Survival, Growth and Ectomycorrhizal Community Development of Container- and Bare-root Grown *Pinus sylvestris* and *Picea abies* Seedlings Outplanted on a Forest Clear-cut

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Abstract

Selection of high quality seedling material is an essential prerequisite for successful reforestation and characteristics of the seedlings produced under different cultivation systems may differ significantly. The aim of the present study was to assess survival, growth and ectomycorrhizal (ECM) community development of containerised and bare-root cultivated *Pinus sylvestris* and *Picea abies* seedlings following their outplanting on a forest clear-cut in Latvia. The experimental plantation 7500 m^2 in size was established in May 2006. Seedlings of four different treatments were arranged in rows in five replicates. Results showed that during four growing seasons (2006-2009) following seedling outplanting, a gradual decrease in seedling survival was observed each year in both tree species and cultivation systems. As a result, after the fourth growing season in a plantation, generally low survival rates were in both tree species and cultivation systems. In *P. sylvestris*, survival rates were similar between containerised and bare-root seedlings (16.7% ± 2.0SE and 14.3% ± 1.2SE, $p > 0.05$, respectively) while in *P. abies* these were significantly lower in containerised seedlings (29.5% ± 3.5SE) than in bare-rooted ones (42.6% ± 4.5SE) ($p < 0.0003$). At the time of outplanting, the height of containerised and bare-root seedlings differed within each of the tree species and similar pattern in seedling height growth remained after the fourth season. Although the communities of ECM fungi detected in a study resembled the ones present in the forest nurseries, a dynamic change from the predominance of *Thelephora terrestris* in the first season to the dominance of *Wilcoxina rehmii* and *Amphinema byssoides* in the later seasons indicated their certain adaptation to the environmental conditions present at the site. The study demonstrated that depending on a tree species the cultivation system may influence both seedling performance in the field and the development of ECM communities.

Key words: ectomycorrhizal fungi, forest nursery, *Pinus sylvestris*, *Picea abies*, seedling cultivation system

Introduction

Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) are the most common tree species in Latvia, where they occupy ca. 46 % of the total forest land (Forest Statistics 2008). Both of these species are also predominantly used in reforestation and in 2007 constituted ca.77 % of all replanted clear-cuts in Latvian state forests (Forest Statistics 2008). Forest nurseries in Latvia produce over 45 million *P. sylvestris* and *P. abies* seedlings yearly using three major standardised cultivation systems: i) containerised system – seedlings are grown in interconnected plastic pots in the greenhouses (50 % of all seedlings are produced using this system), ii) bare-root system – seedlings are grown in an open field beds (25%) and iii) Plug+1 system – seedlings are pre-grown for a year as containerised and then transplanted and cultivated as bare-root (25 %) (personal communication with L.Zvejniece, Deputy Director for production of "LVM Seeds and Plants" in 2009).

Characteristics of the seedlings produced under different cultivation systems may differ significantly. For example, intensive greenhouse cultivation of containerised seedlings may often result in their several times better morphological parameters as compared to bare-root seedlings of similar age (Leugner et al. 2009). Therefore, in recent years, production of bare-root seedlings has decreased in Latvia. However, quality of the bare-root seedlings is often reduced as the result of root damages and/or partial removal during seedling lifting and transplantation in the nursery or at the reforestation site. However, container-grown seedlings are often younger (1–2 years old) than bare-root seedlings (2–4 years old) at the time of their outplanting, and their production is usually more expensive.

Cultivation system may also have a profound effect on root colonisation by symbiotic ectomycorrhizal (ECM) fungi (Menkis et al. 2005) which are known to provide nutritional benefits to their hosts (Parlade and Alvarez 1993, Jonsson et al. 2001) and consequently may affect vitality and quality of the seedlings (Smith and Read 1997). Besides, well developed ECMs may enhance seedling survival and growth in the nursery (Hunt 1992) and in the field (Kropp and Langlois 1990, Le Tacon et al. 1994, Menkis et al. 2007, Menkis et al. 2012). Although the importance of ECMs has been widely acknowledged, the factors determining ECM community structure and species diversity are scarcely understood (Flynn et al. 1998, Tedersoo et al. 2012). Intensive management practices in forest nurseries may often result in both reduced ECM colonisation of seedling roots and species diversity (Arnebrant and Soderstrom 1992, Nilsson and Wallander 2003), while promoting only limited number of ECMs that tolerate such growth conditions (Khasa et al. 2001, Menkis et al. 2005, Menkis and Vasaitis 2011). Consequently, cultivation system that results in seedling material with abundant and diverse ECMs would be desirable.

The aim of the present study was to assess survival, growth and ECM community development of containerised and bare-root cultivated *P. sylvestris* and *P. abies* seedlings following their outplanting on a forest clear-cut in Latvia.

