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Effect of seedling production method on the growth of *Pinus sylvestris* L. on reclaimed post-industrial sites in Poland

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Abstract: Selection of tree species and proper technology of afforestation of post-industrial sites play a crucial role in new ecosystem stability and success of the restoration. There is still current discussion about the effectiveness of different methods in the production of seedlings for extreme site conditions. This study aimed to analyze the effect of the seedling production method on the growth of 6-year-old Scots pine (*Pinus sylvestris* L.) trees planted on reclaimed post-industrial sites in southern Poland.

We compared four methods of seedling production: bare root system, containerized without and with inoculation of ectomycorrhizal fungi *Laccaria bicolor* (Maire) P.D. Orton, and *Hebeloma crustuliniforme* (Bull.) Quél. The experiment was carried out independently on two reclaimed sites: a post-sand-mining site in Bukowno, and a heavy metal polluted site in Miasteczko Śląskie. The effect of the seedling production method on tree growth expressed by height, root collar diameter, above- and belowground biomass of different tree components was analyzed on 240 sampled trees using analysis of variance and general linear model. In addition, the effect of the seedling production method on the root-to-shoot ratio was investigated.

Scots pine tree growth was significantly affected by the seedling production method on heavy metal polluted site. The lowest tree growth parameters were observed in trees planted with bare roots, whereas the largest values were in the case of trees planted from containerized seedlings inoculated with *H. crustuliniforme*. In contrast, on the post-sand-mining site, the treatment effect turned out to be insignificant. The effect on biomass allocation was observed in the case of both sites. Trees prepared with the bare root method differed in greater biomass allocation to the belowground from other treatment groups.

The use of containerized seedlings or additionally inoculated with ectomycorrhizal fungi in heavy metal contaminated sites improves the growth of trees and, although it is more costly and labor-intensive compared to the production of seedlings with a bare root system, should be preferred. In the post-sand-mining area, no effect of the seedling production method on tree growth was observed, and the use of bare root seedlings will be equally effective and additionally less time and cost-consuming.

Keywords: biomass allocation, root-to-shoot ratio, environmental stress, Scots pine plantation, ectomycorrhizal fungi

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Introduction

Seedling establishment and their adaptability to extreme site conditions is a critical phase in the afforestation of all infertile post-agricultural (Sierota & Hilszczańska, 2009) and post-industrial reclaimed lands (Pietrzykowski, 2015). Limitations to seedling growth on these sites include water deficiency, low soil fertility, heavy metal pollution, and reduced soil biological activity (Grossnickle, 2005; Chodak et al., 2013; Pietrzykowski et al., 2014). Successful afforestation depends on the capacity of tree seedlings to capture resources quickly (Duñabeitia et al., 2004a). Therefore, the seedling production method may have large importance for the effectiveness of afforestation processes. It is assumed that seedlings produced in containers have better root protection against mechanical damage and drying out during transport and technological operations, and better availability of nutrients from substrates, as a result, they have limited stress from transplanting compared to seedlings produced in the traditional bare root system (Wilson et al., 2007; Repáč et al., 2014). Moreover, containerized seedlings are characterized by faster growth and higher survival rate than bare root seedlings cultivated in height density in a nursery bed (Wilson et al., 2007; Repáč et al., 2011; Esen et al., 2012). The main disadvantage of producing seedlings in containers is the need for more space due to the size of the containers used, as well as the need for sufficient substrate and transportation facilities (Pinto et al., 2011).

A promising seedling production method is inoculation with ectomycorrhizae (ECM) fungi (Duñabeitia et al., 2004a; Wilson et al., 2007; Esen et al., 2012; Repáč et al., 2014). ECM fungi stimulate the formation of lateral roots and the elongation of root hairs (Karabaghli-Degron et al., 1998; Duñabeitia et al., 2004b; Rupp et al., 1989), which results in both improved absorptions of nutrients, and production of regulators (Reid et al., 1983; Strzelczyk et al., 1994). ECM inoculation results in increased growth and better drought, temperature, and pH-stress tolerance (Kandziora-Ciupa et al., 2013). All these factors improve the establishment, survival, and growth rate of tree seedlings in forest plantations growing in adverse conditions (Duñabeitia et al., 2004a; Ortega et al., 2004; Menkis et al., 2005; Menkis et al., 2011). Moreover, ECM protects young trees against heavy metal toxicity (Morte et al., 2001; Van Tichelen et al., 2001). ECM is especially important for afforestation in areas with a strong impact from industrial emissions (Cudlin et al., 2007). Bojarczuk et al. (2014) found that ECM inoculation of grey poplar micro plants tolerant to heavy metals can facilitate the adaptation of these plants to metal-polluted soils and may be used for afforestation and phytoremediation of polluted land. Polle et al. (2011) found that ECM fungi can also enhance plant tolerance by influencing its defense genes.

In natural conditions, the process of seedling mycorrhization is spontaneous and is very often observed also in nurseries. Controlled inoculation with selected ECM fungi is considered to be a method that mimics natural processes and is suggested as an effective practice for improving the properties of nursery seedling stock (Parladé et al., 2004; Iwański et al., 2006; Rincón et al., 2007). However, some studies (Menkis et al., 2011) have demonstrated that the use of traditional seedling production methods might result in similar or even higher mycorrhization and survival rates than those achieved by controlled ECM inoculation. Moreover, it was reported by Repáč et al. (2011) that ECM inoculation of containerized seedlings of Norway spruce (*Picea abies* (L.) H. Karst), Scots pine (*Pinus silvestris* L.) and European beech (*Fagus sylvatica* L.) did not have a significant effect on survival after planting in the field. Thus, the possibility to avoid expensive controlled ECM inoculation could be an essential procedure in the optimization of seedling production technology.

