SILVA FENNICA

Silva Fennica 42(4) research articles

www.metla.fi/silvafennica · ISSN 0037-5330
The Finnish Society of Forest Science · The Finnish Forest Research Institute

Variation of Ectomycorrhizal Colonisation in Norway Spruce Seedlings in Finnish Forest Nurseries

Enni Flykt, Sari Timonen and Taina Pennanen

Flykt, E., Timonen, S. & Pennanen, T. 2008. Variation of ectomycorrhizal colonisation in Norway spruce seedlings in Finnish forest nurseries. Silva Fennica 42(4): 571–585.

Ectomycorrhizal (ECM) colonisation patterns and seedling growth of containerised spruce seedlings were studied in five typical Finnish forest nurseries by morphotyping and molecular characterisation. ECM colonisation degree of 1-year-old spruce seedlings was below 20% in all studied Finnish forest nurseries. In 2-year-old spruce seedlings the ECM colonisation degree was ca. 50–60% in three of the nurseries, but negligible in others. The ECM fungal species richness varied from 0.1 to 3.8 types per seedling. Altogether seven ECM morphotypes were distinguished. The clearest factors associated with ECM colonisation patterns were nitrogen and phosphorus fertilisation. Particularly fertilisation in the early stage of seedling development appeared to diminish the degree of colonisation and species richness of ECM fungi. Root/shoot ratio was positively correlated with high colonisation degree and species richness of ECM fungi. Higher fertilisation inputs in these overall fertilisation levels did not increase the size of the seedlings. According to these results moderate fertilisation levels particularly in the beginning of seedling cultivation are critical for generating higher root/shoot ratios and sufficient ECM colonisation degree of the roots.

Keywords containerised seedlings, ectomycorrhiza, fungal diversity, nursery, *Picea abies*
Addresses *Flykt* and *Timonen*, University of Helsinki, Department of Applied Chemistry and Microbiology, P.O. Box 27, FI-00014 University of Helsinki, Finland; *Pennanen*, Finnish Forest Research Institute, Vantaa Research Unit, P.O. Box 18, FI-01301 Vantaa, Finland
Received 25 February 2008 **Revised** 4 June 2008 **Accepted** 5 June 2008
Available at http://www.metla.fi/silvafennica/full/sf42/sf424571.pdf

1 Introduction

Every year more than 100 million spruce (*Picea abies* L.) seedlings are produced for planting in commercial nurseries in Finland (Finnish Statistical Yearbook of Forestry 2006). Of these almost 30% are lost within ten years after planting (Rikala 1994). The reasons for seedling death and aborted growth during the first years after planting are not well known. In Finland 98% of the planted spruce seedlings are produced in small containers in nurseries and planted after one or two growing seasons (Finnish Statistical Yearbook of Forestry 2006).

The current nursery culturing methods favour use of relatively high amounts of soluble fertilizers as well as application of biocides. Many ectomycorrhizal (ECM) fungal species are adapted to living in environments with low nutrient levels and particularly elevated amounts of soluble nitrogen have been shown to reduce degree of ECM colonisation and reduce the diversity of ECM species (Alexander and Fairley 1983, Arnolds 1991, Arnebrant and Söderström 1994, Kåren and Nylund 1997). The effect of different fungicides on different ECM fungi varies. E. g. propiconazole, a fungicide registered for use in growing forest tree seedlings, has been shown to inhibit growth of several ECM fungi (Laatikainen and Heinonen-Tanski 2002). Many ECM fungi are also sensitive to flooding and may be adversely affected by the high moisture conditions commonly occurring in nurseries (Stenström 1991). As a result of the current growing practices, it is possible that the ECM colonisation of tree seedlings in the nurseries is severely hampered. Some of the ECM fungi shown to tolerate high levels of nutrients and moisture such as *Hebeloma crustuliniforme* and *Thelephora terrestris* may not be unequivocally beneficial (Perry et al. 1987, Landis 1989, Stenström et al. 1990, Stenström 1991)

ECM colonisation has been shown to be crucial for the survival of the seedlings in the forest sites, because mycorrhizal fungi are able to enhance nutrient and water uptake and inhibit the influence of the pathogenic factors (e. g. Mikola 1973, Duchesne et al. 1989, Schelkle and Peterson 1996, Garbaye and Churin 1997, Werner and Zadworny 2003). ECM inoculations have repeatedly been proven to have beneficial effects on seedling growth and survival after planting, but the results have not always been so straightforward (Stenström and Ek 1990, Stenström et al. 1990, Le Tacon et al. 1992, Garbaye and Churin 1997). Depending on the plant, growth conditions and the properties of the fungal species, the influence of mycorrhizal colonisation on plant growth varies. In an outplanting study of Scots pine the seedlings colonised by different ECM fungi were clearly smaller at the outplanting time, but some ECM species promoted seedling growth so efficiently that after two growing seasons they were as large or much larger than the control seedlings (Stenström and Ek 1990). However, this was not the case with all ECM fungi as some were shown not to have a growth promoting effect (Stenström et al. 1990). Coinoculations therefore have been speculated to benefit the seedling more than single species inoculation (Mikola 1973, Trappe 1977, Kernaghan et al. 2003). Parlade and Alvarez (1993) have also shown promoted Douglas fir growth with coinoculation of many different ECM fungi on peat-vermiculite substrate.