Materials and methods

Study site and experimental design

The study site was at Tireli forest district (managed by “Riga City Forests”) in central Latvia (N56°51’ E23°47’, 10 m above sea level) and represented north temperate forest dominated by *P. abies* which was clear-cut in 2005. Soil at the site was conifer forest podzol, corresponding to *Oxalido-myrtilliosa* forest type, characterised by the following chemical composition (mg/l of soil): N 30, P 87, K 22, Ca 2550, Mg 213, S 28, Fe 850, Mn 6.5, Zn 1.6, Cu 0.2, Mo 0.06, B 0.1. Soil pH in KCl was 5.03 and electrical conductivity – 0.11. To determine those extractable soil parameters, five random soil cores were taken down to ca. 20 cm, pooled together and analysed as a bulk sample. Soil was air-dried for few hours and sieved using a sieve (mesh size 2 x 2 mm). Then, soil electrical conductivity (EC) was determined in distilled water extract (soil – deionized water mixture 1:5) and soil pH – in 1 °M KCl (soil – extractant mixture 1:2.5). Chemical elements present in the soil were extracted using 1 M HCl solution (soil – extractant mixture 1:5) (Rinkis et al. 1987). The concentration of Ca, Mg, Fe, Cu, Zn, and Mn were detected by atomic absorption spectrophotometer with an acetylene-air flame (Page et al. 1982). The amount of N, P, Mo and B was assayed by colorimetry while the concentration of S – by turbidimetry using spectrophotometer. K was measured by a flame photometer with an air-propane/butane flame. Analyses were performed at the Laboratory of Plant Mineral Nutrition, University of Latvia Institute of Biology.

The climate in the area is transitional between maritime and continental, characterised by relatively mild winters and warm summers. Mean annual air temperature is 5.8 °C, but years 2006, 2007, 2008 and 2009 were warmer with average annual temperatures in the area 7.4 °C, 7.4 °C, 8.2 °C and 7.3 °C respectively. Average precipitation in the area is ca. 600–700 mm per year but in 2006 precipitation was only 530 mm or 80 % of usual amount. By contrast, 2007, 2008 and 2009 were characterised by regular annual precipitation with ca. 690 mm, 610 mm and 610 mm, respectively. In the area, the length of vegetation season (temperature +5 °C or higher) is ca. 190 days and the length of the active growth season (temperature +10 °C or higher) is ca. 145 days. Meteorological data were obtained from Latvian Environment, Geology and Meteorology Centre (*www.meteo.lv*).

The experimental plantation 7500 m^2 in size was established in May 2006. Seedling materials used in the present study are shown in Table 1. Seedlings were produced in JSC “Latvijas valsts meži” Strenci forest nursery which is the largest seedling producer in Latvia. In the nursery, containerised seedlings were grown in 85 cm^3 pots in the greenhouse using sphagnum peat as a substrate, and bare-root seedlings were grown in the mineral sandy soil in an open field beds. Sphagnum peat (pH 3.6) was produced by JSC Seda (Seda, Latvia) and contained 70% of milled peat and 30% of block peat with addition of PG Mix 14:16:18 (N, P, K) – 0,8 g/m^3 and lime – 1,8 kg/m^3 (Nollendorfs 2004). Fertilization of all bare-root seedlings and application of pesticides for *P. sylvestris* (for bare-root seedlings – pesticide Dithane was applied eight times during the vegetation season; for containerized seedlings – Amistar was applied four times and Bravo – three times) was done in accordance to standard procedures (JSC Latvijas valsts meži, unpublished data). No pesticides was used in cultivation of *P. abies* seedlings.

Seedlings used in the present study represented standard planting material of each respective cultivation system and their parameters at the time of outplanting are given in Table 1. Before planting, the site was ploughed in 20 rows at intervals of 1.5 m, and in each row seedlings of different treatments (one treatment per row) were planted. Different treatments were arranged in four adjacent rows, resulting in a block which was replicated five times throughout the plantation. Different treatments within each block where arranged randomly.

Table 1. Seedlings used in the present study

Tree species	Age, years	Cultivation system	Standard height/ root collar diameter*	Seed origin	No. of seedlings planted
Pinus sylvestris	2	Bare-root	10-15cm/2mm	Eastern, Aizkraukle region, Jaunjelgava seed orchard	379
	1	Containerised	7-15cm/2mm	Eastern, Jekabpils region, Veznieki seed orchard	365
Picea abies	4	Bare-root	20 cm/4mm	Eastern, Rezekne region	383
	2	Containerised	20 cm/4mm	Northern, Ogre region, Suntazi seed orchard	369

* Standard seedling parameters at the time of outplanting (JSC Latvijas valsts meži, 2011)

Seedling measurements and sampling

In each treatment, height of the seedlings was measured at outplanting in May 2006. Height and survival of the seedlings was also determined after each growing season in October 2006, 2007, 2008 and 2009. After each growing season (except 2009), five randomly selected seedlings of each treatment were collected for root examination. Seedlings were carefully excavated to preserve fine roots, roots were excised from the stems, individually packed into plastic bags, transported to the laboratory, and kept at +4 °C for a maximum period of three weeks before processing.