The most common strategy for afforesting reclaimed sites, especially those with low nutrient content, is to use the succession theory and apply pioneer tree species that have a higher capacity to tolerate unfavorable environmental conditions (Pietrzykowski, 2019). When selecting species composition, in addition to adaptability, the properties and abilities of a tree species to create and transform the soil environment are also important. For these reasons, Scots pine is one of the main tree species used in the reclamation of mining sites in central Europe (Kuznetsova et al., 2010; Pietrzykowski, 2019; Vacek et al., 2021a). This species is characterized by good adaptation to unfavorable habitat conditions as evidenced by its high site index, which often reaches Class I, and in extreme cases, on the most infertile sandy soils, the site index fluctuates between Class II and III (Pietrzykowski, 2019). The high adaptability of Scots pine is also confirmed by Vacek et al. (2021b), who additionally point to high timber production comparable to that achieved in pine commercial forests. In addition, Wąsik et al. (2018) found that selected technical parameters of the wood of pines growing in mining areas were similar to those observed in populations found in undisturbed environments. Scots pine shapes a lower soil pH and provides a higher level of soil organic carbon compared to black pine (*Pinus nigra* Arn.) (Woś et al., 2022).

According to Pietrzykowski (2019), a comprehensive assessment of restoration success, which includes both changes in soil characteristics and stand condition, involves evaluating biomass production. Data on biomass and the growth rate of trees allow assessing the potential and current productivity of the ecosystem, as well as determining the amount of produced wood and biomass for economic purposes. In addition, based on them it is possible to determine the potential for carbon sequestration in the ecosystems formed in mining areas and their impact on reducing the greenhouse effect.

The biomass, especially its allocation to different organs, is crucial for assessing the adaptation of plants to the environment, as well as for studying the impact of silvicultural practices in forest productivity (Gargaglione et al., 2010). A special case of allocation is the root-to-shoot ratio, which, according to "optimal partitioning theory", is an essential morphological tree attribute that indicates how plants compensate for limited resources in the site (Gargaglione et al., 2010; Mašková & Herben, 2018).

The specific objective of this study was to analyze the dynamics of tree growth, biomass allocation, and production concerning four methods of seedling production in six-year-old Scots pine plantations established on reclaimed post-industrial sites in Poland. Therefore, it can be expected that the method of seedling production can affect the growth and allocation of Scots pine biomass, as well as the allometric relationships between basic tree dimensions (diameter, height) and the biomass of individual tree components. We assumed that Scots pine trees grown from containerized seedlings have higher biomass production and biomass allocation to the aboveground part compared to bare-root seedlings. In addition, we assumed that inoculation with ECM fungi further increases biomass production and raises biomass allocation to the aboveground part. To test our hypothesis, experiments with randomized complete block design were established with four methods of seedling production on two reclaimed post-industrial sites in southern Poland.

Materials and methods

Study site and experiment design

The experiment was established on two sites located in southern Poland: PM (50.5153°N, 18.9473°E) – reclaimed open-cast sand mine area in Bukowno, and IE (50.2529°N, 19.3583°E) – a site impacted by industrial heavy metal emissions located near the zinc and lead smelter in Miasteczko Śląskie (Ciszewski et al., 2004; Pająk & Jasik, 2011) (Fig. 1). In both cases, the area was flat terrain located at 280–290 meters above sea level. Both sites have similar climatic conditions, with average annual temperatures of 8.5 °C and 8.6 °C, and total annual precipitation of 736 mm and 756 mm for the PM and IE sites, respectively.

At both locations, the experiment was carried out in a randomized complete block design (Fig. 1). In three blocks, four treatment groups are assigned at random. As a treatment group seedlings cultivated in four different types of nursery systems were used: C – container-grown seedlings without inoculation, L – container-grown seedlings inoculated by *Laccaria bicolor* (Maire) P.D. Orton, H – container-grown

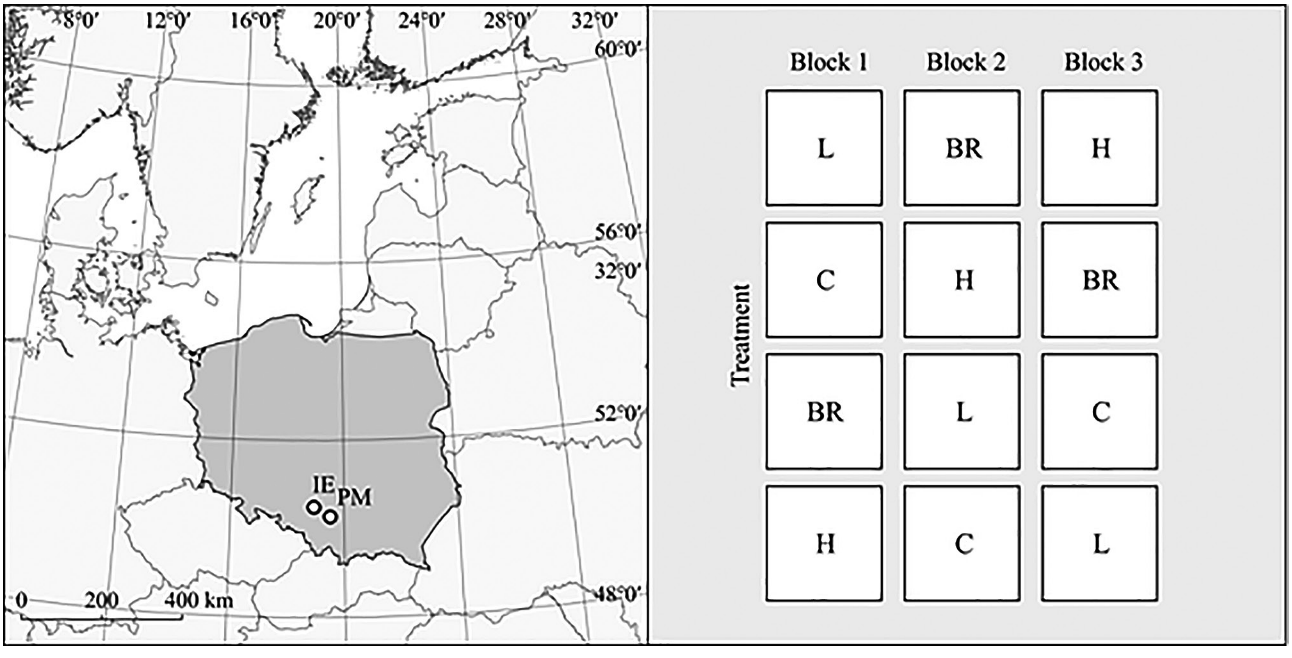

Fig. 1. Location of experimental plantations (left) and scheme of the field experiment design (right). PM – reclaimed open-cast sand mine site in Bukowno; IE – a site impacted by industrial heavy metal emissions in Miasteczko Śląskie; Treatment groups of seedlings: BR – bare root; C – containerized without ECM inoculation; L – containerized with *L. bicolor* inoculation; H – containerized with *H. crustuliniforme* inoculation

seedlings inoculated by *Hebeloma crustuliniforme* (Bull.) Quél, and BR – bare root seedlings grown in open air nursery beds system.

