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ORIGINAL PAPER

Ectomycorrhizal community structure of different genotypes of Scots pine under forest nursery conditions

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Abstract In this paper, we report the effect of Scots pine genotypes on ectomycorrhizal (ECM) community and growth, survival, and foliar nutrient composition of 2-year-old seedlings grown in forest bare-root nursery conditions in Lithuania. The Scots pine seeds originated from five stands from Latvia (P1), Lithuania (P2 and P3), Belarus (P4), and Poland (P5). Based on molecular identification, seven ECM fungal taxa were identified: *Suillus luteus* and *Suillus variegatus* (within the Suilloid type), *Wilcoxina mikolae*, *Tuber* sp., *Thelephora terrestris*, *Cenococcum geophilum*, and Russuloid type. The fungal species richness varied between five and seven morphotypes, depending on seed origin. The average species richness and relative abundance of most ECM morphotypes differed significantly depending on pine origin. The most essential finding of our study is the shift in dominance from an ascomycetous fungus like *W. mikolae* in P2 and P4 seedlings to basidiomycetous Suilloid species like *S. luteus* and *S. variegatus* in P1 and P5 seedlings. Significant differences between Scots pine origin were also found in seedling height, root dry weight, survival, and concentration of C, K, Ca, and Mg in the needles. The Spearman rank correlation coefficient revealed that survival and nutritional status of pine seedlings were positively correlated with abundance of Suilloid mycorrhizas and negatively linked with *W. mikolae* abundance. However, stepwise multiple regression analysis showed that only survival and magnesium content in pine needles were significantly correlated with abundance of ECM fungi, and Suilloid mycorrhizas were a main significant predictor. Our results may have implications for understanding the physiological and genetic relationship between the host tree and fungi and should be considered in management decisions in forestry and ECM fungus inoculation programs.

Keywords Scots pine · Mycorrhizal fungi · Provenance variation · ITS-sequencing · *Suillus* · *Wilcoxina*

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Introduction

Scots pine (*Pinus sylvestris* L.) is, in Europe, one of the most important timber conifer species, with a high commercial and ecological value. Scots pine forests and plantations are a very common forest type in Europe and cover 24% of the total forested area (75 million km^2) (Stanners and Bourdeau 1995). Produced in bare-root nurseries, Scots pine seedlings are predominantly used in standard reforestation and the afforestation of former agricultural and marginally economic lands. In Lithuania and Poland, respectively, around 0.5 and 0.7 million ha of abandoned farmland are designated for afforestation (Milewski 2007; Riepšas 2000). Healthy and vigorous growth of Scots pine seedlings depends largely on the activity of ectomycorrhizal (ECM) fungi, which constitute a large component of microbial biomass in tree roots (Read 1998) and, therefore, may be essential in determining afforestation success. Scots

pines rely heavily on their ECM fungal symbionts to obtain sufficient nutrients from the soil, especially nitrogen and phosphorus, and to enhance resistance to fungal pathogens or toxicity at adverse sites (Read 1998). Colonization of seedlings by various ECM fungi, as the consequence of particular cultivation practices in forest nurseries (Iwański et al. 2006; Leski et al. 2008; Rudawska et al. 2006; Trocha et al. 2006) or achieved by artificial inoculation, both in the nursery (Ortega et al. 2004; Trappe 1977) or in the field (Dunabeitia et al. 2004), may significantly promote survival, and growth of young trees in newly established forest plantations and during afforestation of abandoned farmland or degraded areas (El Karkouri et al. 2002, 2004; Dahlberg and Stenström 1991; Garbaye and Churin 1997; Haselwandter and Bowen 1996; Menkis et al. 2007; Parladé et al. 2004; Pera et al. 1999; Quoreshi et al. 2008; Rincon et al. 2007). Some of naturally occurring ECM fungi can increase survival and growth of forest trees better than artificial inoculations (Cram et al. 1999).