In addition, chemical composition of the needles, seedling root collar diameter and stem volume were determined at the end of an experiment in October 2009. To determine chemical composition of needles, ten current-year needles per plant were taken from ten random plants in each treatment, pooled together within each treatment and analysed as a bulk sample. Sampled needles were oven-dried at 60 °C for two weeks and then finely ground using a ball mill. Samples were dry-ashed in concentrated HNO_3 vapour and re-dissolved in 3% HCl. Nutrient concentrations in extracts were measured by atomic absorption spectrophotometry (Ca, Mg, Fe, Cu, Zn, Mn), colorimetry (N, P, Mo, B) and flame photometry (K, Na) as described by Rinkis et al. (1987). Chemical analyses were carried out at Laboratory of Plant Mineral Nutrition, University of Latvia Institute of Biology and the results are presented in Table 2.

Table 2. Chemical composition of needles (mg/g) of bare-rooted and containerised *P. sylvestris* and *P. abies* seedlings after four growing seasons (October 2009) in a plantation

Chemical element	*Pinus sylvestris*		*Picea abies*	
	bare-root	containerised	bare-root	containerised
N	17.0	13.3	13.7	11.7
P	2.0	2.0	2.4	1.9
K	4.0	4.0	8.0	4.0
Ca	2.8	2.7	4.9	6.0
Mg	2.4	2.0	1.0	1.6
S	1.7	1.9	1.6	1.5
Fe	0.062	0.058	0.058	0.062
Mn	0.052	0.046	0.062	0.072
Zn	0.070	0.046	0.040	0.036
Cu	0.003	0.003	0.003	0.002
Mo	0.0003	0.0007	0.0002	0.0007
B	0.016	0.012	0.012	0.011

ECM morphotyping and molecular identification of fungal taxa

ECM morphotyping of seedling roots was carried out to assess composition and development of ECM communities during three consecutive growing seasons (2006, 2007 & 2008). Each sampled root system was washed in the water to remove the soil. Then, using Leica MZ-7.5 (Wetzlar, Germany) stereomicroscope, 20 single ECM root tips from each plant were randomly collected from different parts of the root system using forceps. ECM tips were recognised by the presence of mantle, external hyphae or rhizomorphs, and the absence of root hair. Sampled ECM root tips were grouped into different morphotypes according to their morphological characteristics (colour, shape, mantle structure, patters of rhizomorphs and extramatrical hyphae) (Agerer 1986-2006). In total, 1200 root tips were examined in the present study. For molecular identification of fungal taxa, 1–10 root tips of each morphotype were placed in 1.5 ml centrifugation tubes and stored at -16 °C.

Representatives of each distinct ECM morphotype were subjected to direct sequencing of internal transcribed spacer of fungal ribosomal RNA (ITS rRNA). DNA extraction and PCR using ITS1F and ITS4 primers (White et al. 1990) were done as in a previous study (Menkis and Vasaitis 2011). Sequencing was performed by Macrogen Inc., Seoul, Korea, utilizing ABI 3730 XL automated sequencers (Applied Biosystems, Foster City, CA, USA). Raw sequence data were analysed using the SeqMan Pro version 9.1.0 software from DNASTAR package (DNASTAR, Madison, WI, USA) and BioEdit version 7.0.5.2 (Hall 1999). Databases at GenBank (Altschul et al. 1997) and UNITE (Koljalg et al. 2005) were used to determine the identity of ITS rRNA sequences. The criteria used for identification were: sequence coverage > 80%; similarity to species level 97–100%, similarity to genus level 94–96 %. Sequences not matching those criteria or lacking taxonomic description in the reference sequences were considered unidentified, assigned to a phylum and given unique names as in Table 3.

Statistical analyses

The impact of the cultivation system on seedling survival and richness of fungal taxa in seedling roots of different treatments and at different time points was compared by chi-square (χ^2) tests calculated from the actual number of observations. Chi-square is a non-parametric test which allows testing differences between two or more actual samples (Mead and Curnow 1983). Differences in height, root collar diameter and stem volume of the seedlings in different treatments and at different time points were analysed by one-way analysis of variance (ANOVA) and Tukey's test which provides confidence intervals for all pairwise differences between means (Chalmers and Parker 1989, Fowler et al. 1998). The statistics were computed using Minitab® statistical software (Minitab® Inc. 2003). Shannon diversity index and qualitative (S_S) Sorensen similarity indices were used to characterise diversity and composition of fungal communities in different treatments, tree species and growing seasons (Magur-

Table 3. Occurrence and relative abundance of different fungal taxa directly sequenced from roots of bare-rooted and containerised *P. sylvestris* and *P. abies* seedlings after the first (2006), second (2007) and third (2008) growing seasons in a plantation