All seedlings were planted in the nursery of the Rudy Raciborskie Forest District. A seed lot collected in 1996 from cones harvested in a mature Scots pine stand was used to produce seedlings (Szabla, 2004). Containerized seedlings were cultivated in V-120 (120 cm^3) containers. The containers were filled with a substrate composed of 70% sphagnum peat (pH = 4.5) and 30% vermiculite. The substrate was sterilized by steam at 90 °C for 2.5 hours. Inoculation and cultivation of seedlings with *L. bicolor* were carried out according to the technology of the French company Robin Pépinières (Szabla, 2004; Kowalski, 2007), and the *H. crustuliniforme* according to Polish technology (Kowalski, 2006). The effectiveness of the above methods of mycorrhization of pine seedlings is over 83% for *L. bicolor* and over 97% for *H. crustuliniforme* (Kowalski, 2007). Plantations were established in the spring of 2000 by planting one-year-old containerized seedlings and two-year-old bare root seedlings cultivated in the traditional open-air nursery beds system. Seedlings with similar biometric features were selected for cultivation. All seedlings were planted by hand using a dibble bar. Both on PM and IE sites, four types of seedlings were planted under the system of three blocks (4 treatment groups × 3 blocks) using 300 seedlings per treatment in each block with spacing 1.2 × 0.8 m.

Soil properties and site reclamation methods

Soil analyses were conducted before the experiment was established. The soil types were classified according to the WRB system (FAO & ISRIC, 2006) as Urbic Anthrosols and Haplic Podzols on the PM and the IE sites, respectively. At each experimental site, two samples of the uppermost mineral soil from 0–15 and 15–30 cm depth were taken. The soil samples (1.0 kg total), which consisted of twelve subsamples for each layer collected in a regular grid across the study site, were analyzed for chemical and physical properties (Table 1). Before analysis, soil samples were air-dried for about one week at room temperature and sieved using a 2mm mesh. The sieved samples were used to measure the particle size composition, determined using a laser diffraction particle sizer (Fritsch Analysette 22, Idar-Oberstein, Germany). In addition, the pH was measured electrochemically with a combination electrode in a suspension with distilled water (1:5, w/v) after 24 h of equilibration. In the fine samples, we measured the content of soil C and N with a LECO CNS True Mac analyzer. The Hg content was determined using

Table 1. Soil physicochemical characteristics

Experimental site	PM		IE	
Soil sampling depth [cm]	0–15	15–30	0–15	15–30
Granulometric fractions				
Sand (2–0.05 mm) [%]	97	98	94	90
Silt (0.05–0.002 mm) [%]	2	1	3	6
Clay (<0.002 mm) [%]	1	1	3	4
Chemical parameters				
$pH_{KCl\ (1:2.5)}$	4.7	4.6	4.0	3.8
C_{org} [%]	0.18	0.14	0.96	1.03
N_t [%]	0.05	0.05	0.04	0.04
P_{av} [mg 10^{-1} kg]	0.010	0.001	0.019	0.028
Na^+ [$cmol_{(+)}$ kg^{-1}]	0.03	0.05	0.04	0.04
K^+ [$cmol_{(+)}$ kg^{-1}]	0.01	0.01	0.01	0.01
Ca^{2+} [$cmol_{(+)}$ kg^{-1}]	0.33	0.29	0.27	0.35
Mg^{2+} [$cmol_{(+)}$ kg^{-1}]	0.10	0.08	0.04	0.04
CEC [$cmol_{(+)}$ kg^{-1}]	0.81	1.44	2.71	4.01
Hh [$cmol_{(+)}$ kg^{-1}]	0.34	1.01	2.34	3.56
Zn [mg kg^{-1}]	6.0	20.5	20.5	16.5
Cu [mg kg^{-1}]	0.4	0.6	0.6	1.0
Pb [mg kg^{-1}]	2.2	3.4	11.3	6.5
Cd [mg kg^{-1}]	0.5	0.3	0.3	0.4
Cr [mg kg^{-1}]	1.3	1.5	2.8	2.7
Ni [mg kg^{-1}]	2.0	0.0	0.0	1.3

PM – reclaimed post-mining site; IE – heavy metal industrial emission impacted site; C_{org} – soil organic carbon; N_t – total nitrogen; P_{av} – phosphorus in a form available to plants; CEC – cation exchangeable capacity; Hh – exchangeable acidity.

a Direct Mercury Analyzer (DMA-80 Hg). The experimental site soils were similar in terms of basic characteristics, including texture, acidity, and nutrient content. The differences between site soils occurring in soil organic carbon and reclaimed soil at PM C_{org} were low (0.14–0.18%), while at IE C_{org} these were higher (0.14–1.03%). The main differences between soils were in terms of lead (Pb) and chromium (Cr) concentrations in the uppermost mineral layer, where at the PM site Pb concentration ranged from 2.2–3.4 mg kg^{-1} and Cr at 1.3–1.5 mg kg^{-1}, while at the IE site Pb was 6.5–11.3 mg kg^{-1} and Cr 2.7–2.8 mg kg^{-1} (Table 1).

The PM site was reclaimed with standard treatments (Pietrzykowski & Krzaklewski, 2007), including re-grading the surface and fertilization (NPK), and green manure in the form of N-fixing lupine (*Lupinus luteus* L.) was also used. Site preparation at IE before afforestation included removal of the upper organic horizons and plowing mineral soil layers.