No reports are currently available about the mycorrhizal status of Norway spruce seedlings in commercial Finnish nurseries. The knowledge about ECM fungal colonisation of Norway spruce is primarily from bare root nurseries where the growing conditions are quite different from those of containerised seedlings (Rudawska 2006, Trocha 2006). Our aim was to examine the mycorrhizal status of containerised spruce seedlings in five Finnish nurseries with different management practices. Based on earlier studies we hypothesized that due to extensive fertilization and use of fungicides both ECM colonisation degree and species richness of ECM fungi would be relatively poor (e. g. Lehto 1989, Kernaghan et al. 2003, Rudawska et al. 2006). We also wanted to examine the ECM fungal species richness, since the proved persistence of the nursery ECM strains after outplanting emphasizes the role of ECM inoculum in the future development of the nursery seedlings (Pennanen et al. 2005).

2 Materials

2.1 Plant Material

Five commercial seedling nurseries from three different breeding zones (Nikkanen et al. 1999) were selected for the survey on the basis of the nurseries having detailed and available records of their seedling growing programs. Spruce seedlings of two age classes, 1-year-old (16 months) and 2-year-old (27 months), were examined from each nursery. 1-year-old seedlings had been grown in 85-cm^3 containers size (PL-81F, Lännen Oyj., Iso-Vimma, Finland) and 2-year-old seedlings in 110-cm^3 containers (PL-64F, Lännen Oyj., Iso-Vimma, Finland) in processed white sphagnum-peat (Kekkilä M6: H 1-3 von Post, limed with 2 kg/m^3 dolomite lime and fertilized with 0.8 kg/m^3 Kekkilä starter fertilizer 6). The peat contained 16-8-16 added NPK respectively and press water pH was 4.3. This widely used premixed fertilizer contains on average 18 mg N and 10 mg P per seedling the exact amount depending on planting technique (Juntunen and Rikala 2001). All nurseries had different fertilisation (Table 1, Fig. 1) and biocide programs (Table 2). However, the used growth media was white Sphagnum peat as in all the Finnish nurseries and the liquid fertilizers were the same in all the studied nurseries. 80% of the Finnish nurseries use the same products for fertilization. In general, the range between fertilization programs in Finnish nurseries is great as some nurseries start the application of liquid fertilizers 11 days and some nurseries 77 days after sowing (Juntunen and Rikala 2001). Thus the five nurseries included in our study represent well the used nursery practices in Finland. Biocides applied in the nurseries were merged into five functional groups: fungicides for grey mold control, other fungicides, insecticides, herbicides, control of liverworts (Table 2). Biocides were applied in accordance with the recommended rates given in the product labels in the different nurseries (Juntunen 2001). 20 seedlings were collected from each nursery in September 2003. Pre examination of seedlings in the nurseries did not show difference in the moisture conditions or root systems of the seedlings at the different locations in the benches of container trays or in the individual containers in the trays. However, to avoid location effect, one seedling was randomly taken from each seedling tray across the bench of trays (10 trays per bench) parallel to the mobile boom sprayers. Altogether two sets of ten seedlings were collected. The seedlings were put into plastic bags and stored in +5 °C. Within one week from sampling roots were gently washed with cold tap water and minimum of 20 pieces of ca.1-cm root samples with at least 250 mycorrhizal tips were cut from each seedling for stereomicroscopic investigation.

2.2 Characterisation of ECM

All root tips were examined by stereomicroscope (Leica MZFLIII or Leica MZ6). Mycorrhizal root tips were grouped into seven morphotype classes (MT1–7) according to gross characteristics of their outer mantles (Agerer 1987–1997).

Table 1. Fertilisation parameters in different nurseries. Results are expressed per seedling.

Seedling age	Nursery	Tot N mg	NO_3-N mg	NH_4-N mg	Urea-N mg	P mg	K mg
1	1	51.7	23.6	5.5	22.6	23.5	78.1
	2	30.9	14.5	3.3	13.0	19.2	53.9
	3	24.5	10.9	2.1	11.5	11.0	46.7
	4	31.1	11.8	2.9	16.4	7.2	33.1
	5	53.6	20.6	32.9	0.0	36.9	57.9
2	1	92.6	41.8	8.8	42.1	32.7	108.9
	2	36.2	24.3	5.0	6.9	20.7	78.1
	3	33.0	17.8	2.6	12.7	18.7	54.0
	4	46.7	17.7	4.4	24.6	10.8	49.7
	5	48.3	20.1	28.2	0.0	31.6	56.4