Fungal taxa	Phylum	Genbank accession No.	*Pinus sylvestris*						*Picea abies*						All
			Bare-root			Containerised			Bare-root			Containerised			
			2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008	
Amphinema byssoides	Basidiomycota	JX907809	-	2.0	-	-	1.7	-	2.8	21.6	55.0	20.0	23.6	19.0	9.8
Amphinema sp.	Basidiomycota	JX907810	1.5	-	24.4	-	-	-	-	-	1.3	-	-	1.0	1.8
Cadophora finlandica	Ascomycota	JX907811	-	-	-	-	-	-	1.0	-	-	-	-	-	0.1
Humaria hemisphaerica	Ascomycota	JX907812	-	5.1	-	-	-	-	-	-	-	-	-	-	0.2
Laccaria proxima	Basidiomycota	JX907813	-	-	-	-	-	-	2.0	-	-	-	-	-	0.3
Phlebiopsis gigantea	Basidiomycota	JX907814	-	-	-	-	-	-	-	-	43.8	-	-	-	2.6
Piloderma sp.	Basidiomycota	JX907815	-	-	-	-	-	-	-	72.7	-	-	-	-	3.7
Rhizopogon rubescens	Basidiomycota	JX907816	-	-	-	10.0	-	-	-	-	-	-	-	-	1.5
Russula velenovskyi	Basidiomycota	JX907817	-	-	5.0	-	-	-	-	-	-	-	-	-	0.3
Suillus luteus	Basidiomycota	JX907818	-	-	-	-	-	3.3	-	-	-	-	-	-	0.1
Suillus variegatus	Basidiomycota	JX907819	-	-	-	6.5	-	-	-	-	-	-	-	-	1.0
Thelephora terrestris	Basidiomycota	JX907820	67.2	92.9	-	80.3	92.2	10.0	81.3	-	-	56.3	74.3	-	56.1
Tylospora asterophora	Basidiomycota	JX907821	1.0	-	-	-	-	-	13.0	-	-	-	-	-	2.1
Unidentified P1	Basidiomycota	JX907822	-	-	3.1	-	-	-	-	-	-	-	-	-	0.2
Unidentified P2	Basidiomycota	JX907823	-	-	-	-	-	27.5	-	-	-	-	-	-	1.2
Unidentified P3	Ascomycota	JX907824	-	-	-	-	6.1	-	-	-	-	-	-	-	0.4
Unidentified S1	Ascomycota	JX907825	-	-	-	-	-	-	-	-	-	-	-	30.0	1.1
Unidentified S2	Ascomycota	JX907826	-	-	-	-	-	-	-	-	-	23.8	-	-	3.5
Wilcoxina rehmii	Ascomycota	JX907827	30.3	-	67.5	3.3	-	59.2	-	5.8	-	-	2.1	50.0	13.9
Shannon diversity index			0.74	0.30	0.87	0.70	0.32	1.01	0.67	0.73	0.75	0.99	0.64	1.07	

ran 1988). The development of ECM communities was analysed using Principal Component Analysis (PCA) in CANOCO 4.5 (ter Braak and Smilauer 1998).

Results

Survival and growth of the seedlings

During four growing seasons (2006–2009) following seedling outplanting, a gradual decrease in seedling survival was observed each year in both tree species and cultivation systems, resulting in relatively low overall survival rates, which generally were lower in *P. sylvestris* than in *P. abies* (Figure 1). In *P. sylvestris*, survival of containerised seedlings was significantly better as compared to bare-root seedlings after 2006 (90.4% ± 2.2 SE and 65.9% ± 2.6 SE, respectively; $P < 0.0001$), 2007 (61.4% ± 1.4 SE and 40.1% ± 1.7 SE, respectively; $P < 0.0001$) and 2008 (34.5% ± 1.8 SE and 20.8% ± 1.5 SE, respectively; $P < 0.0001$) growing seasons, but after 2009 season survival of containerised seedlings decreased more sharply than in bare-root seedlings, resulting in no significant difference between the treatments (16.7% ± 2.0 SE and 14.3% ± 1.2 SE, respectively; $P > 0.05$) (Figure 1A). In *P. abies*, no significant differences in seedling survival was observed between containerised and bare-root seedlings after 2006 (83.2% ± 6.2 SE and 81.5% ± 5.1 SE, respectively; $P > 0.05$), 2007 (60.0% ± 3.8 SE and 61.5% ± 4.7 SE, respectively; $P > 0.05$) and 2008 (39.8% ± 3.2 SE and 45.2% ± 4.8 SE, respectively; $P > 0.05$) growing seasons, but after 2009 season a greater dieback was in containerised seedlings, resulting in their significantly lower survival than in bare-root seedlings (29.5% ± 3.5 SE and 42.6% ± 4.5 SE, respectively; $P < 0.0003$) (Figure 1B). Consequently, after the fourth (2009) growing season, a pronounced decrease in survival was in containerised seedlings of both tree species as compared to the corresponding bare-root seedlings (Figure 1).