The sample tree selection and biomass measurement

Six years after plantations establishment at the end of summer (turn of August/September), ten systematically selected sample trees per treatment in each block were harvested, a total of 240 trees (10 trees × 3 blocks × 4 nursery production systems × 2 sites = 240 trees). After excavation, the height and root collar diameter had been measured, and then each sample tree was divided into an above-ground and below-ground part. The above-ground part of each tree was divided into the stem, branches without needles, and needles, whereas the below-ground part into coarse roots (Ø ≥ 0.5 cm) and fine roots (Ø < 0.5 cm). All components sealed separately in a hermetic container were taken to the laboratory. The collected material was subjected to a two-stage drying process. The first, lasting at least 1.5 months for needles and 2.5 months for woody organs, involved storing the samples in a dry laboratory room. In the second stage, the samples were placed in a drier with forced air circulation (SUP-150W, https://wamed.pl, Poland) at 105 °C and dried until a constant weight is obtained. The air-dried samples were weighed on an electronic scale (WPS 3100/C/2, Radwag, Poland) with an accuracy of 0.02 g.

Based on the dry weight of individual tree components, the biomass of the whole tree and the biomass of above- and below-ground parts were calculated. The allocation of biomass to individual organs, expressed as the share of a given organ in the weight of the entire tree or as the root-to-shoot ratio, was also determined. The root-to-shoot ratio was calculated for all sampling trees by dividing the total dry mass of roots (coarse and fine) by the above-ground dry mass of the tree, which consisted of the stem, branches, and needles.

Data analyses

Effect of seedling production method on the tree growth, biomass production and allocation

ANOVA, followed by Scheffe's post-hoc test, was used to test the effect of the seedling production method on tree size (height, diameter), biomass production and biomass allocation to individual organs, and root-to-shoot ratio.

The analysis was preceded by Shapiro-Wilk normality and Levene's homogeneity of variance tests. Most biomass components and their allocations generally had non-normal distributions. Therefore, to apply parametric tests, a logarithmic transformation of the data was performed. The effect of the method of seedling production was compared separately for trees planted on PM and IE sites.

Allometric relationships of tree biomass

To analyze the difference in tree allometry between the PM and IE sites, the seedling production method, and the biometric features of the individual tree, the General Linear Model (GLM) was used. At the last stage of analysis, allometric equations describing both above- and below-ground component biomass were developed. In general, in the analysis of allometric relations, residual heteroscedasticity occurs, consisting of an increase in the residual variance of the dependent variable with an increase in the values of an independent variable. To obtain the homoscedasticity of residuals, we used the form of the allometric function linearized by finding the natural logarithm Eq. 1:

$$\ln(B) = \ln(\beta_0) + \beta_1 \times \ln(h) + \beta_2 \times \ln(d_0) + \varepsilon \quad (1)$$

where β is biomass component (g), h is tree height (m), d_0 is root collar diameter (cm), ε is a random error of normal distribution, and additive error was assumed for all components.

Logarithmic transformation tends to equalize the variance, however, the transformation also introduces a systematic bias into the calculations (Baskerville, 1972; Sprugel, 1983). Therefore after retransformation, a correction factor *CF* (Eq. 2) was used in the final version of the allometric equations (Eq. 3).

$$CF = 0.5 \times \frac{\Sigma(\ln(B_i) - \ln(\hat{B}_i))^2}{N - 2} \quad (2)$$

$$B = \beta_0 \times h^{\beta_1} \times d_0^{\beta_2} \times e^{CF} \quad (3)$$

Statistical analyses were done using STATISTICA (StatSoft, 1984–2014) and R (R Development Core Team, 2010) software.

Results

Effect of seedling production method on the tree growth, biomass production and allocation

The average height of six-year-old pine trees in the analyzed treatment groups was from 1.61 m (C) to 1.79 m (H) and from 1.09 m (BR) to 1.63 m (L) for the PM and IE sites, respectively (Fig. 2). The average root collar diameter of trees on the PM site ranged from 3.66 cm (BR) to 4.42 cm (H), and on the IE site from 2.59 cm (BR) to 4.41 cm (H). The total biomass of sampled trees ranged from 0.046 kg to 3.151 kg, an average of 1.144 kg on the PM plantation and from 0.023 kg to 4.374 kg with an average of 0.840 kg on the IE site (Fig. 2).

In the case of the PM site treatment effect turned out to be insignificant in the case of nearly all analyzed tree growth parameters, except for root collar diameter (ANOVA, $p = 0.013$; Fig. 2). It was found that seedlings produced in the H system reached 21% larger diameters than those produced in the BR system. The effect of the seedling preparation method on biomass allocation to individual organs was significant for above-ground, below-ground, branch, and coarse root components (ANOVA, $p < 0.0001$; Fig. 3). However, the seedling preparation method did not affect biomass allocation to fine roots, stems,