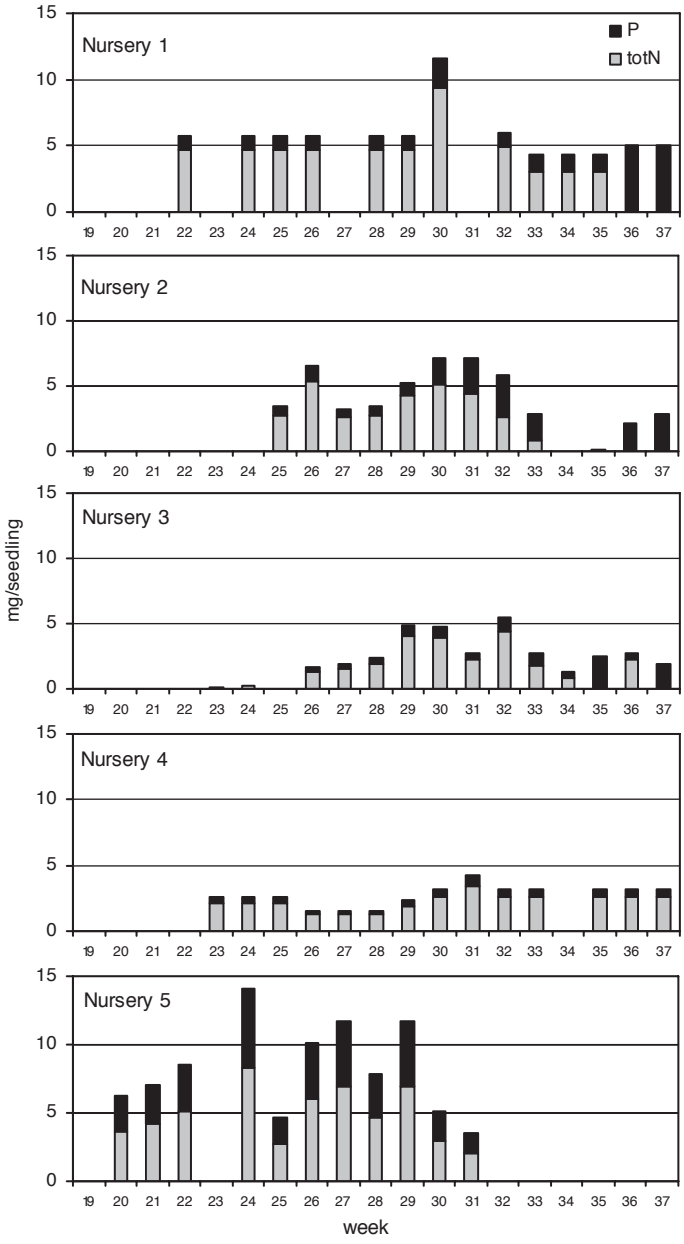

Fig. 1a. Fertilisation program of 1-year-old seedlings in the different nurseries.

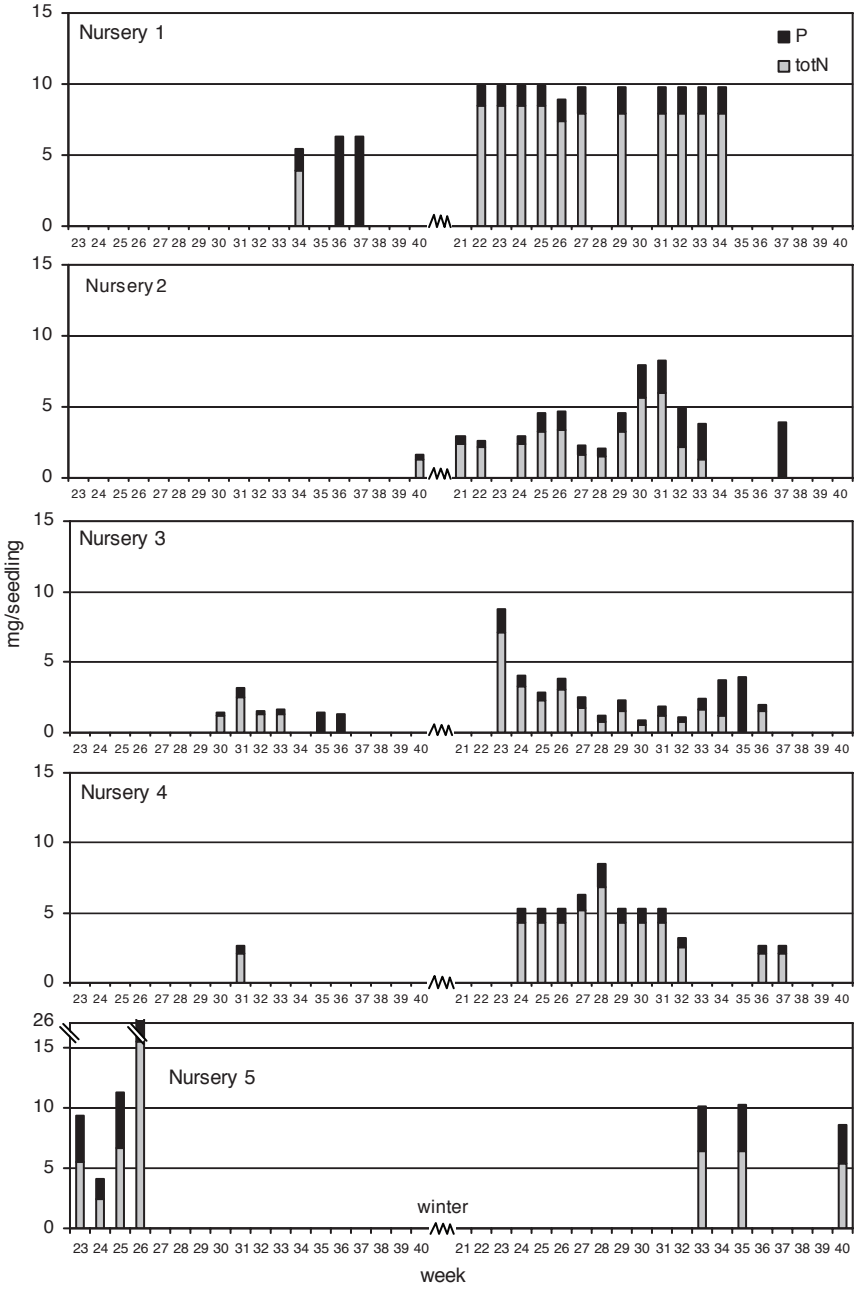

Fig. 1b. Fertilisation program of 2-year-old seedlings in the different nurseries.

Table 2. Biocides used at the different nurseries.