As a widely distributed species, Scots pine has differentiated for adaptive traits among populations (Ledig 1998) which stimulated the early start to breeding programs in different European countries. As a result, Scots pine was the first forest species for which different provenance studies were performed (Langlet 1971), and the first IUFRO provenance test of the species was established in 1907 (Giertych 1991). Seed provenance, usually defined as the source of seeds in a geographical or habitat context, may reflect genotypic variation within species; there has been, therefore, some interest in determining the effect of this variation on ectomycorrhiza formation (Peterson and Bradbury 1999). Most early studies revealed a significant difference in percentage of root tips colonized by ECM fungi between seed sources of different conifers including *Pseudotsuga taxifolia* or *Pseudotsuga menziesii* (Linnemann 1960; Wright and Ching 1962), *Pinus elliottii* (Marx and Bryan 1971; Rosado et al. 1994), *Pinus contorta* and *Pinus ponderosa* (Cline and Reid 1982; Karst et al. 2009), *Pinus banksiana* (Navratil 1986), *Pinus taeda* (Dixon et al. 1987), *Picea sitchensis* (Walker et al. 1986), *Picea mariana* (Thomson et al. 1990), and *Larix laricina* (Zhu and Navratil 1987). More recently, a genetic basis for natural mycorrhization was demonstrated for Norway spruce clones (Korkama et al. 2006) and some angiosperm trees, like poplars (Gehring et al. 2006; Khasa et al. 2002; Tagu et al. 2001, 2005) and willows (van der Heijden and Kuyper 2001).

Despite the extensive geographical distribution and ecological and economic relevance of Scots pine, surprisingly little information exists about the genetic variation of this tree relative to the degree of ECM fungi colonization. To our knowledge, the only provenance trials with Scots pine that have taken into account ECM symbiosis were done in Sweden by Lundeberg (1968) and showed that relative susceptibility of seedlings to colonization by ECM fungi varied significantly among provenances following outplanting. In these early studies, however, the ECM community structure of Scots pine was not examined in detail, as is now possible with modern molecular methods (e.g., Iwański et al. 2006). Knowledge about ECM species associated with a tree species over its natural distribution may provide important information for ecological studies and management decisions in forest ecosystems. To address this issue, we studied Scots pine of different geographic origin, using a common-garden experiment with five populations in a bare-root nursery in Lithuania. We hypothesized that the seed origin of the host plant would influence the quality and/or quantity of indigenous ECM fungal community in the early stage of development of Scots pine under forest nursery conditions.

Materials and methods

Description of the study site and experimental design

The study was performed in a bare-root forest nursery of Vilnius University Botanical Garden (54°43′N, 25°24′E). The Scots pine seeds for the trial originated from five open-pollinated natural stands, from regions with different light, temperature, and moisture regimes (Table 1). The nursery test design was five complete blocks with five plots (1× 1 m) per seed origin, randomly allocated in each block. Nursery soil (pH_{KCl}=6.39) was characterized as Haplic Podzol with a 9-cm-deep alluvial horizon, enriched with a 23-cm-deep illuvial horizon. *P. sylvestris* required no stratification, and the seeds were therefore planted directly, by hand, into the nursery soi1 for germination. Seeding was performed in the end of April 2004. A 5-by-5-cm sowing stencil was used. Seedlings in the first and second growing season were manually irrigated and weeded. No fertilizers were applied throughout the study.

In September 2006, after 2 years of growth under nursery conditions, five randomly selected seedlings were harvested from each block (25 seedlings per seed origin, 125 seedlings in total) for analysis of growth parameters, chemical composition of the needles, and mycorrhizal assessment. Plant height was measured, and the shoots and roots (after mycorrhizal evaluation) were dried at 65± 2°C for 24 h.

