https://doi.org/10.17221/198/2020-JFS

Influence of mycorrhizal preparation on seedling growth and *Armillaria* infestation

František Lorenc[1]*, *Jan Lubojacký*[1], *Tomáš Tonka*[2]

[1]*Forestry and Game Management Research Institute, Jíloviště-Strnady, Czech Republic*
[2]*Faculty of Agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic*

**Corresponding author: lorenc@vulhm.cz*

Citation: Lorenc F., Lubojacký J., Tonka T. (2021): Influence of mycorrhizal preparation on seedling growth and *Armillaria* infestation. J. For. Sci., 67: 155–164.

Abstract: The influence of three types of treatment on seedling survival percentage, growth and *Armillaria* infestation of *Fagus sylvatica* L. (FAGUS), *Abies alba* Mill. (ABIES) and *Picea abies* (L.) H. Karst. (PICEA) seedlings were tested in this study: (*i*) inoculation with the Ectovit® preparation containing ectomycorrhizal fungi (INOCUL), (*ii*) Ectovit® preparation + Conavit® fertilizer (INOCUL + FERTILIZ) and (*iii*) the untreated group (CONTROL). The selected sample contained 100 seedlings per each tree species and treatment type (900 seedlings in total). Besides that, 18 months after planting, 10 living seedlings per each species and treatment (90 seedlings in total) were sampled to evaluate root dry mass and *Armillaria* infestation. The data were statistically evaluated by frequency analysis, analysis of variance and Kruskal-Wallis test. The overall seedling survival percentage was very low, probably due to extreme drought and high temperatures, with significantly lower results for the ABIES INOCUL + FERTILIZ and PICEA INOCUL + FERTILIZ groups. All tested growth characteristics (seedling height increment, root collar diameter increment, seedling shoot dry weight, root dry mass) were significantly higher in PICEA seedlings. Root collar diameter increment showed significant differences within each species and inconsistent results. *Armillaria* was detected only in the PICEA CONTROL group as rhizomorphs identified as *A. ostoyae*. The results suggest that the artificial mycorrhizal preparation can be an efficient method of preventing *Armillaria* infestation, especially in spruce seedlings.

Keywords: ectomycorrhiza; honey fungus; inoculation; roots; silviculture; nutrients

Due to global climate change, European forests are facing changes in average values of climatic factors and increasingly more extreme weather conditions such as longer droughts, storms and floods (Lindner et al. 2008). These conditions activate numerous biotic harmful factors including pathogenic fungi (Haavik et al. 2015). Honey fungus (hereinafter referred to as *Armillaria*) belongs to the most significant wood-destroying fungi. It thrives in longer growing periods connected with higher temperatures, and it benefits from host trees stressed by lower precipitation (Lindner et al. 2008). Spruce stands of non-original composition (Černý 1988) on oligotrophic or mesotrophic sites, sites with soil compaction and pH values < 5 are the most threatened ones (Lindner et al. 2008). The symptoms of *Armillaria* infestation include resin flow, white rot typically affecting roots and lower parts of the trunk, butt swell, light grey-green to yellow-green colour of needles and later their cast, presence of sporocarps near infested trees, presence of whitish membranous mycelium (syrrotium) under the bark and presence of brown to black cord-like rhizomorphs on roots and near them (Soukup 2005). *Armillaria* belongs to the *Basidiomycota* division and comprises approximately 70 species (Sipos et al. 2018). In Europe, there are seven *Armillaria*

Supported by the National Agency for Agricultural Research (NAZV) of the Ministry of Agriculture of the Czech Republic, Project No. QK1820091.

https://doi.org/10.17221/198/2020-JFS

species: *Armillaria ostoyae* (Romagn.) Herink, *A. mellea* (Vahl) P. Kumm, *A. borealis* Marxmüller & Korhonen, *A. gallica* Marxmüller & Korhonen, *A. cepistipes* Velen., *Desarmillaria tabescens* (Scop.) R.A. Koch & Aime and *D. ectypa* (Fr.) R.A. Koch & Aim (Guillaumin et al. 1993; Zicha 2019).

Inoculation with mycorrhizal fungi has a positive effect on plant survival rate and growth, and it increases resistance to various abiotic factors and biotic harmful agents (Gryndler et al. 2004; Pešková, Tuma 2010). Successful inoculation requires ectomycorrhizal fungi to be able to make ectomycorrhiza with the host plant easily and quickly, to adapt to the site conditions and be resistant to stress. There is no universally suitable symbiont for a given tree species (Mejstřík 1988). The positive effect of artificial inoculation with mycorrhizal fungi for trees is considerably strong especially under unfavourable conditions including droughts (Garbaye, Churin 1997; Ortega et al. 2004; Pešková, Tuma 2010). With regard to ongoing climate change, it can be expected that artificial mycorrhizal treatment can become an increasingly important method of supporting seedlings cultivated in forest nurseries and in establishing new forest sites by reforestation and afforestation. Nevertheless, there is no verifiable growth stimulation in many cases (Castellano 1996; Holuša et al. 2015), and the effect of artificial inoculation can differ even when the same treatment is applied to trees of the same species (Holuša et al. 2009; Pešková, Tuma 2010; Repáč et al. 2011). The influence of artificial mycorrhizal inoculation on *Armillaria* infestation in case of plants living in symbiosis with ectomycorrhizal fungi (i.e. most tree species including beeches, firs and spruces) has been tested thoroughly only with the Scots pine (*Pinus sylvestris* L.) (Kowalski, Wojnowski 2009). Other studies only reported about individual cases of the presence of *Armillaria* rhizomorphs without their species identification and statistical evaluations (Tučeková et al. 2009; Pešková, Tuma 2010).