Applied biocides	Active ingredient	Nurseries									
		1		2		3		4		5	
		1-y	2-y	1-y	2-y	1-y	2-y	1-y	2-y	1-y	2-y
Fungicides for grey mold											
Euparen M [b)]	tolylfluanid	x	x	x		x	x				
Rovral [a)]	iprodione									x	x
Scala	pyrimethanil									x	
Teldor	fenhexamid										x
Topsin M [a)]	thiophanate-methyl	x	x					x	x		
Other fungicides											
Benlate	benomyl	x									
Bayleton25	triadimefon								x		
Tilt 250 EC [a)]	propiconazole						x			x	x
Bravo 500	chlorothalonil										x
Insecticides											
Metasystox R [a)]	oxydemeton-methyl			x	x	x	x				
Roxion [a)]	dimethoate			x	x						
Decis 25 EC [a)]	deltamethrin									x	x
Gori 920	permethrin						x				
Herbicides											
Gardoprim	triazine	x	x								
Matrigon	clopyralid		x								
Control of liverworts											
Mogeton WP [a)]	quinoclamine	x	x		x	x	x				

[a)] Products registered for forest nursery use
[b)] Product is registered for forest nursery use, but will be removed from register 31.10.2008

Examples of roots with unclear mycorrhizal status or morphotype were bleached and stained with trypan blue to visualise fungal structures (Phillips and Hayman 1970). 10–20 samples of all ECM morphotypes were stored in ethanol at –20 °C for molecular characterisation.

DNA extraction from ECM root tips was carried out according to Vainio et al. (1998). In short, single root tips were homogenised with a glass rod in Eppendorf tubes containing 8 µl of lysis buffer and quartz sand. Extracts were then incubated at 65 °C for 30 min. DNA was purified by 200 µl phenol/chlorophorm/isoamyl alcohol (25:24:1, v/v/v) and 200 µl chlorophorm/isoamyl alcohol (24:1, v/v). 20% Polyethylene glycol in 2.5 M NaCl was used for precipitation. Samples were incubated on ice for 20 min, centrifuged and resulting pellets were washed with 70% cold ethanol before drying. Dried DNA extracts were resuspended in TE buffer (6:1). The polymerase chain reactions (PCRs) were performed according to Anderson et al. (2003) on a PTC-100^{TM} (MJ Research, Inc.). Primer pair ITS1-F (Gardes and Bruns 1993) with a GC clamp 5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3' and ITS2 (White et al. 1990) were used in order to amplify partial ITS products of fungi. Success rate for PCR-amplification was verified on 1% agarose gels in 1× TAE buffer prior to denaturing gradient gel electrophoresis (DGGE) performed with D-GENE gel system (Bio-Rad, Hercules, USA) as described in Korkama et al. (2006). Altogether, amplification products of 104 ECM root tips were separated in DGGE gels. Bands showing different mobilities were processed for sequencing as in Korkama et al. (2007). In short, bands were excised and put into 100 µl of sterile water overnight. The water solution was used as a template in PCR as described by Anderson et al. (1993). PCR-DGGE cycles were continued 1–5 times and obtained single-banded samples were amplified (25 cycles), purified (High Pure PCR Product Purification Kit, Roche, Mannheim,

Germany) and sequenced with SEQ 8000 DNA analysis system using Quick Start Kit (Beckman Coulter Inc. USA). In many cases, direct sequencing from DGGE gels does not produce single-banded products or good quality sequences and thus when needed, representatives (1–3) of each fungal amplification product with different mobility were sequenced directly from PCR product as described by Korkama et al. (2006) or were cloned with pGEM-T® Easy Vector cloning kit (Promega Inc., Madison WI). For direct sequencing and cloning, PCR amplification was performed with ITS1F-ITS4 (White et al. 1990) primer pairs. Cloned sequences were sequenced at Haartman Institute Sequencing Unit (University of Helsinki, Finland).

2.3 Data Analysis

Acquired sequences were manually checked and edited using VectorNTI® Suite programme package, version 7, for PC (InforMax, Invitrogen, MD, USA) and compared to sequences in the Unite (Koljalg et al. 2005) and DDBJ/EMBL/GenBank databases with Blastn. The differences between ECM colonisation degrees were tested by Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) and post hoc analysis by Dunn's multiple comparisons test (InStat package, GraphPad Software Inc., San Diego, CA, USA). Relative abundances of ECM morphotypes were subjected to Detrended Correspondence Analysis (DCA) using PC-ORD, version 5 (McCune and Mefford 2006). The environmental matrix consisted of measured characteristics of the seedlings and amounts of applied nutrients and biocides.

3 Results

The ECM colonisation degree of 1-year-old seedlings in the different nurseries varied from 0% to 19.4% (Fig. 2). Although the ECM colonisation degree in nurseries 1 and 5 was neglible and 16% in nurseries 2–4 the differences were not statistically significant due to extensive variation in colonisation between individual samples. In 2-year-old seedlings the average ECM colonisa-