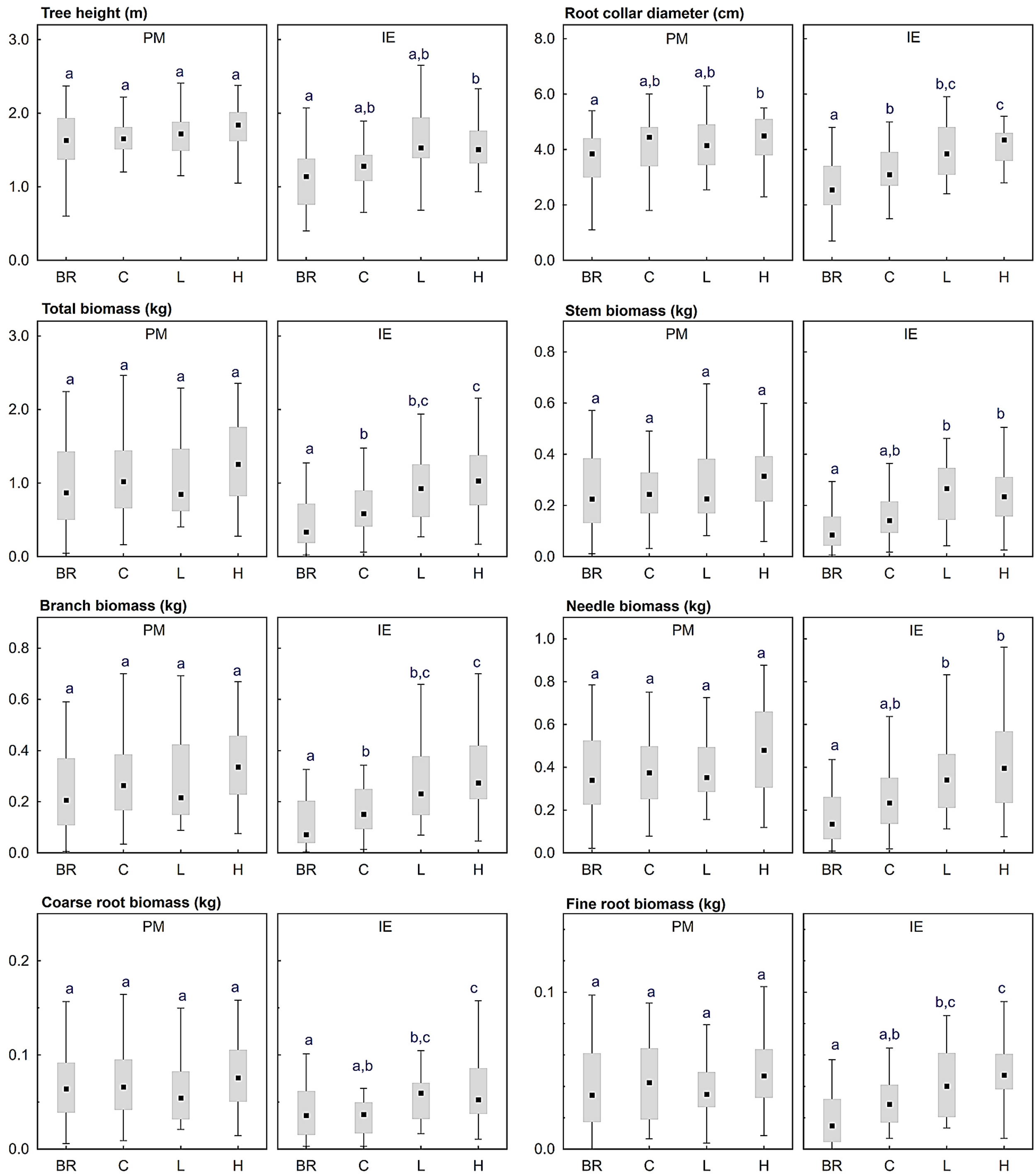

Fig. 2. Effect of seedling preparation method (BR – bare root; C – containerised without ECM inoculation; L – containerised with *L. bicolor* inoculation; H – containerised with *H. crustuliniforme* inoculation) on the growth parameters of six-years-old trees planted on PM – reclaimed open-cast sand mine area and IE – a site impacted by industrial heavy metal emissions

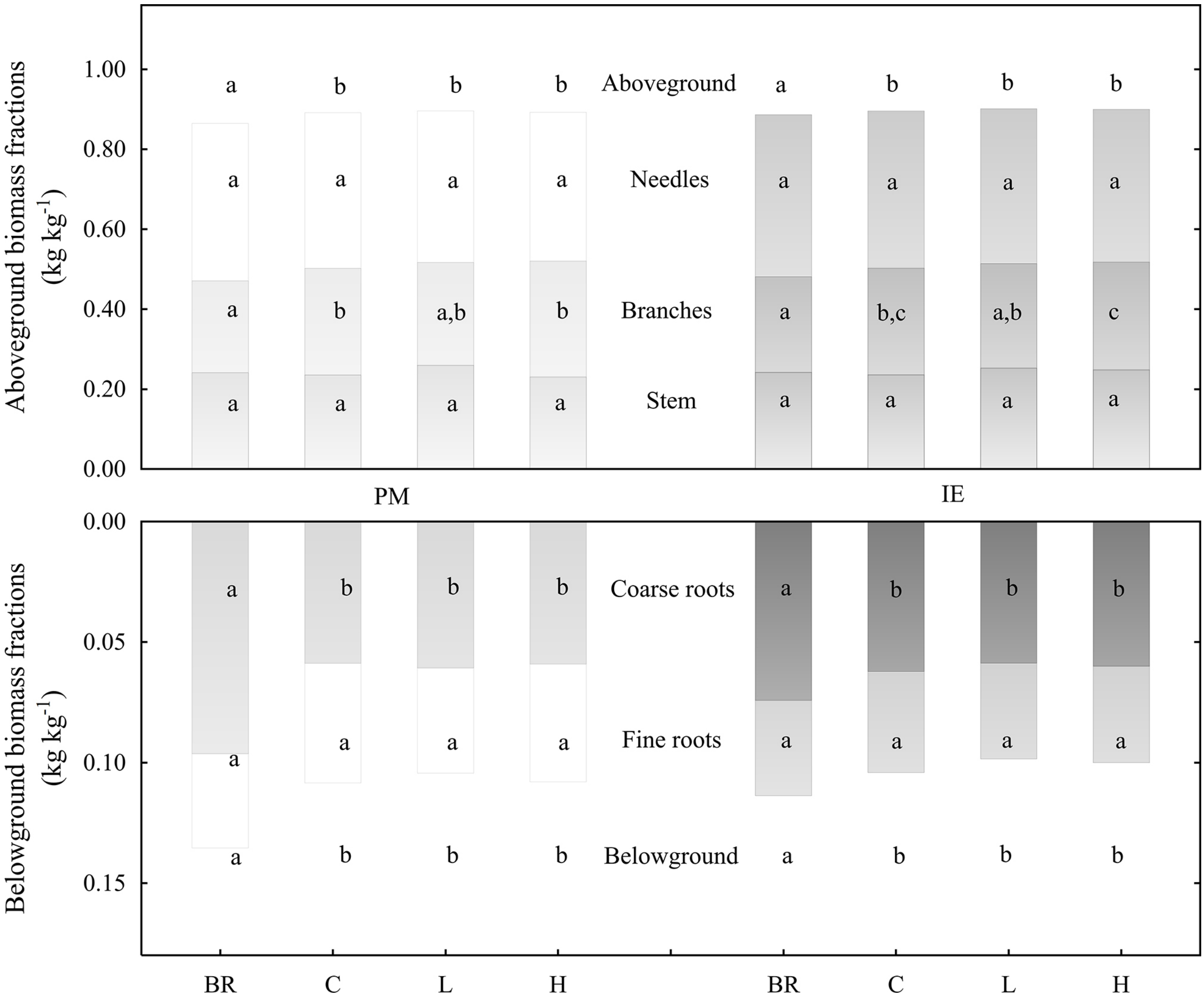

Fig. 3. The effect of seedling preparation method (BR – bare roots; C – containerised without ECM inoculation; L – containerised with *L. bicolor* inoculation; and H – containerised with *H. crustuliniforme* inoculation) on the biomass allocation for trees growing on PM – reclaimed open-cast sand mine area, and IE – a site impacted by industrial heavy metal emissions