Plant tissue analysis

The foliar carbon content and nutrient composition were determined in three samples from five which were harvested (to reduce the costs of the analysis). One-year-old Scots pine needles were rinsed in demineralized water to remove

Table 1 Latitude, longitude, mean annual temperature (MAT), timing of growth cessation (in growing degree days >5°C), mean effective temperature sum (Σ*T*+5°C) (in degree days, d.d.; threshold of daily mean temperature +5°C), and mean annual precipitation (MAP) at the site of the Scots pine seed origins

Origin, symbol	Latitude	Longitude	MAT (°C)	Growing d.d. >5°C	ΣT+5°C, d.d.	MAP (mm)
Smiltene, Latvia (P1)	57°14′	25°52′	5.4	179	967	775.0
Telšiai, Lithuania (P2)	55°57′	22°15′	5.3	183	970	692.0
Labanoras, Lithuania (P3)	55°16′	25°50′	5.8	187	1,085	650.0
Mogilev, Belarus (P4)	53°55′	30°21′	5.4	191	1,031	595.0
Rychtal, Poland (P4)	50°46′	18°05′	8.2	203	1,665	650.0

adhering particles, dried 65±2°C for 24 h, and weighted and milled in automatic mortar (Retech MM200 with agate jars and balls, Sigma). Milled, pine needles, approximately 2.5 g dry weight from each sample, were digested in a mixture of spectrally pure concentrated acids: HNO_3 and $HClO_4$ in a proportion of 4:1 (*v*:*v*). The obtained solution was evaporated to about 1 ml and next diluted with doubly distilled water to a volume of 25 ml. The carbon and nitrogen content in Scots pine seedling needles were measured (in milled samples), using the elemental combustion system 4010. The remaining macroelements were measured by atomic absorption spectroscopy (Varian 220 FS) with atomization in an air-acetylene flame. The accuracy of the analyses was checked against standard reference material, namely, pine needles SRM 1575 and tomato leaves SRM 1573a (National Institute of Standards and Technology [http://ts.nist.gov/MeasurementServices/ReferenceMaterials/ARCHIVED_CERTIFICATES/archived_certificates.htm]).

Analysis of mycorrhizal community structure

After transportation to the lab, the seedlings were stored at 4°C until processing. During analysis in the lab, the growth medium was washed off from root system, and overall mycorrhizal development was examined, as described elsewhere (Aučina et al. 2007; Rudawska et al. 2006). On average, approximately 250 root tips were counted per seedling. ECM morphotypes were described based on macroscopic observations (color of the mantle, presence of rhizomorphs, extramatrical hyphae, and cystidia) and compared to a database at the Laboratory of Mycorrhizal Research at the Institute of Dendrology (Agerer 2001; Aučina et al. 2007; Iwański et al. 2006; Leski et al. 2008; Rudawska et al. 2006; Trocha et al. 2006). Live roots were identified on the basis of their turgid appearance. The numbers of live mycorrhizas of each morphotype were recorded. The relative abundance of each morphotype (number of root tips of each morphotype/total number of mycorrhizas) was calculated for each sample.

Selected samples of morphotypes were stored in 2% cetyltrimethylammonium bromide buffer at room temperature no longer than 2 weeks for further analysis. Complete methods for molecular identification of mycorrhizas are presented in Aučina et al. (2007). Briefly, DNA was extracted from one root tip per sample using the miniprep method (Gardes and Bruns 1996), and fungal symbionts were identified using polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) with ITS-1 and ITS-4 primers (White et al. 1990). ITS products (10 µL) were cut with *Hinf*I, *Mbo*I, or *Taq*I restriction enzymes (EurX, Poland). The amplified products and the restriction fragments (restriction fragment length polymorphism (RFLPs)) were electrophoresed on 1.5% and 2% high-resolution agarose gels (Prona), respectively, stained with ethidium bromide, and photographed under ultraviolet light using a Polaroid or CDD camera. The size of the RFLP fragments was determined using Taxotron® software (SAS Pasteur Institute, Paris, France) and compared with a databank maintained by the Laboratory of Mycorrhizal Research at the Institute of Dendrology. Two or three samples of each unique RFLP pattern were sequenced (with the exception of the *Cenococcum* type). Sequencing was performed with a CEQ 20000XL automatic sequencer using primers ITS1 and ITS4 and the Beckman Coulter DTCS Quick Start chemistry kit. ITS-PCR products were sequenced for the forward and reverse directions and merged into a contig using BioEdit and ClustalW software. Consensus sequences were constructed, with manual editing of ambiguous readings, and compared to published sequences in the GenBank or UNITE database (Kõljalg et al. 2005) using the BLAST tool. Species-level identification of mycorrhizas was defined as the sharing of >98% ITS region sequence identity with the reference sequence, over a length of at least 450 bp.