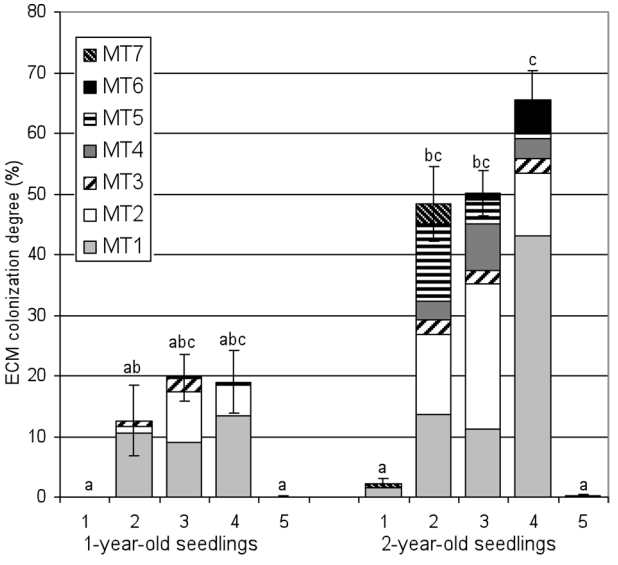

Fig. 2. Average ectomycorrhizal (ECM) colonisation degrees of the containerised spruce seedlings in the five different nurseries. Mycorrhizal morphotype (MT). Statistically significant differences between columns are shown with different letters above the columns. Error bars show SE.

Table 3. Fungal sequences retrieved from typical ectomycorrhizal morphotypes observed in spruce seedlings.

Class	Morphotype description	Accession no	Closest GenBank match	Identities	Similarity	E value
MT1	Light smooth mantle	EU427323	*Thelephora terrestris*, DQ068970	630/630	100%	0.0
MT2	Light scarce mantle, fluffy external hyphae	EU427325	*Laccaria proxima*, AY750156	667/672	99%	0.0
		DGGE match [a)]	*Laccaria laccata*, UDB000106	667/668	99%	0.0
		EU427324	*Tylospora asterophora*, AF052557	620/626	99%	0.0
MT3	White mantle, rhizomorphs	EU427326	*Hebeloma velutipes*, AF430254	615/619	99%	0.0
MT4	Grayish mantle, yellow rhizomorphs	EU427327	*Amphinema byssoides*, AY838271	569/573	99%	0.0
		EU427328	Uncultured ECM fungus, EF521222	525/541	97%	0.0
MT5	Dark brown/black mantle, white root tip	EU427329	*Hymenoscyphus* sp., AJ292200	473/479	99%	0.0
MT6	Chocolate brown smooth mantle	EU427330	*Thelephora terrestris*, DQ068970	634/634	100%	0.0
MT7	Black mantle with extensions	EU427331	*Cenococcum geophilum*, AY394919	533/533	100%	0.0

[a)] Identical single DGGE band with culture collection Laccaria laccata F-NC02

Table 4. Growth parameters and ectomycorrhizal status in different nurseries. Results are expressed per seedling. Values followed by different letters are statistically significantly different. The values have been compared within each age class.

Seedling age	Nursery	Shoot height cm	Total DW g	Root/shoot ratio	ECM ECM fungal species richness
1	1	17.9 ± 0.79 a	0.7 ± 0.07 a	0.55 ± 0.048 b	0.0 a
	2	23.0 ± 1.35 b	1.4 ± 0.10 b	0.50 ± 0.024 ab	1.1 ± 0.31 ab
	3	19.3 ± 0.66 ab	1.6 ± 0.11 b	0.63 ± 0.039 b	2.0 ± 0.30 b
	4	22.5 ± 1.32 b	1.4 ± 0.17 b	0.59 ± 0.055 b	1.5 ± 0.43 b
	5	20.3 ± 0.39 ab	1.7 ± 0.05 b	0.37 ± 0.025 a	0.1 ± 0.10 a
2	1	21.2 ± 1.20 a	1.7 ± 0.20 a	0.45 ± 0.025 ab	0.4 ± 0.31 a
	2	22.2 ± 1.18 a	2.1 ± 0.15 ab	0.67 ± 0.050 c	3.8 ± 0.36 b
	3	25.0 ± 0.73 ab	2.3 ± 0.13 ab	0.62 ± 0.033 bc	3.7 ± 0.40 b
	4	26.2 ± 1.73 ab	3.0 ± 0.34 bc	0.62 ± 0.049 bc	3.6 ± 0.37 b
	5	31.0 ± 0.85 b	4.1 ± 0.33 c	0.31 ± 0.018 c	0.1 ± 0.10 a

± Standard error, n = 10

tion degree was clearly higher than in 1-year-old seedlings varying from 0 to 66%. ECM colonisation degree of spruce roots in nurseries 2–4 was statistically significantly higher than that in nurseries 1 and 5 where the ECM colonisation degree remained under 5%.

Seven morphotypically distinct types of ECM root tips were found in the 30 789 analysed mycorrhizal tips (Table 3, Fig. 2). The number of operational taxonomical units (OTUs) observed in DGGE gels was ten. MTs 1 and 6 of light and dark mantled ECM respectively, were both formed by