and needles (ANOVA, p > 0.05, Fig. 3). Significant differences (Scheffé's test, p < 0.05) in allocation were observed between seedlings produced in the BR system and the others, i.e. C, L, and H (Fig. 3).

In contrast, on the IE site, both the tree root collar diameter, height, total biomass, and biomass of particular tree components were found to be significantly affected by the seedling production method (ANOVA, p < 0.0001; Fig. 2). The lowest values were observed in the case of trees planted with BR, whereas the largest values were for trees planted from containerized seedlings inoculated with *H. crustuliniforme*. Trees planted from containerized seedlings without ECM inoculation and inoculated by *L. bicolor* generally attain average values for the tree growth parameters and the differences between treatment groups C and L, except for the tree height, were not significant (Scheffé's test, p > 0.05; Fig. 2). The method of seedling production affected all tree growth parameters, however, the effect on biomass allocation to individual tree parts was observed only for branches and coarse roots. The Scheffé's post-hoc test indicated that trees grown from seedlings prepared with the BR method differed significantly in biomass allocation from other seedling treatment groups (Fig. 3). At both the PM and IE sites, trees grown from seedlings prepared with the BR method had a lower biomass allocation to the above-ground part and consequently a higher allocation to the below-ground part. However, the difference in allocation to the below-ground part resulted from a significant allocation to coarse roots, whereas there were no observed significant differences in allocation to fine roots. Similarly, differences in biomass allocation to the above-ground part were due to significant allocation to branches, while allocation to the stem and needles was insignificant between the analyzed variants of the experiment (Fig. 3).

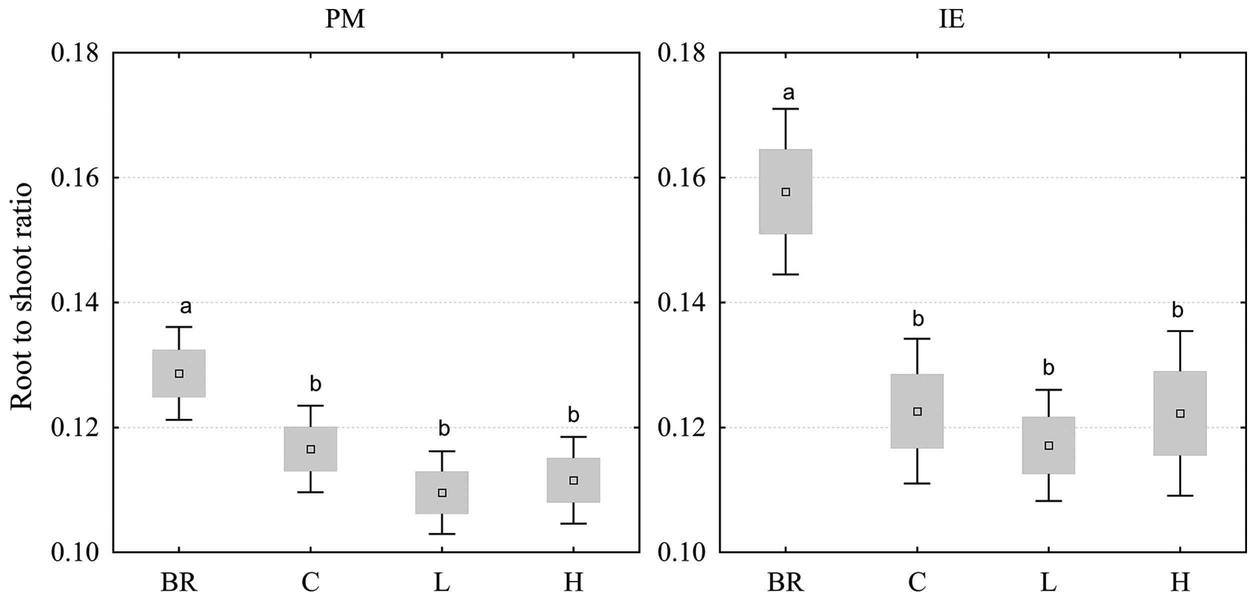

Fig. 4. Effect of seedling preparation method (BR – bare roots; C – containerised without ECM inoculation; L – containerised with *L. bicolor*, and H – containerised with *H. crustuliniforme* inoculation) on root to shoot ratio for trees growing on PM – reclaimed open-cast sand mine area and IE – a site impacted by industrial heavy metal emissions

Both in the case of PM and IE sites was found a significant difference in the root-to-shoot ratio between trees cultivated from the BR and the other treatment groups (Fig. 4), however, the ECM inoculation did not significantly affect (ANOVA and Scheffé's test) root-to-shoot ratio for containerized seedlings (Fig. 4).

Allometric relationships of tree biomass

Using the General Linear Model, it was found that both the variability of total biomass and individual biomass components are in covariance mainly with tree root collar diameter and only in a very small part with seedling preparation method (only coarse root biomass, $p < 0.05$) and site (only belowground biomass, $p < 0.05$), although the method of seedling preparation affected tree heights, diameters and all analyzed tree biomass characteristics, especially in the case of IE site (Table 2). Except for coarse roots, some differences in biomass allometry between treatment groups become insignificant after removing the effect of tree height and diameter (Fig. 5). Therefore it was possible to develop uniform generalized allometric equations for both analyzed sites and treatment groups with tree height and diameters as independent variables without loss of explained variance. Developed equations explain 73% of the variance in the case of fine roots up to 94% for above-ground and total biomass (Table 3).