The goal of this study was to explore whether and how much the mycorrhizal treatment increases the ability to resist to the *Armillaria* infection level in cases of three tree species which are highly important in forestry: European beech – *Fagus sylvatica* L. (hereinafter referred to as FAGUS), European silver fir – *Abies alba* Mill. (hereinafter referred to as ABIES) and Norway spruce – *Picea abies* (L.) H. Karst. (hereinafter referred to as PICEA). Based on the results, the study aims to evaluate efficiency and applicability of an artificial mycorrhizal preparation in forestry practice. For this purpose, the survival rate, basic growth characteristics and the presence of *Armillaria* infestation were compared in seedlings treated with: (*i*) mycorrhizal preparation, (*ii*) mycorrhizal preparation + fertilizer and (*iii*) untreated (control) seedlings planted in a site with the strong *Armillaria* infection level.

MATERIAL AND METHODS

Material. The study was carried out in a research site near the municipality Moravský Beroun – Čabová (Olomouc Region, Czech Republic; 49°48'20''N, 17°29'30''E). In the years 1981–2010, the average annual temperature of the site was 7.8 °C, average annual precipitation was 708 mm (ČHMÚ 2020). The research period was characterised by relatively high temperatures and low precipitation, especially in April–August 2018 (ČHMÚ 2020, Figure 1). The bedrock is mostly composed of laminated shales. The soil type is dystric Cambisol (CENIA 2010–2020). The site is located in a former spruce stand with the strong *Armillaria* infection level which was also confirmed by the frequent presence of their sporocarps in autumn 2018 and 2019.

The following bare-root seedlings were planted at the site: FAGUS, ABIES and PICEA (Table 1). Two thirds of these seedlings of each species were inoculated by the Ectovit® preparation, containing ectomycorrhizal fungi (hereinafter referred to as INOCUL). In a half of the inoculated seedlings, the Conavit® fertilizer was also applied to planting holes and to the roots during the planting (hereinafter referred to as INOCUL + FERTILIZ). One third of all seedlings was not treated (hereinafter referred to as CONTROL) (Table 1).

Methods. In the period of 21st–22nd June 2018, 100 seedlings for each of the 9 combinations of the tree species and treatment were selected, i.e. 900 plants in total. Approximately 14–17 seedlings in 6–7 rows in the centre of the research site were marked for each combination. Studied parameters of the marked seedlings included the seedling survival percentage, seedling height (as cm from the root collar, i.e. from the soil surface to the top of the terminal bud) and root collar diameter (with a calliper to the nearest 0.1 mm; measured on the seedling stem at the point of contact with the soil surface). Measurements were repeated on 11th–12th October 2018 and 24th–30th October 2019.

https://doi.org/10.17221/198/2020-JFS

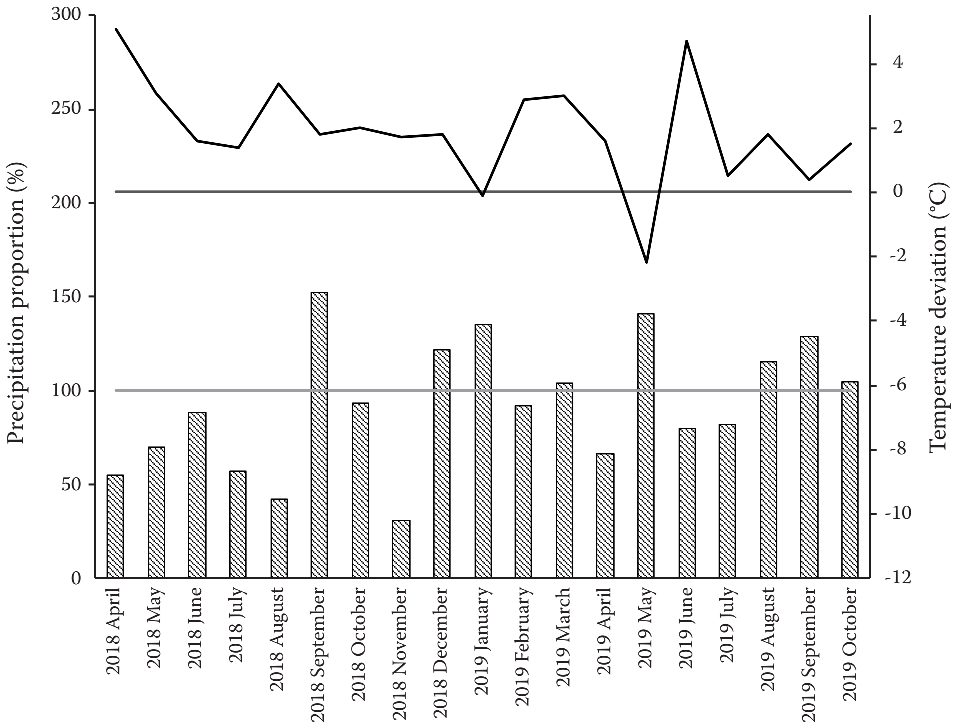

Figure 1. Temperature deviation from normal (°C) and percent of normal precipitation (%) in the research period in Olomouc region in 1981–2010 (ČHMÚ 2020)

