of nitrogen in the soil. Ectendomycorrhizas on conifer seedlings are especially abundant in nurseries where high doses of nitrogen are used [45]. The latter mycorrhizas were found also on *Picea abies* seedlings growing in nurseries [46, 47]. After seedlings' outplanting from nurseries, ectendomycorrhizas were observed to readily replace other mycorrhizal fungi. The fungi can thrive on roots only when a soil's environment lacks competitive species [48].

Occurrence of Mites

Mites are included for the predominant representatives of mesofauna in forest soils. They perform a number of significant tasks, and their occurrence is important because of soil-forming processes in which they participate considering numerous connexions with other soil organisms, e.g. ectomycorrhizal fungi. Saprophage Oribatida mites most

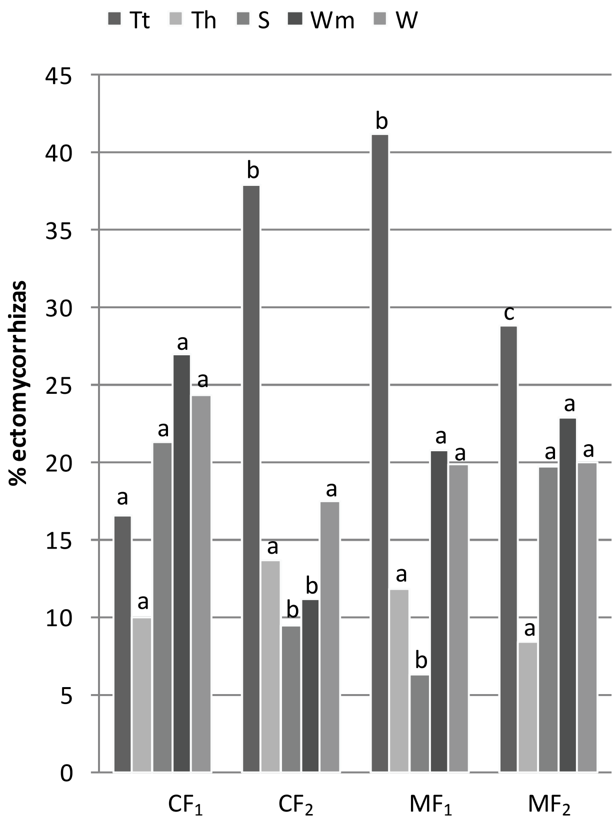

Fig. 3. Frequencies of mycorrhizal morphotypes of European larch seedlings (Tt – *Thelephora terrestris*, Th – Thelephoraceae, S – *Suillus* sp., Wm – *Wilcoxina mikolae*, W – *Wilcoxina* sp., n=5, p<0.05, data with the same letter do not differ significantly).

often dominate among mites in forest soils. They compose about 70% of all mites [49], and their density (e.g. in Scots pine greenwood) can range from 49,220 to even 258,810 individuals·m^{-2} [50]. Literature confirms that these mites can feed on ectomycorrhizal fungi [51, 52]. In addition, soil fauna can impact fungal growth by grazing [53, 54], which may split the hyphal connections. Soil animals can also directly relocate nutrients by defecation, and transfer microbial propagules into fresh substrates [55, 56].

In this experiment, the density of mites ranged from 1,080 to 7,630 individuals·m^{-2}, in the first year of the study at treatment CF_1 and in the second year at treatment MF_1, respectively (Table 3). The increase in density of mites was noted in treatments CF_1, MF_1, and CF_2 during a two-year cycle of investigation, and differences between the first and second years were significant.

Distinct changes were noted in gatherings of mites due to mulching: instead of Actinedida, which were predominant in treatment C (their percentage ranged 87-90% of all Acari) Oribatida occurred (on average 62-74%). Predacious Gamasida, as well as Tarsonemida, were less numerous in the investigated soils as compared to the above-mentioned orders of mites. Mulching was the only factor influencing the occurrence of oribatid mites. Influences of structure-forming additives for composts on these mites were not detected. On the other hand, such influence was noted in the case of Gamasida.

Results indicate that after treatment of mulching, a moderate increase of the general density of mites was obtained due to saprophage Oribatida. A slightly better result of edaphon introduction was obtained previously in the investigation on Scots pine cultivation in the same forest nursery [9], but a considerably better effect was found in white birch cultivation [10]. This measure was also considerably more advantageous in the experiment on Scots pine cultivation conducted simultaneously under identical pluvio-thermal conditions – the density of mites in the second year of investigation was maximum and ranged from 22,020 to 37,480 individuals·m^{-2} [57]. A moderate increase of the density in European larch cultivation, as compared to that of Scots pine, can be caused by different ecological conditions that are created by these plantations. On the other hand, improvement of the biological condition of soils in European larch cultivation after treatment of mulching was distinct and statistically confirmed, although the increase of bioactivity was lower than in cases of Scots pine [14] and white birch [10].

Conclusions

1. The 0-15 cm surface layer of the soils of mulched plots were characterized by lower pH values and higher contents of $C_{org.}$ and N_{total}, as well as concentrations of K, P, Zn, Cu, and Mn than non-mulched plots.
2. The sort of organic fertilizer did not affect significantly seedling growth both in the first as well as in the second growing seasons. The influence of mulching with forest ectohumus on seedling growth was significant.
3. Mycorrhizal structure was well developed. This factor was promising regarding improvement of European larch seedling adaptation on post-agricultural land. Sewage sediments used along with sawdust and pine bark did not negatively influence ectomycorrhizae development of juvenile seedlings.
4. Forest edaphon inoculation – by mulching – increased the density of mites, especially saprophage Oribatida, which can indicate the positive effect of this measure on the increase in biological activity in the studied soils.

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