Statistical analysis

The chemical composition of seedlings, growth parameters, and species richness were analyzed by two-way analysis of variance (ANOVA), with pine origin as a fixed factor and block as the random factor (the normality assumption of data and homogeneity of variance were tested through the

Shapiro–Wilk test and Leven test, respectively). Post hoc comparisons of means were made using Tukey's honestly significant difference (HSD) test, with a significance level of $P<0.05$. No homogeneity of variance was found, and differences between Scots pine origins in relative abundance of morphotypes were therefore tested using the Kruskal–Wallis and Mann–Whitney *U* tests. Prior to analysis, the relative abundance of mycorrhizal morphotypes was arcsine square root transformed. Computations were performed using the statistical software package Statistica 5.5.

Multivariate community analyses of mycorrhizal fungi were carried out with PAST 1.89 software (Hammer et al. 2001), based on square root transformed data. The data matrix consisted of 125 samples (seedlings) with relative abundance of each ECM fungal species within each sample. The Bray–Curtis coefficient was used to produce a dissimilarity matrix of species composition between any two samples (seedlings). To visualize differences (based on the Bray–Curtis matrix) in the mycorrhizal community structure relative to Scots pine origins, nonmetric multidimensional scaling ordination (NMDS) was used. One-way analysis of similarity (ANOSIM) was performed to test differences in relative abundance of mycorrhizal fungal species among pine origins. ANOSIM is a nonparametric, multivariate permutation test, analogous to the parametric, univariate ANOVA, and is particularly applicable when analyzing species data that do not meet the assumptions required for multivariate ANOVA (Clarke and Green 1988).

Individual correlation using Spearman rank correlation, as well as multiple regression were applied to assess relationships between growth parameters, survival and chemical composition of pine seedlings, and relative abundance of five mycorrhizal morphotypes present on seedlings from all origins. Stepwise multiple regressions with backward elimination were used after screening potential independent variables (relative abundance of ECM fungi) for significant autocorrelation. All dependent variables were log-transformed to improve linearity. Relative abundance of mycorrhizal morphotypes was log $(n+1)$ transformed.

Results and discussion

Little is known about the ECM community of Scots pine relative to genetic variation of the host. This investigation produced results supporting the hypothesis that the seed origins of the host plant influence the indigenous ECM fungal community on the root system of Scots pine under forest nursery conditions. Seedlings from all seed sources were exposed to the same indigenous community of ECM fungi that reside in the soil of the tested nursery. The efficiency of this nursery in ECM colonization of young seedlings of Scots pine has previously been established (Aučina et al. 2007). Similarly, in the present experiment, regardless of the seed source, all seedlings were fully colonized. All distinguished mycorrhizas were classified into seven morphotypes. Our previous paper gives detailed descriptions and the basis of identification of these ECM morphotypes (Aučina et al. 2007). Molecular studies revealed the following ECM species: *Suillus luteus* and *Suillus variegatus* (both identified within the Suilloid type), *Wilcoxina mikolae*, *Tuber* sp., *Thelephora terrestris*, and *Cenococcum geophilum*. The orange smooth type, initially designated as an "uncultured basidiomycete" (based on the closest BLAST match to uncultured basidiomycete isolate AZ969603) in the present paper was renamed Russuloid type, as identified from morphological features and based on 83% similarity to the sporophore of *Russula parazurea* (DQ422007). *T. terrestris* mycorrhizas (100% similarity to FJ532478) were not noted in our previous study. The Suilloid/*Wilcoxina* type was characterized by double colonization with *S. luteus* and *W. mikolae*. Such multi-species morphotypes are common under nursery conditions