Table 2. General Linear Model to evaluate the effects of tree height (*h*), root collar diameter (d_0), seedling production method (SPM) and site on the biomass of individual tree components. The significance of the effects was evaluated using the Wald statistic (W) at a level of $\alpha = 0.05$

Dependent variable	Statistics	GLM independent variable					
		Intercept	h	d_0	SPM	Site	SPM*Site
Total biomass	*W*	531.7	11.3	623.0	8.7	0.1	11.1
	p	<0.0001	0.001	<0.0001	0.033	0.783	0.011
Aboveground biomass	*W*	4320.3	8.7	692.2	3.1	11.4	17.6
	p	<0.0001	0.0033	<0.0001	0.0787	0.0098	0.0005
Branch biomass	*W*	104.3	0.0	448.9	2.3	0.0	6.8
	p	<0.0001	0.995	<0.0001	0.509	0.943	0.079
Needle biomass	*W*	199.7	0.3	382.2	5.7	3.7	10.2
	p	<0.0001	0.616	<0.0001	0.129	0.053	0.017
Belowground biomass	*W*	781.6	5.4	410.0	0.0	29.2	5.8
	p	<0.0001	0.0204	<0.0001	0.9325	<0.0001	0.1233
Coarse root biomass	*W*	2.8	8.9	286.8	70.0	0.1	7.8
	p	0.097	0.003	<0.0001	<0.0001	0.780	0.050
Fine root biomass	*W*	6.1	0.1	195.4	1.3	0.6	8.1
	p	0.014	0.725	<0.0001	0.732	0.455	0.043

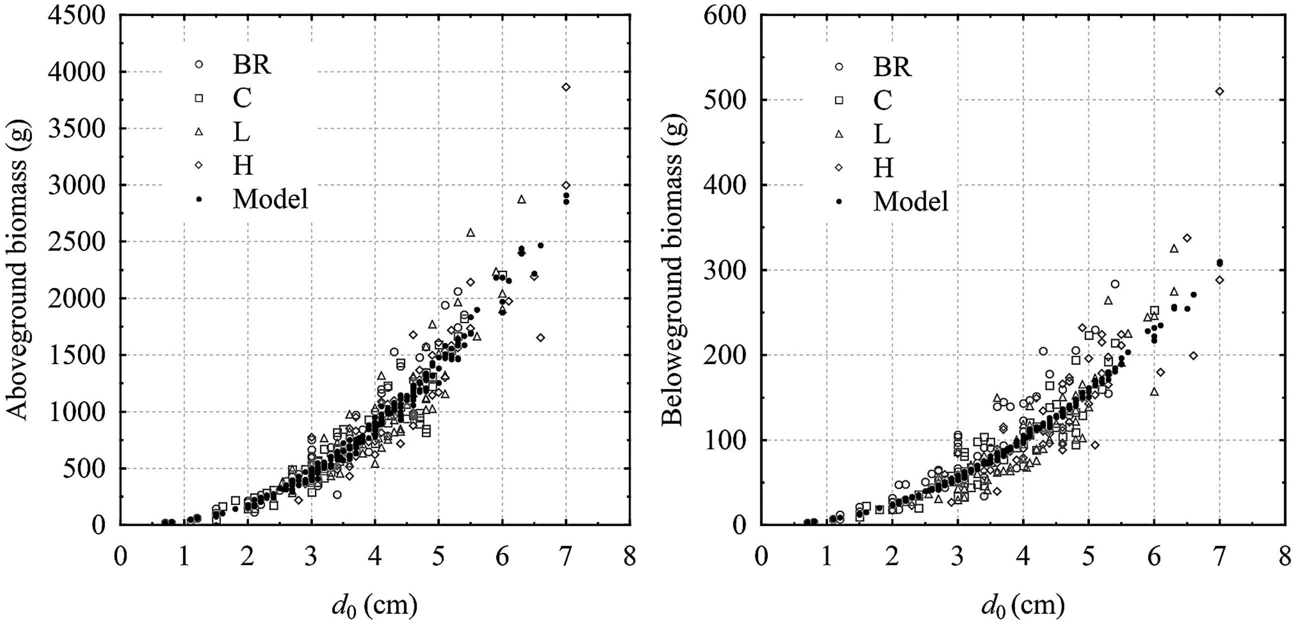

Fig. 5. Dependence of aboveground and belowground biomass on the root collar diameter (d0) for trees growing from bare roots (BR), containerized without ECM inoculation (C), containerized with *L. bicolor* (L) and *H. crustuliniforme* (H) inoculation

Table 3. Parameters (β_0, β_1, β_2) and basic statistics (R^2_{adj} – adjusted coefficient of determination, *CF* – correction factor) of biomass allometric equations

Biomass component	Parameter	Value of parameter	Standard error	*t*-statistic	*p*-level	R^2_{adj}	CF
Total biomass	β_0	12.3066	0.2992	8.38	<0.0001	0.938	0.0214
	β_1	0.3293	0.0752	4.37	<0.0001		
	β_2	1.9383	0.0719	26.95	<0.0001		
Aboveground biomass	β_0	9.5761	0.3039	7.43	<0.0001	0.937	0.0221
	β_1	0.3555	0.0763	4.65	<0.0001		
	β_2	1.9418	0.0730	26.58	<0.0001		
Needles biomass	β_0	9.6477	0.3999	5.67	<0.0001	0.885	0.0382
	β_1	0.1786	0.1005	1.78	0.0770		
	β_2	1.9681	0.0961	20.47	<0.0001		
Branch biomass	β_0	9.3223	0.4023	5.55	<0.0001	0.911	0.0386
	β_1	−0.0261	0.1011	−0.26	0.7962		
	β_2	2.4731	0.0967	25.58	<0.0001		
Belowground biomass	β_0	3.2585	0.3952	2.98	0.0031	0.879	0.0373
	β_1	0.1552	0.0993	1.56	0.1195		
	β_2	1.9062	0.0950	20.06	<0.0001		
Coarse root biomass	β_0	0.6849	0.4726	−0.81	0.4240	0.819	0.0533
	β_1	0.4599	0.1187	3.87	0.0001		
	β_2	1.53232	0.1136	13.49	<0.0001		
Fine root biomass	β_0	5.1727	0.5735	2.86	0.0046	0.733	0.0774
	β_1	−0.2314	0.1458	−1.59	0.1140		
	β_2	2.3092	0.1517	15.22	<0.0001		