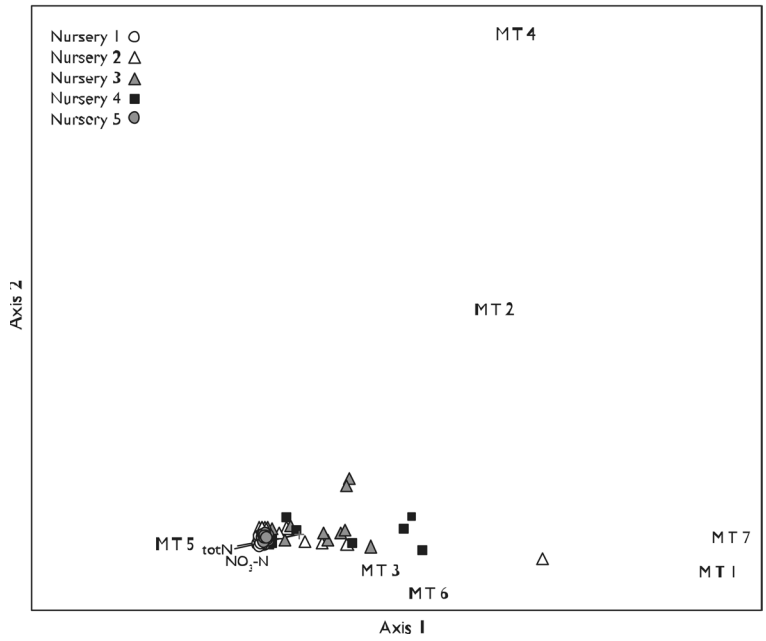

Fig. 3a. Detrended Correspondence Analysis (DCA) graph of 1-year-old seedlings and their relation to different fertilisers and biocides (eigenvalue axis 1 = 0.243, axis 2 = 0.087, variance = 0.404). Only the meaningful environmental factors are marked on the figure. Mycorrhizal morphotype (MT).

genetically identical *Thelephora terrestris* strains (Table 3). The same sequence was also detected from two distinct OTUs on DGGE gels. MT 2 ECM root tips contained mostly *Laccaria* spp. of two different kinds, but two out of ten contained also *T. terrestris* and three tips contained *Tylospora* sp., *Hebeloma* sp. and *Amphinema byssoides*. MTs 3, 4, 5 and 6 consisted of one species each (Table 3). However, two different strains of *Amphinema byssoides* were found in MT 4. Both *T. terrestris* and *Laccaria spp.* fruiting bodies were observed in the nurseries.

The total number of detected ECM fungal species increased from the ca. three species in the 1-year-old seedlings to ca. six fungal species in the 2-year-old seedlings in nurseries 2–4. In these nurseries the average ECM fungal species richness in individual 1-year-old seedlings varied between one and two and in the 2-year-old seedlings it remained just below four (Table 4). In the nurseries with low ECM fungal colonisation degree also the average species richness remained below one in both types of seedlings.

The total dry weight of both 1-year-old and 2-year-old seedlings was statistically significantly smallest in Nursery 1 (Table 4). The root/shoot ratio was well below one in all seedlings regardless of their age or nursery. The lowest root/shoot ratios were found in Nursery 5. The high amount of total N and nitrate in the nurseries appeared to have most effect on mycorrhizal status of 1-year-old seedlings (Fig. 3a). Particularly ECM fungal species richness reacted adversely to increasing N concentrations. MT2 and MT4 separated along axis 2 because the occurrence of these morphotypes was higher in the nursery 3 compared to the other nurseries. The clearest explaining factors for ECM colonisation patterns of 2-year-old seedlings originating from the nurseries 1 and 5 were nitrogen and phosphorus fertilisation (Fig. 3b). Occurrence of all morphotypes was relatively even among the nurseries 2, 3 and 4. However, MT6 was not found at all at the nursery 4 and it was the only ECM morphotype found at Nursery 5. Nevertheless, the applied biocides with the exception of insecticides also correlated positively

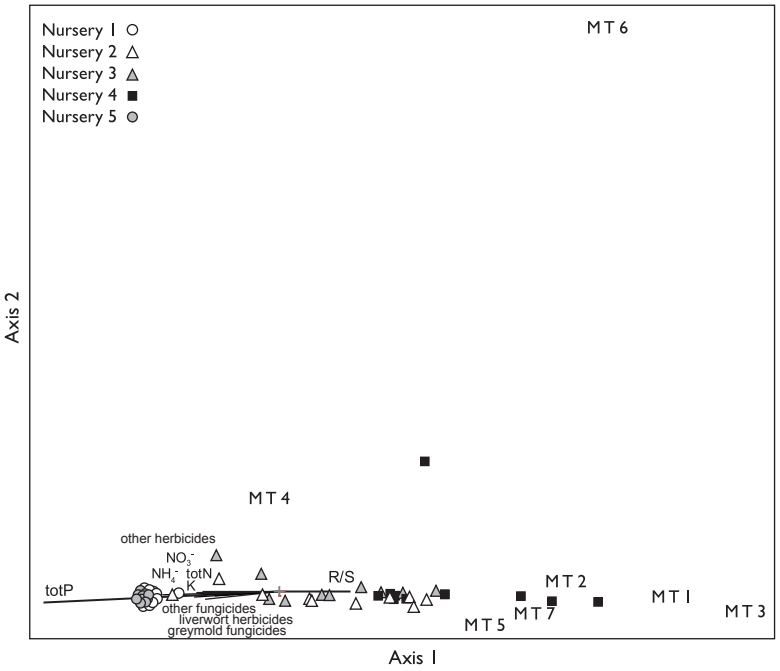

Fig. 3b. Detrended Correspondence Analysis (DCA) graph of 2-year-old seedlings and their relation to different fertilisers and biocides (eigenvalue axis 1 = 0.392, axis 2 = 0.174, variance = 1.245). Only the meaningful environmental factors are marked on the figure. Mycorrhizal morphotype (MT).

with seedlings without ECM fungal colonisation. Both colonisation degree and species richness of ECM fungi was low in the seedlings associated with high amounts of nutrients and biocides. Interestingly the root/shoot ratio increased along with increasing ECM colonisation degree.