Table 2 Relative abundance of ectomycorrhizal morphotypes and average fungal species richness on *Pinus sylvestris* seedlings of different origins after 2 years of growth in a bare-root nursery (values are mean ± standard error, $n=25$)

Origin	Relative abundance (%)							Average species richness
	Suilloid type	Suilloid/ *Wilcoxina* type	*Wilcoxina* type	*Thelephora terrestris*	Tuber type	Russuloid type	*Cenococcum geophilum*	
P1	61.6±4.5a	4.1±1.2ab	29.8±3.7b	–	0.8±0.4ab	3.7±1.7b	–	3.4±0.2b
P2	23.7±2.7c	6.78±1.7ab	58.5±4.2a	1.4±1.4a	0.02±0.02b	5.2±1.2b	4.4±2.0b	4.5±0.2a
P3	39.2±5.6bc	8.5±2.6a	34.3±5.3b	–	3.3±1.2a	13.9±3.8a	0.8±0.8c	4.2±0.2ab
P4	31.1±3.0bc	8.9±0.8a	56.9±3.0a	0.1±0.1a	0.2±0.1b	2.8±0.6b	–	4.2±0.2ab
P5	44.9±4.6b	1.5±0.5b	29.2±4.2b	0.2±0.2a	1.7±0.6ab	4.1±1.2b	18.4±3.9a	4.7±0.2a
Origin (P>F)	0.0001	0.004	0.0001	0.463	0.006	0.001	0.0001	0.0003

Within a column, values with different letters are significantly different ($P<0.05$; Mann–Whitney *U* test for relative abundance and Tukey's test for average species richness)

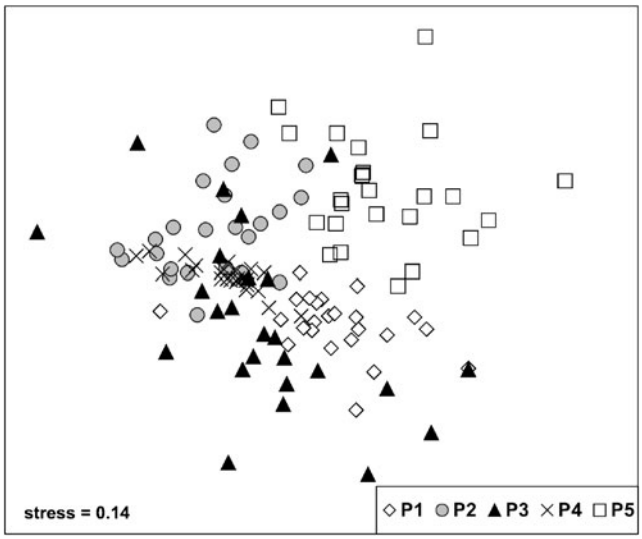

Fig. 1 Two-dimensional nonmetric multidimensional scaling ordination plot of ectomycorrhizal fungal communities, based on Bray–Curtis coefficient. Each point is a representation of ectomycorrhizal fungi composition on Scots pine seedlings from five different origins P1, P2, P3, P4, and P5 (details of the pine origins in Table 1)

(Aučina et al. 2007; Rudawska personal communication) and reflect a succession of colonization of Scots pine seedlings from ectendomycorrhizal *Wilcoxina* to ECM Suilloid fungi.