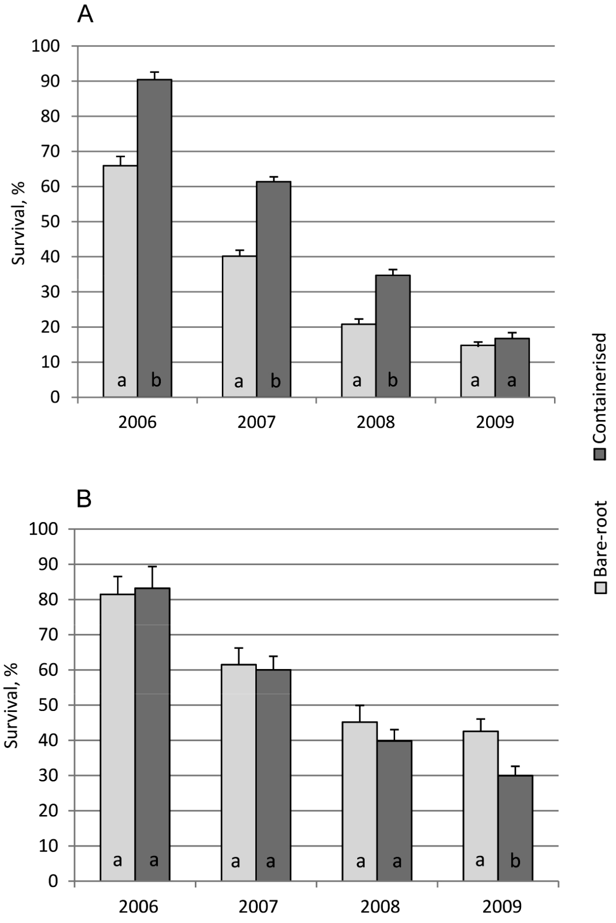

Figure 1. Survival of the tree seedlings during four growing seasons: (**A**) *Pinus sylvestris*; (**B**) *Picea abies*. Within the same year, statistically significant differences between the treatments in chi-squared tests are designated by different letters. Error bars indicate standard error of the mean

At the time of outplanting, the height of containerised and bare-root seedlings differed within each of the tree species (Figure 2). In *P. sylvestris*, the outplanting height of containerised seedlings was significantly higher as compared to bare-root seedlings (15.9cm ± 0.1 SE and 10.9 cm ± 0.1 SE, respectively; $P < 0.0001$) and similar trend in seedling height persisted after each of the following growing seasons: 2006 (26.7cm ± 0.3 SE and 15.6 cm ± 0.3 SE, respectively; $P < 0.0001$), 2007 (40.4 cm ± 1.8 SE and 26.3 cm ± 1.9 SE, respectively; $P < 0.0001$), 2008 (51.3 cm ± 1.8 SE and 44.7 cm ± 2.3 SE, respectively; $P < 0.0001$) and 2009 (82.2 cm ± 4.0 SE and 57.4 cm ± 3.8 SE, respectively; $P < 0.0001$) (Figure 2A). In *P. abies*, in contrast, the outplanting height of containerised seedlings was significantly smaller as compared to bare-root seedlings (24.5cm ± 0.2 SE and 30.2 cm ± 0.3 SE, respectively; $P < 0.0001$) (Figure 2B) and certain fluctuations in growth of the seedlings of different treatments was observed after each year. A better growth of containerised seedlings as compared to bare-root seedlings was observed during the two first seasons in the plantation resulting in similar height of the seedlings in both treatments after 2006 season (34.2 cm ± 0.4 SE and 33.6 cm ± 0.4 SE, respectively; $P > 0.05$) and in significantly higher height of containerised seedlings after 2007 season (43.1 cm ± 1.2 SE and 36.8 cm ± 0.9 SE, respectively; $P < 0.0001$). However, during the third and fourth seasons, containerised seedlings grew poorer as compared to bare-root seedlings what resulted in similar height of the seedlings in both treatments after 2008 season (59.5 cm ± 1.4 SE and 60.3 cm ± 1.3 SE, respectively; $P > 0.05$) and in significantly lower height of containerised seedlings after 2009 season (75.6 cm ± 2.3 SE and 82.5 cm ± 1.9 SE, respectively; $p<0.03$) (Figure 2B).

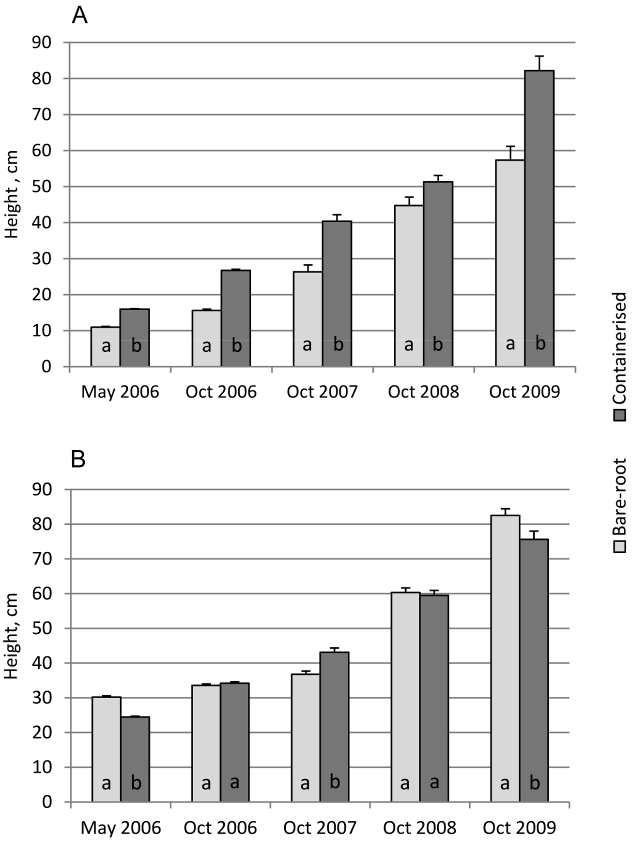

Figure 2. Height of the tree seedlings at outplanting (May 2006) and after the first (Oct 2006), second (Oct 2007), third (Oct 2008) and fourth (Oct 2009) growing seasons: (**A**) *Pinus sylvestris*; (**B**) *Picea abies*. Within the same year, statistically significant differences between the treatments in one-way ANOVA are designated by different letters. Error bars indicate standard error of the mean