Table 1. Summary of used seedlings and treatments

Tree species	FAGUS	*Fagus sylvatica* L. Two-year-old seedlings undercut as one-year-old seedlings, planted on 20th–30th April 2018.
	ABIES	*Abies alba* Mill. Five-year-old seedlings undercut as two-year-old seedlings, planted on 1st–10th May.
	PICEA	*Picea abies* (L.) H. Karst. Three-year-old seedlings transplanted from a plastic greenhouse to a nursery as one-year-old seedlings, planted on 10th–20th April 2018. Application of Vaztak Active insecticide (microemulsion with alpha-cypermethrin 50 g·L^{-1} as active ingredient; BASF SE, Ludwigshafen, Germany) in dosage of 5 l·ha^{-1}, applied with a back sprinkler as a protection against the large pine weevil (*Hylobius abietis* L.) in June 2018.
Treatment	INOCUL	Inoculation of seedlings by Ectovit® preparation (Symbiom, s.r.o., Lanškroun, Czech Republic) before planting. During inoculation, the whole root surface was immersed in the mycorrhizal solution made of 3 kg of the Ectovit® product and 50 L of water. The preparation contains the mycelium of 4 ectomycorrhizal fungi on agar medium: *Amanita muscaria* (L.) Lam., *Hebeloba crustuliniforme* (Bull.) Quél., *Laccaria proxima* (Boud.) Pat. and *Paxillus involutus* (Batsch) together with basidiospores of two ectomycorrhizal fungi as part of the peat component: *Pisolithus arhizus* (Scop.) Rauschert and *Scleroderma citrinum* Pers.
	INOCUL + FERTILIZ	Inoculation of seedlings by Ectovit® preparation + application of Conavit® fertilizer (Symbiom, s.r.o., Lanškroun, Czech Republic) to planting holes and to the roots during planting with dosage of 50 ml per plant. The fertilizer contains extracts from marine organisms, natural humates, ground minerals and natural keratin; it is rich in N, P, K, Ca, Mg and it also contains B, Mn, Cu and Zn.
	CONTROL	Seedlings without inoculation and fertilization.

FAGUS – *Fagus sylvatica*; ABIES – *Abies alba*; PICEA – *Picea abies*; INOCUL – inoculation; INOCUL + FERTILIZ – inoculation + fertilization; CONTROL – untreated group

https://doi.org/10.17221/198/2020-JFS

Table 2. Results of the chemical analyses of soil samples

Tree species	Treatment	DW (%)	pH (H_2O)	pH (KCl)	C_{tot}	N_{tot}	S_{tot}	Na_{av}	K_{av}	Mg_{av}	Ca_{av}	Zn_{av}	P_{av}	Mn_{av}	Fe_{av}	Al_{av}
					(mg·kg^{-1} dry weight)											
	INOCUL	96.3	4.01	3.23	108 438	5 149	605	10.46	124.31	54.5	159.45	5.87	1.95	92.36	100.83	1 030.88
FAGUS	INOCUL + FERTILIZ	96.99	4.36	3.41	56 935	3 238	390	12.96	103.27	55.46	196.19	4.4	3.72	279.26	21.78	802.43
	CONTROL	95.84	4.05	3.19	99 612	5 033	600	9.07	148.83	58.25	228.99	6.56	2.41	238.39	88.51	1 145.33
	INOCUL	94.51	3.81	3.15	135 489	6 664	810	15.41	156.72	90.83	307.96	10.8	2.91	312.42	136.97	1024.57
ABIES	INOCUL + FERTILIZ	97.21	3.99	3.13	53 652	2 935	380	6.71	112.9	36.11	100.95	3.21	2.86	86.09	45.08	941.66
	CONTROL	96.67	3.96	3.11	87 631	4 564	590	8.23	124.02	63.69	251.18	6.25	3.61	115.34	94.56	976.70
	INOCUL	95.12	3.94	3.15	168 056	7 935	900	12.35	168.54	83.01	378.48	7.16	5.71	59.79	133.89	1 046.56
PICEA	INOCUL + FERTILIZ	95.1	4.12	3.24	135 763	6 655	760	19.80	127.31	103.39	539.26	8.39	13.8	117.83	119.99	875.75
	CONTROL	96.74	4.23	3.46	116 919	6 323	670	18.00	162.23	93.46	340.31	8.26	12.31	159.95	75.67	704.46

DW – dry weight; tot – total; av – available form; FAGUS – *Fagus sylvatica*; ABIES – *Abies alba*; PICEA – *Picea abies*; INOCUL – inoculation; INOCUL + FERTILIZ – inoculation + fertilization; CONTROL – untreated group

For statistical analysis, seedling height increment was calculated as a difference in seedling height between October 2019 and October 2018, and root collar diameter increment was calculated as a difference in root collar diameter also between October 2019 and October 2018.

In order to evaluate the *Armillaria* infestation, 10 living seedlings of each category were observed, i.e. 90 seedlings in total, on 25th October 2019. The *Armillaria* rhizomorphs were detected and sampled in a thorough examination of root balls under laboratory conditions. Seedling roots and rhizomorphs were cleaned with water in the laboratory and put into a 35% ethanol fixative solution. After molecular analysis, samples were dried in a dryer at 105 °C and then the seedling shoot dry weight and root dry mass (for root diameters < 1, 1–2, 2–5 and > 5 mm, and in total) were measured.