4 Discussion

The aim of nurseries is to produce viable seedling material. One parameter used for tree seedling viability in Canada and USA is the ECM colonisation degree of the seedlings (Castellano and Molina 1989). Mycorrhizal inoculation is known to affect to the adaptation ability of tree seedlings to the regeneration area particularly in the case of exotic species (e. g. Mikola 1970, Garbaye and Churin 1996, Duñabeitia et al. 2004, Menkis et al. 2007). However, intensive use of fertilizers and biocides in nurseries may result in low ECM colonisation degree. One technique to combine mycorrhizal inoculations with relatively high fertilisation level is to use exponential fertilizer delivery (Quoreshi and Timmer 1998, Quoreshi and Timmer 2000). This means that high nutrient additions early in the growing season should be avoided and nurseries should progressively acclimatize ECM fungi to higher nutrient inputs. Two of the five Finnish nurseries (nurseries 1 and 5) surveyed in this study started fertilisation already in May and had higher fertilisation inputs than the other nurseries. These two nurseries also had lowest colonisation degree and species richness of ECM fungi. In practise, mycorrhizas were almost totally absent in these two nurseries, even on 2-year old seedlings. It has been estimated that 50% is the minimum degree of ECM colonisation that significantly increases survival and growth of planted seedlings (Marx et al. 1982). Considering this, the ECM colonisation degree in the studied nurseries was rather poor. The average ECM colonisation degree of the 1-year old seedlings was 20% or less and exceeded 50%

only in the 2-year old seedlings in one nursery. This result is very similar to the nursery survey performed by Lehto (1989) in which she reported that the ECM colonisation degree for containerized Scots pine seedlings was 54%.

Biocides appeared also to have an effect on ECM colonisation efficiency. Many fungicides are selective for pathogenic fungi of which many belong to different phylogenetic group than most ECM, e.g. to Zygomycetes. However in general, biocides can cause multitude of complex reactions on target and also on non-target organisms (Castellano and Molina 1989). Because the selection of used biocides was highly variable, for example there were several biocides which were used only in one nursery, we combined the biocides into five functional groups. The use of fungicides and herbicides were negatively correlated with ECM status of the seedlings. Only the use of insecticides did not seem to affect seedlings. Aleksandrowicz-Trzcinska (2000) reported that the use of fungicides triadimefon, thiophanate-methyl and chlorothalonil on Scots pine seedlings had no effect on root colonisation by *Hebeloma crustuliniforme*, whereas tolylfluanid decreased the presence of this ECM strain in the roots. Nevertheless, all the abovementioned fungicides inhibited the mycorrhizal colonisation of roots by other naturally emerging ECM fungi in control treatments without fungicides. Chlorothalonil and propiconazole inhibited ECM fungal growth most efficiently when a suite of common ECM fungi were tested (Laatikainen and Heinonen-Tanski 2002). However, there was large variation of responses between the different fungal species as e. g. growth of *Cenococcum geophilum* appeared to be stimulated by propiconazole. The high amounts of nutrients were also associated with the low ECM status and low root/shoot ratio of the seedlings. As both high fertilisation levels and fungicides are simultaneously used at commercial nurseries and both had similar effects on the ECM status of the roots it is impossible to properly separate the effect of biocides from the effect of the amounts of nutrients.

All the ECM taxa observed in this study have commonly been observed in both European and North American nurseries (Lehto 1989, Stenström et al 1990, Tammi et al. 2001, Kernaghan et al. 2003, Menkis et al. 2005, Trocha et al. 2006). *Thelephora terrestris* was the most commonly observed ECM fungal species in spruce roots. This agrees with the ability of *Thelephora* spp. to dominate under conditions of poor aeration, water-logging and high fertilization rates (Castellano and Molina 1989, Kernaghan et al. 2003). Although *Thelephora* spp. are adapted well to the nursery conditions and also commonly colonise Norway spruce roots many years after transplanting (Korkama et al. 2006) they may not be the optimal ECM inoculum for good seedling establishment and growth in the field. *Thelephora terrestris* was present in all the ECM fungal combinations in the Scots pine experiment where ECM fungal inoculation failed to promote tree growth after transplanting into the field (Stenström et al. 1991). *Thelephora terrestris* has also been tested as a single inoculum and with all the tested tree partners, with the exception of oak, it failed to promote the development of the seedlings (Castellano and Molina 1989). In a study of different Norway spruce clones *Thelephora terrestris* was more often associated with slow growing clones than fast growing clones. When compared with *Suillus bovinus Thelephora terrestris* was less efficient in N retrieval (Bending and Read 1995). Nevertheless, in a short term experiment of 52 days *Thelephora terrestris* was shown to promote growth and nutrient uptake particularly in drought conditions (Lehto 1992). The other ECM fungal species found in Finnish nurseries correlate with the species found also in Canadian studies about containerised nursery seedlings (Kernaghan et al. 2003). *Laccaria* spp. are commonly found in nurseries and are known for their ability for plant growth promotion at least with Douglas fir (Selosse et al. 2000). However, there is also a report of growth reduction as result of *Laccaria* spp. inoculation of Scots pine (Stenström et al. 1990). *Cenococcum geophilum* is known to be particularly important in improving drought resistance of the host (Pigott 1982, Jany et al. 2003). Menkis et al. (2007) reported that *C. geophilum* was beneficial for the growth of the Scots pine after planting and *Piceirhiza bicolorata*, a member of the *Hymenoschyphus*-aggregate, beneficial for development of Norway spruce. Both *C. geophilum* and *Tylospora* spp. appear to help the growth of Norway spruce seedlings in Finland (Pennanen et al. unpublished). In

a study by Fransson et al. (2000) these two ECM taxa were found to favor fertilized spruce forest sites compared to unfertilized control plots and thus it seems that their ability to stand higher nutrient concentrations enables their occurrence in nurseries. In general, persistence of nursery ECM fungi in the seedlings after outplanting implies that the role of ECM inoculum obtained already in the nurseries may be significant for the seedlings future development (Dahlberg 1990, Pennanen et al. 2005).