Discussion

The results show that the effect of the seedling production method on tree biomass growth and allocation in Scots pine plantations was different on PM and IE sites. On the PM site, the effect of seedling preparation is not significant, whereas on the IE site both containerization and ECM inoculation provided better conditions for the development of Scots pine seedlings than the bare root system. Positive effects of the containerization and ECM inoculation on the development of seedlings have been reported in Norway spruce and pine species, amongst others by Repáč (1996), Brunner & Brodbeck (2001), Ahonen-Jonnarth et al. (2003), Duñabeitia et al. (2004a), Rincón et al. (2007), Dumins & Lazdina (2022). However, in other studies inoculation did not affect or even reduced seedling growth (Rincón et al., 2005; Repáč, 2007; Díaz et al., 2009; Menkis et al., 2011; Repáč et al., 2014). Seedling response to inoculation can be affected by several factors, including the type of inoculum, inoculation pattern, interspecific and intraspecific host-fungus variation, seedling production practices, and environmental conditions (Díaz et al., 2009; Duñabeitia et al., 2004a; Flykt et al., 2008). Mycorrhiza formation permits a higher level of water uptake from dry soils and protects against heavy metal toxicity (Morte et al., 2001; Van Tichelen et al., 2001; Ahonen-Jonnarth et al., 2003). Moreover, Ahonen-Jonnarth et al. (2003) found that ectomycorrhizal mycelia may prevent the leaching of base cations and aluminum, deciding that aluminum did not affect P uptake in mycorrhizal plants. The aforementioned indications point to the great advantages of artificially mycorrhizal seedlings. It should be noted, however, that seedlings produced with the bare root method in a traditional nursery beds system are characterized by a high degree of spontaneous colonization with mycorrhizal fungi (Rudawska & Leski 2021). Thus, the differences observed in our study may be mainly due to the use of containers in seedling production.

In our study, significant differences were observed in biomass allocation to branch and coarse roots and thus in the root-to-shoot ratio between bare root and containerized seedlings both in the PM and IE sites. A root-to-shoot ratio is an essential morphological attribute, that is a measure of seedling water loss and water uptake capability at the time of planting (Burdett, 1990; Grossnickle, 2005). However, from the point of view, successful afforestation, which depends on the capacity to capture resources quickly, biomass allocation to roots is very important. Root resistance to water uptake is variable, depending upon the number of new roots a seedling develops just after planting (Grossnickle, 2005). New unsuberized roots permit a more efficient uptake of water (i.e. lower resistance to water flow) than suberized roots (Chung & Kramer, 1975; Sands et al., 1982). Therefore, biomass allocation to small, unsuberized roots is substantially more critical than biomass allocation to large suberized roots. In the case of our experiment, both at the PM and the IE sites the effect of the seedling preparation method was visible in the case of biomass allocation to coarse roots, whereas biomass allocation to fine roots was not significant. Therefore, in this aspect, neither containerization nor ECM inoculation was effective. Several studies have shown that growth substrate and ECM inoculation affect the root-to-shoot ratio (Repáč, 1996; Brunner & Brodbeck, 2001; Flykt et al., 2008). In an experiment with Norway spruce seedlings, Repáč et al. (2014) found that inoculation increased the root biomass. Similar results were also reported by Repáč (1996) and Flykt et al. (2008), but opposite results were presented by Brunner & Brodbeck (2001), who found that fungal inoculation with *H. crustuliniforme* or *L. bicolor* significantly enhanced shoot and total biomass compared to non-inoculated controls, and thus reduced root-to-shoot ratio. In our experiment, inoculation with *H. crustuliniforme* and *L. bicolor* did not significantly affect the root-to-shoot ratio compared to the variant without inoculation, while all containerized seedlings differed significantly from those produced in the bare root system.

At the experimental site (IE) exposed to strong industrial emissions from a zinc and lead smelter, visible effects on biomass growth were found as a result of seedling containerization and ECM inoculation. This result indicates the importance and profitability of seedling preparation for the growth of Scots pine in the case of extreme site conditions exposed to heavy metals deposition. Simultaneously the effect of the seedling production method and especially ECM inoculation is nearly invisible even at the post-mining site with poor water conditions. Studies conducted on mycorrhizae in plants growing on heavy metal contaminated sites have reported that ECM, especially heavy metal tolerant fungi, have evolved a pollutant tolerance to protect plants and therefore may be involved in phytoremediation (Khan et al., 2000; Van Tichelen et al., 2001; Colpaert et al., 2011).

Conclusions

Environmental conditions are an important factor modifying the growth and biomass production of trees grown from seedlings produced in different nursery systems. Under extremely unfavorable conditions of high heavy metal contamination, differences in the growth of seedlings produced in different ways were revealed. Thus, the choice of the optimal method of seedling production should take into account the target environmental conditions in which the seedlings will be planted. Our results indicate that in an environment contaminated with heavy metals, the use of containerized seedlings and also additionally inoculated with mycorrhizal fungi will be more favorable compared to bare-root seedlings. On the other hand, in a non-fertile habitat, but without exposure to toxic substances, the method of seedling production did not matter for the subsequent growth of trees, making it possible to freely choose the cheaper solution.

Despite the impact on biomass production, habitat did not modify the biomass allocation of trees grown from seedlings produced in different nursery systems. A greater allocation of biomass to the root system was found in trees grown from bare-root seedlings, while a smaller allocation was found when containerized seedlings were planted. This indicates that bare-root trees are likely to be subjected to greater nutrient and water deficiency, in contrast to containerized seedlings. To counteract these deficiencies, additional fertilization or other root system protection treatments can be considered when planting bare-root seedlings.

The method of seedling production and environmental conditions do not affect the allometric relationship between biomass and basic tree dimensions such as diameter and height. The biomass of pine can be estimated from the same allometric equations regardless of these factors.

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