Identification of the *Armillaria* species on seedlings was carried out using the molecular analysis of rhizomorph samples detected on selected seedlings. The CTAB-PVP method was used for DNA isolation (Williams et al. 1993). Specific sections of isolated DNA were amplified by the polymerase chain reaction (PCR) using ITS1 and ITS4 primers, following PCR re-amplification with *Armillaria* specific AR1 and AR2 primers (Lochman et al. 2004). The PCR products were sequenced in an external laboratory (SEQme s.r.o., Dobříš, Czech Republic). Sequenced samples were compared with the BLAST (Bethesda, MD, USA) database of sequenced biological samples, and based on identified similarities, individual *Armillaria* species were determined.

The soil from root balls of selected seedlings was mixed, and composite samples were made for individual categories (i.e. 9 composite samples in total). Analyses of these composite soil samples included: dry weight, pH in H_2O, pH in KCl, concentration of C, N and S in total, and concentration of Na, K, Mg, Ca, Zn, P, Mn, Fe and Al in available form (Table 2). The analysis was carried out in the Testing Laboratories of the Forestry and Game Management Research Institute following standard operating procedures (SOP) elaborated in accordance with the respective ČSN/EN/ISO standards.

Statistical evaluation was processed in the STATISTICA program (Version 10, 2010) with the significance level of α = 0.05. Each tree species was tested separately. The seedling survival percentage and number of seedlings with *Armillaria* rhizomorphs were evaluated by the frequency analysis of

https://doi.org/10.17221/198/2020-JFS

observed values and their comparison with expected values (χ^2). Growth characteristics (seedling height increment, root collar diameter increment, seedling shoot dry weight, root dry mass) were tested for the normal distribution by the Shapiro-Wilk test. In case of the normal distribution, the significance of differences between individual treatment forms was determined by the analysis of variance (ANOVA) and subsequently the Tukey HSD test was applied for multiple comparisons. For data not showing the normal distribution (seedling height increment in all tree species, root collar diameter increment in all tree species, seedling shoot dry weight in ABIES, root dry mass in ABIES), the non-parametric Kruskal-Wallis test (K-W) was used, and subsequently Dunn's test (z) for multiple comparisons (Table 3).

RESULTS

Seedling survival percentage was significantly lower in ABIES and PICEA (Table 3), especially in the INOCUL + FERTILIZ category (Table 4).

There were no significant differences in seedling height increment, seedling shoot dry weight, and root dry mass between individual types of treatment in individual tree species (Table 3, Figure 2).

Table 3. Results of the statistical tests for assessed variables

Variable	Tree species	N	Value of the test	P	Significantly different treatments
Seedling survival percentage (%)	FAGUS	300	χ^2: 0.1818	0.91	
	ABIES	300	χ^2: 11.5319	**	INOCUL > INOCUL + FERTILIZ; CONTROL > INOCUL + FERTILIZ
	PICEA	300	χ^2: 19.7813	***	CONTROL > INOCUL + FERTILIZ; INOCUL > INOCUL + FERTILIZ
Increment of the seedling height (cm)	FAGUS	169	K-W: 4.4969	0.11	
	ABIES	115	K-W: 1.2168	0.54	
	PICEA	188	K-W: 1.5697	0.46	
Increment of the root collar diameter (cm)	FAGUS	129	K-W: 17.5205	***	CONTROL > INOCUL; z = 4.1754***
	ABIES	123	K-W: 12.2831	**	CONTROL > INOCUL; z = 3.2272**; CONTROL > INOCUL + FERTILIZ; z = 2.4961*
	PICEA	185	K-W: 25.0741	***	INOCUL > CONTROL; z = 4.6355***; INOCUL > INOCUL + FERTILIZ; z = 3.5409**
Seedling aboveground dry weight (g)	FAGUS	30	F: 1.5678	0.23	
	ABIES	30	K-W: 5.5819	0.06	
	PICEA	30	F: 1.8720	0.17	
Root dry mass (g)	FAGUS	30	F: 1.6058	0.22	
	ABIES	30	K-W: 2.2168	0.33	
	PICEA	30	F: 3.0928	0.06	
No. of seedlings with *Armillaria* rhizomorphs	FAGUS	30	χ^2: 0.0000	1.00	
	ABIES	30	χ^2: 0.0000	1.00	
	PICEA	30	χ^2: 16.0000	***	CONTROL > INOCUL; CONTROL > INOCUL + FERTILIZ