Seedlings from different seed sources differed in the numbers of associated symbionts: seven morphotypes were found on seedlings from P2 and P5, six on seedlings from P3 and P4, and five on seedlings of P1 origin. Differences in average species richness (per seedling) among Scots pine origins were statistically significant and was the highest on P5 and the lowest on P1 (Table 2). Block effect was not significant (two-way ANOVA, P=0.755). Five morphotypes were common to all pine origins (Suilloid type, Suilloid/*Wilcoxina* type, *W. mikolae*, *Tuber* sp., and the Russuloid type). *T. terrestris* was noted in low abundance on P2, P4, and P5 and was absent on P1 and P3, possibly because of poor competitiveness with other ECM symbionts present in this nursery, namely, the Suilloid and *Wilcoxina* mycorrhizas. *C. geophilum* was found with seedlings of P2, P3, and P5 origin. The species diversity of ECM fungi observed in this nursery on Scots pine seedlings of different geographical origin fits well with the range described for Scots pine seedlings from other bare-root forest nurseries (Iwański et al. 2006; Menkis et al. 2005).

The relative abundance of most ECM morphotypes (with the exception of *T. terrestris*) differed significantly depending on pine origin (Mann–Whitney U test, P<0.05; Table 2). The highest abundance of the Suilloid/*Wilcoxina* type was found on seedlings from P3 and P4. *Tuber* sp. and the Russuloid type were present in significantly greater abundance on P3 seedlings than on seedlings of other pine origins. *C. geophilum* appeared in greatest abundance on seedlings of P5 origin. The significant differences in abundance of certain mycorrhizas (e.g., Russuloid and *C. geophilum*) depending on seed origin are somewhat puzzling, and we can only speculate that they have their basis in the genotype of the host tree. Genetic pedigree of the host was also found as responsible for the varying ECM community structure of the fast and slow growing Norway spruce clones (Korkama et al. 2006). The abundance of Suilloid morphotypes and *W. mikolae* across seedlings from all seed sources did not exhibit the same pattern. The Suilloid type was most abundant on P1, intermediate on P5, and the least abundant on seedlings of P2 origin. Conversely, the *W. mikolae* was most abundant on P2 and P4, and less abundant on P1, P2, and P5 seedlings.

Such mutual fluctuations seem to indicate a host-genotypic effect in response to colonization by these ECM fungi. In previous studies, the genotypic effect of the host plant on the ability to form ectomycorrhizas was primarily quantitatively assessed as colonization level or number of mycorrhizas (Cline and Reid 1982; Karst et al. 2009). Moreover, these studies were performed under laboratory or greenhouse conditions, and extrapolations to field conditions are difficult. Only recently, Korkama et al. (2006) reported variation in richness and abundance of ECM communities for eight Norway spruce clones of the same age, planted in a clear-cut area. To our knowledge, our results are among the first to arise from sequencing of ECM fungal rDNA directly from root tips to obtain species-specific information about

Table 3 Growth and survival of *Pinus sylvestris* seedlings of different origins after 2 years of growth in a bare-root nursery (values are mean ± standard error, n=25)

Origin	Seedling ht (cm)	Dry weight (g)			Survival (%)
		Roots	Stem	Needles	
P1	16.9±0.80a	0.71±0.10a	0.77±0.07a	1.60±0.11a	55.7±2.10b
P2	16.2±0.77a	0.42±0.06b	0.80±0.09a	1.57±0.14a	30.1±0.70d
P3	18.6±0.62a	0.49±0.05ab	0.80±0.06a	1.57±0.13a	43.6±0.25c
P4	13.1±0.61b	0.55±0.07ab	0.79±0.07a	1.49±0.16a	29.7±2.13d
P5	16.9±0.73a	0.38±0.03b	0.67±0.05a	1.69±0.13a	60.0±1.23a
Origin P>F	0.0001	0.008	0.645	0.897	0.0001

Within a column, values with different letters are significantly different (P<0.05; Tukey's test)

Table 4 Foliar nutrient composition of *Pinus sylvestris* seedlings of different origins after 2 years of growth in a bare-root nursery (values are mean ± standard error, $n=15$)