The root collar diameter of the seedlings was measured ones after the growing season 2009, showing that in *P. sylvestris* it was significantly larger in containerised seedlings than in bare-root seedlings (1.7 cm ± 0.09 SE and 1.1 cm ± 0.08 SE, respectively; $p < 0.0001$) while in *P. abies* it did not differ significantly between the seedlings of different treatments (1.4 cm ± 0.03 SE and 1.5 cm ± 0.03 SE, respectively; $p > 0.05$). Consequently, in *P. sylvestris* the stem volume was significantly greater in containerised seedlings than in bare-root seedlings (312.5cm^3 ± 55.2 SE and 105.8 cm^3 ± 23.7 SE, respectively; $p < 0.005$) while in *P. abies* it did not differ significantly between the seedlings of different treatments (179.6 cm^3 ± 11.9 SE and 207.2 cm^3 ± 12.2 SE, respectively; $p > 0.05$). However, the chemical analysis of the needles determined after 2009 season has showed generally lower amounts of all chemical elements in containerised *P. sylvestris* and *P. abies* seedlings as compared to the corresponding bare-root seedlings (Table 2). An exception to this was Mo element which was found in several times larger amounts in containerised seedlings than in bare-rooted ones of both tree species (Table 2).

Composition and development of ectomycorrhizal communities

During three growing seasons (2006–2008) following plantation establishment, a total of 14 ECM morphotypes was detected on *P. sylvestris* and 12 – on *P. abies* (data not shown), but ITS rRNA sequencing of representative root tips of each individual ECM morphotype revealed the presence of 19 fungal taxa of which 8 (42.1%) were exclusively on *P. sylvestris*, 6 (31.6%) – on *P. abies* and 5 (26.3%) were common to both tree species (Table 3).

Regarding the cultivation system, 8 (42.1%) taxa were exclusively on bare-root seedlings, 7 (36.8%) – on containerised seedlings and 4 (21.1%) were common to seedlings of both cultivation systems. The detected community was composed of 13 (68.4%) basidiomycetes and 6 (31.6%) ascomycetes. Identification at least to genus level was successful for 14 (73.7%) taxa while 5 (26.3%) taxa remained unidentified. Within each season, cultivation system and tree species, the detected number of different fungal taxa in seedling roots ranged between 3–5 (Table 3) and their richness as revealed by chi-square test did not differ significantly when compared between any of these datasets ($p > 0.05$). The Shannon diversity indices were generally low and rather similar when compared between seedlings of different cultivation systems of each respective tree species and growing season (Table 3). Despite that in majority of cases a certain pattern of Shannon diversity was observed during three consecutive seasons for respectively containerised and bare-rooted *P. sylvestris* seedlings, and containerised and bare-rooted *P. abies* seedlings as follows: relatively low diversity after the first 2006 season (0.70 and 0.74, and 0.99 and 0.67); decreased diversity after the second 2007 season (0.32 and 0.30, and 0.64 and 0.73); recovered and increased diversity after the third 2008 season (1.01 and 0.87, and 1.07 and 0.75) (Table 3). Sorensen similarity indices of fungal communities between respectively containerised and bare-rooted *P. sylvestris* and containerised and bare-rooted *P. abies* were moderate after 2006 season (0.50 and 0.50), high after 2007 season (0.67 and 0.67) and low to moderate after 2008 season (0.25 and 0.57). Furthermore, the PCA analysis showed that during 2006–2007 seasons (except for bare-root *P. abies* 2007) the fungal communities of both tree species and cultivation systems were largely similar and were dominated by the ECM basidiomycete *Thelephora terres-*

tris (Figure 3) (Table 3). However, after the next season, a dynamic change in fungal communities was observed in both tree species and cultivation systems resulting in subdivision of bare-root *P. abies* community which was dominated by ECM basidiomycete *Amphinema byssoides*, from the fungal communities present in other three treatments which were dominated by the ECM ascomycete *Wilcoxina rehmii* (Figure 3).

cultivation systems on seedling performance in the field and that this should be considered while performing similar studies in the future.

One of the possible reasons for the observed differences in survival and growth of the seedlings from different cultivation systems may be differences in their root architecture. Bernier et al. (1995) suggested that root architecture may influence early survival and