N – sample size; P – significance level (*, **, ***P < 0.05, 0.01, 0.001); K-W – Kruskal-Wallis test; F – value of the Analysis of Variance (ANOVA); z – Dunn's test; FAGUS – *Fagus sylvatica*; ABIES – *Abies alba*; PICEA – *Picea abies*; INOCUL – inoculation; INOCUL + FERTILIZ – inoculation + fertilization; CONTROL – untreated group

https://doi.org/10.17221/198/2020-JFS

Table 4. Mean ± standard deviation and values for assessed variables

Variable	Tree species	Treatment	*N*	Value
Seedling survival percentage (%)	FAGUS	INOCUL	100	56
		INOCUL + FERTILIZ	100	60
		CONTROL	100	60
	ABIES	INOCUL	100	57
		INOCUL + FERTILIZ	100	28
		CONTROL	100	56
	PICEA	INOCUL	100	77
		INOCUL + FERTILIZ	100	35
		CONTROL	100	80
Increment of the seedling height (cm)	FAGUS	INOCUL	51	0.75 ± 2.42
		INOCUL + FERTILIZ	59	2.00 ± 3.95
		CONTROL	59	1.45 ± 3.32
	ABIES	INOCUL	57	3.28 ± 4.05
		INOCUL + FERTILIZ	28	2.46 ± 3.20
		CONTROL	30	3.30 ± 3.43
	PICEA	INOCUL	76	11.62 ± 8.53
		INOCUL + FERTILIZ	35	10.11 ± 7.09
		CONTROL	77	11.08 ± 10.76
Increment of the root collar diameter (cm)	FAGUS	INOCUL	38	0.04 ± 0.04
		INOCUL + FERTILIZ	48	0.07 ± 0.05
		CONTROL	43	0.11 ± 0.09
	ABIES	INOCUL	48	0.11 ± 0.13
		INOCUL + FERTILIZ	23	0.08 ± 0.06
		CONTROL	52	0.14 ± 0.10
	PICEA	INOCUL	75	0.62 ± 0.25
		INOCUL + FERTILIZ	31	0.42 ± 0.25
		CONTROL	79	0.42 ± 0.24
Seedling aboveground dry weight (g)	FAGUS	INOCUL	10	2.89 ± 1.13
		INOCUL + FERTILIZ	10	3.26 ± 1.81
		CONTROL	10	4.21 ± 2.07
	ABIES	INOCUL	10	6.58 ± 3.19
		INOCUL + FERTILIZ	10	5.33 ± 3.86
		CONTROL	10	9.05 ± 4.39
	PICEA	INOCUL	10	69.72 ± 28.60
		INOCUL + FERTILIZ	10	55.60 ± 24.62
		CONTROL	10	48.01 ± 22.80
Root dry mass (g)	FAGUS	INOCUL	10	2.85 ± 1.64
		INOCUL + FERTILIZ	10	3.83 ± 2.49
		CONTROL	10	4.52 ± 2.07
	ABIES	INOCUL	10	3.83 ± 1.93
		INOCUL + FERTILIZ	10	3.91 ± 3.12
		CONTROL	10	5.81 ± 3.65
	PICEA	INOCUL	10	27.32 ± 11.38
		INOCUL + FERTILIZ	10	21.43 ± 9.18
		CONTROL	10	16.57 ± 8.23
No. of seedlings with *Armillaria* rhizomorphs	FAGUS	INOCUL	10	0
		INOCUL + FERTILIZ	10	0
		CONTROL	10	0
	ABIES	INOCUL	10	0
		INOCUL + FERTILIZ	10	0
		CONTROL	10	0
	PICEA	INOCUL	10	0
		INOCUL + FERTILIZ	10	0
		CONTROL	10	8

FAGUS – *Fagus sylvatica*; ABIES – *Abies alba*; PICEA – *Picea abies*; INOCUL – inoculation; INOCUL + FERTILIZ – inoculation + fertilization; CONTROL – untreated group

https://doi.org/10.17221/198/2020-JFS

Root collar diameter increment was statistically significantly different in (1) FAGUS (Table 3): statistically significantly higher in CONTROL compared to INOCUL (Tables 3 and 4); (2) in ABIES (Table 3): statistically significantly higher values in CONTROL compared to INOCUL (Tables 3 and 4) as well as CONTROL compared to INOCUL + FERTILIZ (z; $P < 0.05$; Table 3 and 4); (3) and in PICEA (Tables 3): significantly higher values of INOCUL compared to CONTROL (Tables 3 and 4) and INOCUL compared to INOCUL + FERTILIZ (Tables 3 and 4).

Out of 90 selected seedlings (10 of each category), the *Armillaria* presence was detected just as rhizomorphs only on roots and butts of eight seedlings – and all of them were from PICEA CONTROL (Table 4). Molecular analyses identified the rhizomorphs as *Armillaria ostoyae*.

The lowest concentrations of nutrients in the soil (in total) were identified in ABIES INOCUL + FERTILIZ and FAGUS INOCUL + FERTILIZ, while the highest ones were found in PICEA INOCUL + FERTILIZ and PICEA CONTROL (Table 2).

DISCUSSION

In this study seedling survival percentage was generally very low due to extremely unfavourable climatic conditions in the Olomouc Region in the planting year: in 2018, the average annual temperature was 1.7 °C higher and average annual precipitation reached only 79% in comparison with the long-term normal of 1981–2010 (ČHMÚ 2020). A noticeable negative effect of drought and high temperatures on seedling survival percentage contrary to the influence of artificial mycorrhizal inoculation was also mentioned by Pešková and Tuma (2010). In this study the lowest seedling survival percentage was documented in the categories ABIES INOCUL + FERTILIZ and PICEA INOCUL. As distinct from our results, preceding studies reported the higher survival percentage of inoculated seedlings in *Fagus sylvatica*, *Abies alba*, *Picea abies* (Tučeková et al. 2009), *Tilia cordata* Mill., *Acer campestre* L. and *Quercus robur* L. (Fini et al. 2016). In case of *Hebeloma crustuliniforme* inoculation, the higher seedling survival percentage was