Origin	Concentration (%)						C/N
	C	N	P	K	Ca	Mg	
P1	48.0±0.31a	1.84±0.08a	0.19±0.02a	0.83±0.03a	0.44±0.01ab	0.15±0.004a	26.18±1.16a
P2	44.51±0.11b	1.87±0.16a	0.2±0.01a	0.74±0.01ab	0.38±0.03b	0.13±0.005b	25.61±1.91a
P3	47.34±0.32a	1.73±0.08a	0.19±0.01a	0.70±0.02abc	0.47±0.01a	0.14±0.004ab	27.51±1.00a
P4	44.8±0.12b	1.70±0.02a	0.17±0.01a	0.56±0.04c	0.48±0.02a	0.12±0.003b	27.89±0.22a
P5	44.3±0.36b	1.89±0.07a	0.2±0.003a	0.68±0.05bc	0.36±0.01b	0.14±0.01ab	25.46±1.13a
Origin (P>F)	0.0001	0.495	0.406	0.004	0.012	0.0001	0.497

Within a column, values with different letters are significantly different ($P<0.05$; Tukey's test)

variations in abundance of ECM fungi among Scots pine seedlings of different origin, under the natural conditions of a forest nursery.

Our study's most essential finding is the shift in dominance from an ascomycetous fungus like *W. mikolae* in P2 and P4 seedlings to basidiomycetous Suilloid species like *S. luteus* and *S. variegatus* in P1 and P5 seedlings. Previous experiments with Scots pine seedlings showed that northern populations are characterized by earliest growth cessation and higher carbon allocation below ground than central populations (Lundeberg 1968; Oleksyn et al. 1992). This difference may be one reason that the most northern population, P1, hosted the highest quantity of Suilloid mycorrhizas. It is generally assumed that ECM morphotypes with high fungal biomass in the mantle sheathing of the short roots (e.g., Suilloid mycorrhizas) require more carbon from the host tree than do thin-mantled and smooth types with less external mycelium (e.g., *W. mikolae*; Colpaert et al. 1992; Gorissen and Kuyper 2000; Saikkonen et al. 1999). It is worthy of note that the abundance of Suilloid mycorrhizas may be regulated not only by the internal status of host plant nonstructural carbohydrates but also by external delivery of highly complicated carbon polymers from forest litter (Aučina et al. 2007).

We used the NMDS and ANOSIM analysis to evaluate the influence of pine seed source on ECM fungal communities. The NMDS ordinations of the mycorrhizal communities showed partial separation based on different Scots pine origins (Fig. 1). The most separated are sample points for P5 origin, and the P2 and P3 origins in the NMDS diagram are scattered and not well defined. Despite the apparent overlap in the NMDS, the ANOSIM showed a significant difference between pine origins (global $R=0.4748$, $P<0.0001$, permutation=10,000) and for all pairwise origins ($R=0.2372$–0.7952, P always <0.0001; detailed results not presented).

Along with variation in quantitative and qualitative traits of ECM community structure, we found significant differences between Scots pine origin in seedling height, root dry

Table 5 Spearman's rank correlations between relative abundance of ectomycorrhizal morphotypes and growth parameters and foliar nutrient composition of *Pinus sylvestris* seedlings (data presented only for morphotypes for which significant correlations were noted)

Growth parameter, nutrient	ECM type					
	Suilloid type			Wilcoxina type		
	R	*t*(N-2)	*P*	*R*	*t*(N-2)	*P*
Height	−0.03	−0.24	0.809	−0.18	−1.54	0.128
Roots dw	0.09	0.81	0.419	−0.04	−0.36	0.724
Stem dw	−0.17	−1.46	0.150	0.14	1.21	0.229
Needles dw	−0.04	−0.34	0.735	−0.01	−0.10	0.918
Survival	0.29	2.57	0.012	−0.42	−3.95	0.0002
C	0.32	2.85	0.006	−0.21	−1.83	0.071
N	0.24	2.08	0.041	−0.23	−2.06	0.042
P	0.03	0.23	0.818	−0.12	−1.05	0.297
K	0.27	2.35	0.021	−0.19	−1.64	0.106
Ca	0.07	0.57	0.571	−0.03	−0.25	0.803
Mg	0.58	6.10	0.0001	−0.61	−6.63	0.0001