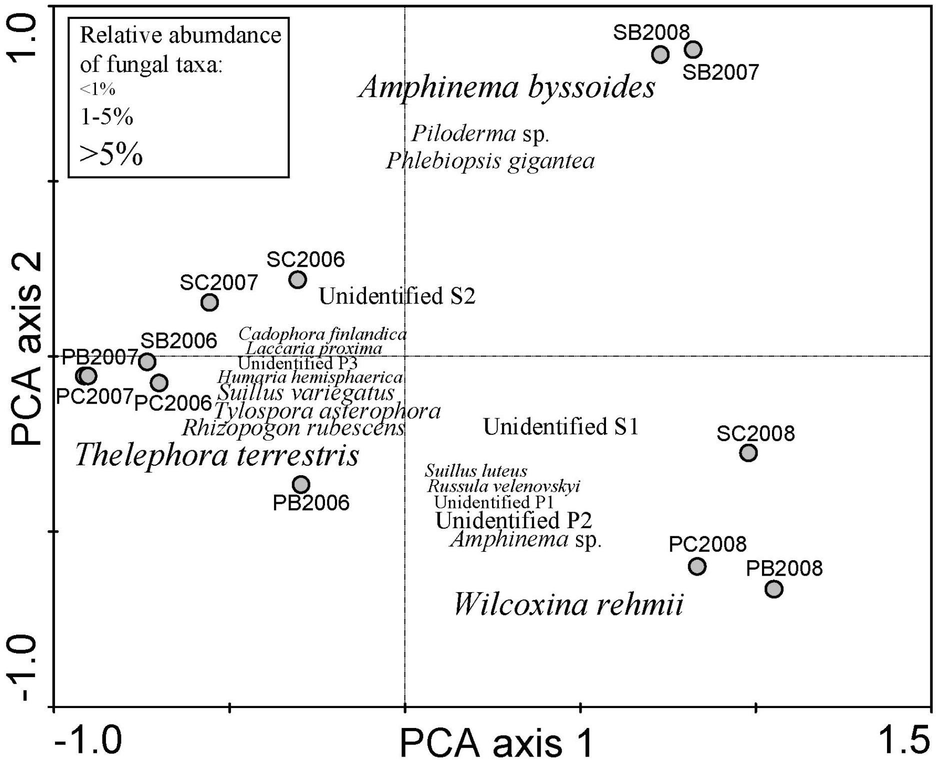

Figure 3. Ordination diagram based on principal component analysis of fungal communities in roots of *Pinus sylvestris* and *Picea abies* seedlings from different treatments (PB – *P. sylvestris* bare-root; PC – *P. sylvestris* containerised; SB – *P. abies* bare-root; SC – *P. abies* containerised) and three growing seasons (2006, 2007 and 2008). Taxonomic names correspond to a position in the ordination (centred) and their font size corresponds to a relative abundance as indicated in the upper left corner of the diagram and Table 3

Discussion and conclusions

Several similar studies have previously reported that containerised seedlings may often exhibit faster growth and better survival than bare-root seedlings after their outplanting in the field or in the nursery (Leugner et al. 2009, Vaario et al. 2009, Menkis et al. 2011). In agreement with these studies, the present study has also demonstrated that during the first three growing seasons following seedling outplanting, containerised seedlings showed generally similar or better survival and growth as compared to bare-root seedlings of both tree species. However, after the fourth growing season their survival and growth (except for growth of *P. sylvestris*) has decreased and this was particularly notable for *P. abies* (Figure 1, 2). The latter may therefore suggest that possibly longer period of time is required to reveal the effects of different

growth of the seedlings more than other parameters since it may influence the capacity of the seedlings to produce new roots that extend outside the original root system into the surrounding soil. During the cultivation in forest nurseries, roots of containerised seedlings are usually more compressed and compacted within growth containers as compared to roots of bare-root seedlings as these develop more naturally. However, roots of containerised seedlings are often characterised by better primordia and larger nutrient reserves (Leugner et al. 2009). Besides, differently from the bare-root seedlings, roots of containerised seedlings to a large extent remain intact following seedling outplanting and this may also favour seedling early establishment and growth in the field. In support, Grossnickle (2005) reported that containerised seedlings can have greater root growth than bare-root seedlings during first growing seasons. In the present study, differences in age between containerised and bare-root seedlings could also affect the development of roots and therefore growth and mineral nutrition of the seedlings of different treatments. As containerised seedlings were younger (Table 1) and consequently had relatively smaller roots, these were likely growing faster during the first years in the plantation. However, the results also suggest that depending on the site conditions such effects might be short-lived as already after the fourth season in the plantation in addition to the survival and growth parameters the nutritional status (as revealed by the chemical composition of the needles) of bare-root seedlings was far better as compared to containerised seedlings of both tree species (Table 2). Such results might be connected to water regime present at the study site because it has been reported that performance of containerised seedlings was better when they were planted on regular humidity sites (Grossnickle 2005). In the present study, however, the site corresponded to *oxalido-myrtilliosa* forest type characterised by average fertility and temporarily excessive humidity in the soil.