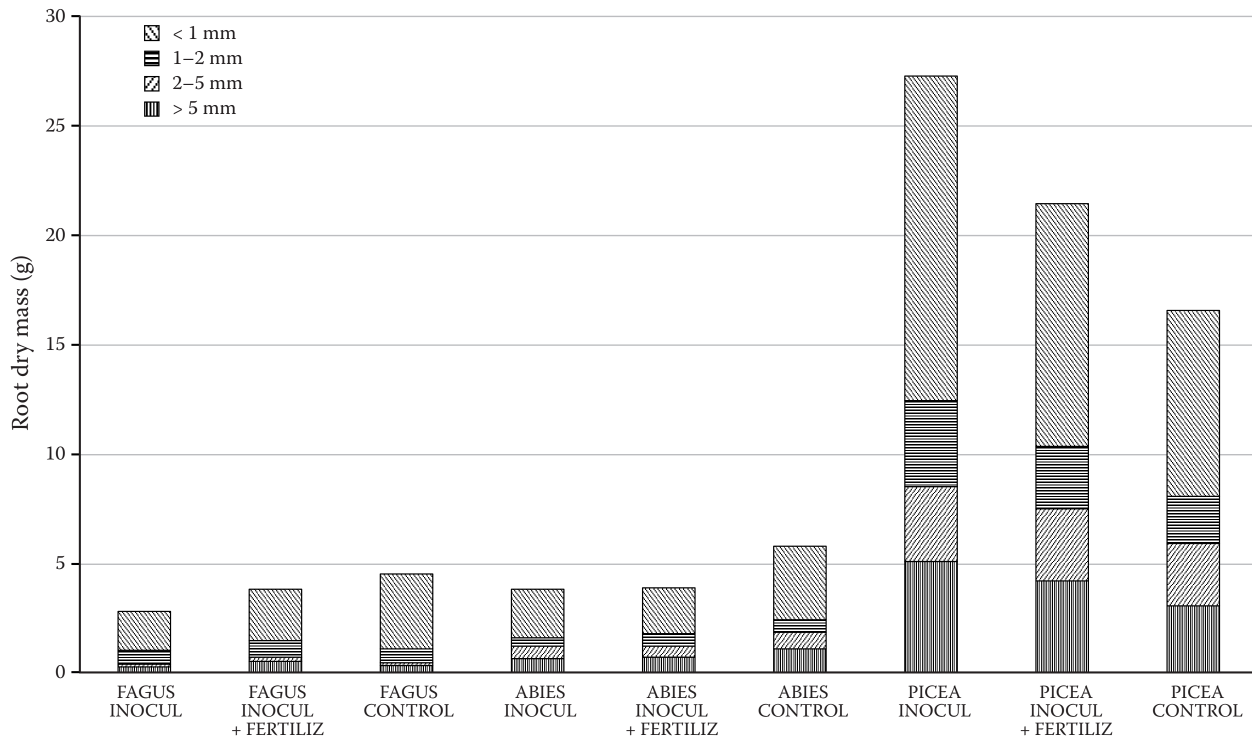

Figure 2. Weight of root dry mass of selected plants according to the root diameter

FAGUS – *Fagus sylvatica*; ABIES – *Abies alba*; PICEA – *Picea abies*; INOCUL – inoculation; INOCUL + FERTILIZ – inoculation + fertilization; CONTROL – untreated group

https://doi.org/10.17221/198/2020-JFS

also seen in *Pinus sylvestris* (Kowalski, Wojnowski 2009). In our study, categories with the lowest seedling survival percentage had either the lowest (ABIES INOCUL + FERTILIZ) or the highest (PICEA INOCUL + FERTILIZ) concentration of nutrients in their soil (Table 1). Both the deficiency and overabundance of nutrients in soil can have a negative impact on plant physiology and it may result in their withering or even dieback (Holub 2006), which may have been the case for some seedlings. A considerable influence of the environmental conditions on the seedling survival percentage was also mentioned by Kowalski and Wojnowski (2009). A negative influence of fertilizers or mycorrhiza on the seedling survival percentage is not expected as it has not been documented in any of the existing studies (Khasa et al. 2001; Fini et al. 2016). The seedling survival percentage in our study was thus influenced by climatic and edaphic conditions, whereas mycorrhizal inoculation and fertilizer did not have a substantial effect on their survival.