dw dry weight

weight, and survival rate (Table 3), without a significant influence of block (two-way ANOVA, $P = 0.183$, 0.232, and 0.324, respectively). Seedling height was significantly lower for P4 (most eastern origin) in comparison to the other pine origins. This finding is in accordance with previous provenance studies indicating that the best-growing races of Scots pine originate from the lowlands of Central Europe, particularly from northern and western Poland and from the Baltic countries. On the other hand, easternmost provenances are associated with plants that are less reliable and adaptable to new sites (Giertych 1991). The dry weight of roots was significantly higher for seedlings from origin P1 than P5. The survival rate of seedlings was highest for P5, intermediate for P1 and P3, and lowest for P2 and P4. These results indicate that one of the best origins in our studies is P5, from Rychtal in Poland. In many other respects, this origin is associated with superior Polish Scots pine provenances (Giertych 1991).

Tukey's HSD test revealed higher significant differences in the concentration of C, K, Ca, and Mg in the needles of seedlings from different origins (Table 4). The highest concentration of C was noted for P1 and P3. The K and Mg concentrations were highest in seedlings of P1 origin, whereas the Ca concentration was greatest in P3 and P4 seedlings. Block effect was significant only for Mg concentration (two-way ANOVA, $P = 0.008$).

No significant relationships between growth parameters of seedlings (height, stem, needle, and root dry weight) and abundance of observed mycorrhizal fungi were found (Spearman rank correlation, Table 5). Though in our studies heritability was not estimated, such results may suggest that the observed differences in seedling height and root biomass are under control of the host genotype. This assumption is supported by laboratory and field experiments with diverse European Scots pine populations that clearly showed that height, diameter, and biomass were all significantly related to latitude of origin and thus tree genotype (Giertych 1979; Giertych and Oleksyn 1981, 1992; Oleksyn et al. 1992, 1999). In contrast to the growth parameters, pine seedling survival was significantly positively correlated with abundance of Suilloid mycorrhizas and significantly negatively linked with *W. mikolae* relative abundance (Table 5). A positive rank correlation was found between all macroelement concentrations (significant for C, N, and K) and abundance of Suilloid mycorrhizas. Negative rank correlation was found between tested macroelement concentrations and abundance of *W. mikolae* mycorrhizas (significant for N and Mg).

However, the results from stepwise multiple regression analysis showed that only survival and magnesium content in pine needles were significantly correlated with abundance of ECM fungi ($R^2 = 0.36$, $p < 0.001$ and $R^2 = 0.32$, $p < 0.01$, respectively). In case of seedlings survival, significant components of beta were only found for abundance of Suilloid mycorrhizas ($\beta = 0.35$, $p < 0.001$) and Suilloid/*Wilcoxina* mycorrhizas ($\beta = -0.4$, $p < 0.001$). The abundance of Suilloid mycorrhizas was the only variable that was a significant predictor for magnesium content in pine needles ($\beta = 0.56$, $p < 0.001$). The greater survival of seedlings with abundant Suilloid mycorrhizas may be connected with the morphological appearance of this morphotype, including thick mantle, abundant fan-shaped extramatrical hyphae, and highly differentiated hydrophobic rhizomorphs. *W. mikolae*, which forms smooth mycorrhizae, devoid of external structures, is evidently less efficient in water and nutrient absorption.

As with all research, the questions tested led to essential cause-and-effect results, further demonstrating that the system in question (tree genotypes and ECM community in nursery common-garden experiment) is complex and will require additional investigations. A key outcome of this study was that Scots pine seedlings of different origins, even from a not very expansive area, are characterized by different community structure of ECM fungi under forest nursery condition. This result may have implications for understanding the physiological and genetic relationship between the host tree and fungi and should be considered in management decisions in forestry and ECM fungus inoculation programs.

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