In addition, survival, growth and nutrition of the seedlings may also depend on composition and activity of symbiotic ECM fungi (Smith and Read 1997). In the present study, the communities of ECM fungi observed after the first growing season in a plantation were generally similar to the ones previously described from forest nurseries (Menkis et al. 2005, Flykt et al. 2008, Menkis and Vasaitis 2011) and largely differed from ECMs present in older natural (Stankevičienė et al. 2008) and intensively managed stands (Ozolinčius et al. 2007) grown in the area, while the dynamic changes in the later seasons, i.e. from the predominance of *T. terrestris* to the dominance of *W. rehmii* and *A. byssoides*, were likely driven by the host specificity and/or ECM inoculum availability, and indicated certain adaptation of ECM communities to the environmental conditions present at the site (Dahlberg and Stenström 1991, Gagné et al. 2006, Menkis et al. 2007). On the other hand, sampling and analysis of relatively small proportion of root-tips and plants in the plantation might be partially responsible for the observed rapid shift in composition and abundance of fungal taxa. Dominant taxa of the present study (genera *Thelephora*, *Wilcoxina* and *Amphinema*) were previously shown to be an early stage and widespread fungi (Horton and Bruns 2001). Among these, the basidiomycete *T. terrestris* was reported to be the most common ECM fungus in the forest nurseries worldwide (Marx et al. 1984). However, despite its adaption to environmental conditions of the nursery i.e. to high levels of nutrients and moisture (Perry et al. 1987), it often fails to support seedling establishment in the field (Ivory and Munga 1983, Lee 1992). *Wilcoxina* species belong to a group of E-strain fungi that were found to be commonly associated with the tree seedlings in soils following site disturbance and therefore could be important for seedlings in overcoming the replanting stress (Yu et al. 2001, Menkis et al. 2010). *A. byssoides* is known as an efficient root coloniser of *P. abies* seedlings and may play an important role in seedlings survival and establishment following their outplanting (Menkis et al. 2007, Vaario et al. 2009, Menkis et al. 2011). In the present study, the observed predominant establishment of *A. byssoides* in roots of bare-root seedlings of *P. abies* after the second and third growing seasons might be also associated with their better survival and growth in the following seasons (Figure 3) (Table 3). Among other fungi, the presence of *Phlebiopsis gigantea* in ECM roots of *P. abies* seedlings (Table 3) further supported the hypothesis about the multi-trophic nature of this wood-decay fungus (Vasiliauskas et al. 2007, Menkis et al. 2012).

In conclusion, as the production of containerised seedlings is increasing in Europe and production of bare-root seedlings is declining (Flykt et al. 2008), the results of the present and related studies may suggest that caution should be taken when selecting seedlings for outplanting on dry and/or humid habitats as on these containerised seedlings may perform poorer than bare-root seedlings (Leugner et al. 2009). On the other hand, seedling production using Plug+1 system (see Introduction) could be an alternative as using this cultivation system seedling roots develop more naturally as compared to containerised seedlings and have better primordia as compared to bare-root seedlings. Besides, their production is less expensive. However, in order to obtain more comprehensive picture about the impacts of different cultivation systems on seedling performance following their outplanting in the field, more related studies are needed in the region, encompassing different soil conditions, tree species and cultivation systems.

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РОСТ И РАЗВИТИЕ ЭКТОМИКОРИЗНЫХ СООБЩЕСТВ САЖЕНЦЕВ СОСНЫ ОБЫКНОВЕННОЙ И ЕЛИ ОБЫКНОВЕННОЙ, ВЫРАЩЕННЫХ В КОНТЕЙНЕРАХ И С ОТКРЫТОЙ КОРНЕВОЙ СИСТЕМОЙ ПОСЛЕ ПОСАДКИ НА ВЫРУБКАХ

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Резюме

Выбор посадочного материала высокого качества является необходимым условием для успешного лесовосстановления. Однако саженцы, производимые при различных системах культивации, могут значительно отличаться. Цель данного исследования – оценка выживаемости, роста и развития эктомикоризного сообщества саженцев сосны обыкновенной и ели обыкновенной, выращенных в контейнерах и с открытой корневой системой, после их посадки на лесосеке в Латвии. В мае 2006 года была создана экспериментальная плантация размером 7500 м^2. Саженцы, выращенные с использованием четырех различных методов культивирования, были высажены рядами в пяти повторностях. Результаты показали, что в течение четырех вегетационных периодов (2006–2009) после посадки наблюдалось постепенное снижение выживаемости саженцев обоих видов деревьев и обеих культивационных систем. Констатирована относительно низкая общая выживаемость саженцев после четвертого сезона: для сосны обыкновенной наблюдалась схожая выживаемость между саженцами выращенными в контейнерах и саженцами с открытой корневой системой (соответственно, 16,7 % ± 2.0SE и 14,3 % ± 1.2SE, p> 0,05), а для ели обыкновенной выживаемость была значительно ниже у контейнерных саженцев (29,5 % ± 3.5SE), чем у саженцев культивированных с открытой корневой системой (42,6 % ± 4.5SE) (p <0,0003). Во время посадки отмечены различия между высотой саженцев выращенных с использованием различных технологий для обоих видов деревьев, и аналогичная тенденция сохранилась также через четыре года после посадки. Хотя сообщества эктомикоризных грибов, обнаруженных в данном исследовании, напоминали сообщества присутствующие в лесных питомниках, динамические изменения от преобладания *Thelephora terrestris* в первом сезоне к доминированию *Wilcoxina rehmii* и *Amphinema byssoides* в последующие сезоны заявили об определенной адаптации к условиям данного эксперементального участка. Исследование показало, что в зависимости от вида деревьев, система культивирования может повлиять как на выживаемость лесопосадок, так и на развитие эктомикоризных сообществ.

Ключевые слова: эктомикоризные грибы, лесные питомники, сосна обыкновенная, ель обыкновенная, культивационная система.