In ABIES, root collar diameter increment was significantly higher in CONTROL in comparison with the others, while in FAGUS, root collar diameter increment was significantly higher in CONTROL compared to INOCUL. PICEA INOCUL, where the increment of root collar diameter was significantly higher in comparison with the other treatments, had a significantly lower concentration of available phosphorus in soil in comparison with other PICEA categories and on the other hand, it had the highest total concentrations of nitrogen, carbon and sulphur (Table 1). A positive effect of the increased concentration of nitrogen in soil on tree growth is widely known; it has been documented for example in *Picea abies* by Utriainen and Holopainen (2001). Root collar diameter increment may have been positively influenced by the overall concentration of nitrogen in the soil. Nevertheless, this relationship was not valid for ABIES and FAGUS trees because the concentration of nutrients in the soil including nitrogen was considerably lower with the INOCUL + FERTILIZ treatment (Table 1). A significant influence on seedling growth may have been that of microclimatic conditions in the specific location of planting, as mentioned for instance by Holuša et al. (2015). Results of the Ectovit® influence on the growth of the given tree species were therefore ambiguous. In previous studies where this preparation was used, Holuša et al. (2009) observed a significant positive influence on the seedling height, root collar diameter and maximum root length of *Picea abies* seedlings in a site with the high *Armillaria* infection level. On the other hand, no significant influence of the Ectovit® preparation on seedling growth was found in other studies including Pešková and Tuma (2010), who examined the *Picea abies* seedlings, Repáč et al. (2011), who studied bare-root and container-grown seedlings of *Picea abies*, *Pinus sylvestris*, *Larix decidua* Mill., *Fagus sylvatica* and *Acer pseudoplatanus* L., or Lorenc and Novotný (2020), who study *Quercus robur* L. seedlings. Using the ectomycorrhizal preparation VAMBAC®, Holuša et al. (2015) observed its significant positive influence on treated seedlings and young stands of *Quercus robur* in terms of the seedling height, root collar diameter, maximum root length and root dry mass, but only in some of the sites. Similarly, Tučeková et al. (2009) used the same preparation on *Picea abies*, *Abies alba* and *Fagus sylvatica* seedlings and reported a significant increment of seedling height, and root collar diameter increment only in some of the five research sites and only in certain time periods (Tučeková et al. 2009). It is therefore obvious that in many cases artificial inoculation does not necessarily stimulate seedling growth.

Armillaria ostoyae rhizomorphs were found only on seedling roots of one category in our study, the PICEA CONTROL group. Although other *Armillaria* species can also be commonly found in spruce stands (Holuša et al. 2018), only sporocarps of the *A. ostoyae* which predominantly attack spruces (*Picea* spp.) (ÚKZÚZ 2020) were present at the research site during the sampling. With other types of treatment of the PICEA seedlings, no rhizomorphs were found, probably due to the mycorrhizal preparation treatment which helped seedlings resist the *Armillaria* infestation. Tučeková et al. (2009) observed lower amounts of rhizomorphs on inoculated seedlings not only in *Picea abies*, but also in *Abies alba*, while the *Fagus sylvatica* seedlings had *Armillaria* rhizomorphs both on inoculated and control seedlings. The *Armillaria* syrrotium was recorded by Pešková and Tuma (2010) on three untreated seedlings and one *Picea abies* seedling treated with the mycorrhizal preparation. A lower level of *Armillaria* infestation was confirmed in *Pinus sylvestris* seedlings inoculated with *Hebeloma crustuliniforme* (Kowalski, Wojnowski 2009). Artificial inoculation with suitable mycorrhizal fungi can thus help seedlings to resist the *Armillaria* infection level.

https://doi.org/10.17221/198/2020-JFS

CONCLUSION

Our study confirms that artificial mycorrhizal inoculation is significant especially in sites with a high infection level of pathogenic organisms including the *Armillaria* fungi as it can help protect seedlings of potentially threatened tree species. However, there can also be a considerable influence of the climatic and microclimatic conditions at the site, nutrient concentration in soil, quality of the mycorrhizal product and fertilizer, physiological condition of seedlings during planting, planting time and cultivation method. As a consequence of these factors, the positive influence of artificial inoculation with mycorrhizal fungi may not manifest itself.

Acknowledgment: The authors would like to thank Ing. Karel Lehnert, Ing. Josef Neplech and Ing. Jan Adamov for field work, and Jana Fojtíková and Monika Boušková for their help with laboratory work.

REFERENCES

Castellano M.A. (1996): Outplanting performance of mycorrhizal inoculated seedlings. In: Mukerji K.G. (ed.): Concepts in Mycorrhizal Research. Dordrecht, Kluwer Academic Publishers: 223–301.

CENIA (2010–2020): Národní geoportál INSPIRE. CENIA, česká informační agentura životního prostředí. Available at: https://geoportal.gov.cz/web/guest/home;jsessionid=3533A0A21202CBECB773840607898B24 (accessed Dec 6, 2018). (in Czech).

Černý A. (1988): Parazitické dřevokazné houby. Prague, Státní zemědělské nakladatelství: 104.

ČHMÚ (2020): Portál ČHMÚ. Český hydrometeorologický ústav. Available at: http://portal.chmi.cz/ (in Czech)

Fini A., Ferrini F., Seri M., Amoroso G., Piatti R., Robbiani E., Frangi P. (2016): Effect of fertilization and mycorrhizal inoculation in the nursery on post-transplant growth and physiology in three ornamental woody species. In: Groening G., Moore G.M., Rayner J.P., Moore E.E.F. (eds): 29th International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014). Acta Horticulturae (ISHS), 1108: 47–54.

Garbaye J., Churin J.L. (1997): Growth stimulation of young oak plantations inoculated with the ectomycorrhizal fungus *Paxillus involutus* with special reference to summer drought. Forest Ecology and Management, 98: 221–228.

Gryndler M., Baláž M., Hršelová H., Jansa J., Vosátko M. (2004): Mykorhizní symbióza: O soužití hub s kořeny rostlin. Prague, Academia: 366. (in Czech).

Guillaumin J.J., Mohammed C., Anselmi N., Courtecuisse R., Gregory S.C., Holdenrieder O., Intini M., Lung B., Marxmüller H., Morrison D., Rishbet J., Termorshuizen A.J., Tirró A., van Dam B. (1993): Geographical distribution and ecology of the *Armillaria* species in western Europe. European Journal of Forest Pathology, 23: 321–341.

Haavik L.J., Billings S.A., Guldin J.M., Stephen F.M. (2015): Emergent insects, pathogens and drought shape changing patterns in oak decline in North America and Europe. Forest Ecology and Management, 354: 190–205.

Holub V. (2006): Vliv abiotických a antropogenních faktorů na dřeviny. In: Gregorová B., Tábor I., Jančařík V., Černý K., Holub V., Rom J., Šumpich J., Kloudová K. (ed): Poškození dřevin a jeho příčiny. Prague, Agentura ochrany přírody a krajiny: 64–88. (in Czech).

Holuša J., Pešková V., Vostrá M., Pernek M. (2009): Impact of mycorrhizal inoculation on spruce seedling: Comparision of 5-year experiment in forests infested by honey fungus. Periodicum Biologorum, 111: 413–417.

Holuša J., Pešková V., Lorenc F. (2015): The impact of artificial mycorrhizal inoculation on the growth of common oak seedlings and development of mycorrhiza: Inoculation may not positively affect growth of seedlings. Periodicum Biologorum, 117: 519–526.

Holuša J., Lubojacký J., Čurn V., Tonka T., Lukášová K., Horák J. (2018): Combined effects of drought stress and *Armillaria* infection on tree mortality in Norway spruce plantations. Forest Ecology and Management, 427: 434–445.

Khasa P.D., Sigler L., Chakravarty P., Dancik B.P., Erickson L., McCurdy D. (2001): Effect of fertilisation on growth and ectomycorrhizal development of container-grown and bare-root nursery conifer seedlings. New Forests, 22: 179–197.

Kowalski S., Wojnowski L. (2009): Armillaria root rot dynamics in the experimental plantation with Scots pine (*Pinus sylvestris* L.) seedlings non-mycorrhized and mycorrhized with the fungi *Hebeloma crustuliniforme* and *Laccaria bicolor*. Sylwan, 153: 31–38.

Lindner M., Garcia-Gonzalo J., Kolström M., Green T., Reguera R., Maroschek M., Seidl R., Lexer M.J., Netherer S., Schopf A., Kremer A., Delzon S., Barbati A., Marchetti M., Corona P. (2008): Impact of Climate Change of European Forests and Options for Adaptation. Report to the European Commision Directorate-General for Agriculture and Rural Development. Brussels, Agriculture and Rural Development: 173.

Lochman J., Sery O., Mikes V. (2004): The rapid identification of European *Armillaria* species from soil samples by nested PCR. FEMS Microbiology Letters, 237: 105–110.

Lorenc F., Novotný R. (2020): Vliv živin v půdě na úspěšnost umělé inokulace ektomykorhizními houbami u sazenic dubu letního (*Quercus robur* L.). Zprávy lesnického výzkumu, 65: 50–56. (in Czech)

https://doi.org/10.17221/198/2020-JFS

Mejstřík V. (1988): Mykorhizní symbiózy. Prague, Academia: 150. (in Czech)

Ortega U., Duñabeitia M., Menendez S., Gonzalez-Murua C., Majada J. (2004): Effectiveness of mycorrhizal inoculation in the nursery on growth and water relations of *Pinus radiata* in different water regimes. Tree Physiology, 24: 65–73.

Pešková V., Tuma M. (2010): Ověření vlivu mykorhizního preparátu na růst a vývoj smrkových sazenic na LS Jablunkov. Zprávy lesnického výzkumu, 55: 211–220. (in Czech)

Repáč I., Tučeková, I. Sarvašová I., Vencurik I. (2011): Survival and growth of outplanted seedlings of selected tree species on the High Tatra Mts. windthrow area after the first growing season. Journal of Forest Science, 57: 349–358.

Sipos G., Anderson J.B., Nagy L.G. (2018): Armillaria. Current Biology, 28: R293–R305.

Soukup F. (2005): *Armillara ostoyae* (Romagn.) Herink václavka smrková. Lesnická práce, 84 (Vol. 10, Supplement): I–IV. (in Czech)

Tučeková A., Longauerová V., Leontovyč R. (2009): Poznatky z testovania mykorhizovaného preparátu Vambac na smreku (*Picea abies* L.) v oblasti s dlhodobo zvýšeným stavom Armillaria sp. In: Mykorhiza v lesích a možnosti její podpory: Sborník referátů. Frýdek-Místek, Česká lesnická společnost: 52–58. (in Czech)

ÚKZÚZ (2020): Rostlinolékařský portál. Ústřední kontrolní a zkušební ústav zemědělský. Available at: http://eagri.cz/public/app/srs_pub/fytoportal/public/#rlp|domu|uvod (accessed March 12, 2020).

Utriainen J., Holopainen T. (2001): Influence of nitrogen and phosphorus availability and ozone stress on Norway spruce seedlings. Tree Physiology, 21: 447–456.

Williams J.G.K., Hanafey M.K., Rafalski J.A., Tingey S.V. (1992): Genetics analysis using RAPD markers. Method in Enzymology, 260: 335–348.

Zicha O. (ed.) (1999–2019): BioLib. Available at: http://www.biolib.cz (accessed Feb 24, 2020)

Received: November 30, 2020
Accepted: January 15, 2021