Root/shoot ratios were negatively correlated with fertilization levels but positively correlated with colonisation degree and species richness of ECM fungi. This is in contrast to the study by Quoreshi and Timmer (2000) where mycorrhizal inoculation had no effect on shoot/root ratio. Although Quoreshi and Timmer did not observe an effect in shoot/root ratio they noticed that nutrient uptake was increased significantly reflecting higher absorption capacity of nutrients by mycorrhizal root systems. Khasa et al. (2001) have suggested that similar size of the seedlings can be obtained by application of lower fertilizer rate (67%) and selected mycorrhizal fungi rather than 100% fertilizer rate without mycorrhizal fungi. This may explain the similar or higher total seedling dry weight in the nurseries with lower fertilisation, but higher ECM colonisation degree.

Shoot height of the seedlings was statistically different in two nurseries but variation was not related to the different amounts of nutrients used in the nurseries. This implies that the addition of large amounts of nutrients does not increase the height of the seedlings. However the largest amounts of nutrients in the studied nurseries (nurseries 1 and 5) were close to the regular amounts of nutrients used in most Finnish nurseries since on average, 46 mg and 75 mg N per seedling is applied for 1- and 2-year old spruce seedlings, respectively (Juntunen and Rikala 2001). In Sweden, Rytter et al. (2003) performed fertilization experiments for Norway spruce seedlings and concluded that it may be possible to reduce the customary use of fertilizers in Swedish forest nurseries by 50% without any major negative impact on plant size or nutrient status. Controlling of fertilization allowed the production of Norway spruce seedlings with large root/shoot ratios and they stated that large roots, at least in some circumstances, may be more important than a large shoot for future height growth development. Rytter et al. (2003) did not study ECM colonisation of the root systems, but our results show that high root/shoot ratio of the nursery seedlings is associated with higher ECM colonisation degree and ECM fungal species richness. Korkama et al. (2006) suggested that for growth of planted spruce seedlings, more important characters than size and morphology of the roots might be its function and activity, which are largely dependent on ECM community structure (Agerer 2001). Thus it seems evident that both size of the root system and ECM colonisation are the key parameters behind the performance of the planted spruce seedlings and these aspects should be taken into account in nursery cultivation.

5 Conclusions

The nursery spruce seedlings cultivated by common practises had usually at least some ECM colonisation in their root systems. However, the average ECM colonisation degree of 1-year old seedlings was clearly lower than the recommended 50% in all the studied nurseries. For 2-year old seedlings the situation was a bit better with ECM colonisation degree of approximately 50% in three of the five nurseries. According to this study moderate rather than high overall fertilisation levels are adequate for good seedling growth. Restrained fertilisation doses have the additional value of cost efficiency and preserving the mycorrhizal potential of the roots. Nurseries should particularly avoid using high amounts of nutrients in the beginning of cultivation and progressively acclimatize ECM fungi to higher nutrient inputs.

Acknowledgements

Finnish Forester Foundation is thanked for financial support. We warmly thank the nurseries for collaboration in this project. We are also grateful to Marja Poteri and the anonymous referees for their helpful comments to the manuscript.

References

Agerer, R. 1987–1997. Colour atlas of ectomycorrhizae. Einhorn-Verlag, Germany, Schväbisch Gmund, Germany.

— 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11: 107–114.

Aleksandrowicz-Trzcinska, M. 2000. The effect of fungicides on mycorrhizal and non-mycorrhized seedlingd of Scots pine II. The formation of mycorrhizae. Folia Forestalia Polonica. Series A: Forestry 42: 15–25.

Alexander, I.J. 1983. Effects of N fertilization on populations of fine roots and mycorrhizas in spruce humus. Plant and Soil 71: 49–53.

Anderson, I.C., Campbell, C.D. & Prosser, J.I. 2003. Diversity of fungi in organic soils under a moorland – Scots pine (Pinus sylvestris L.) gradient. Environmental Microbiology 5: 1121–1132.

Arnebrant, K. 1992. Effects of different fertilizer treatments on ectomycorrhizal colonization potential in 2 Scots pine forests in Sweden. Forest Ecology and Management 53: 77–89.

Arnolds, E. 1991. Decline of ectomycorhizal fungi in Europe. Agricultural Ecosystems and Environment 35: 209–244.

Bending, G.D. & Read, D.J. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. New Phytologist 130: 401–409.

Castellano, M.A. & Molina, R. 1989. Mycorrhizae. In: Landis, T.D., Tinus, R.W., McDonald, S.E. & Barnett, J.P. (eds.). The container tree nursery manual, Volume 5. Agricultural Handbook 674. U.S. Department of Agriculture, Forest Service, Washington, DC. p. 101–167.

Dahlberg, A. 1990. Effect of soil humus cover on the establishment and development of mycorrhiza on the containerized pinus sylvestris L. and pinus contorta ssp. latifolia Engelm. after outplanting. Scandinavian Journal of Forest Research 5: 103–112.

Duchesne, L.C., Ellis, B.E. & Peterson, R.L. 1989. Disease suppression by the ectomycorrhizal fungus Paxillus involutus: contribution of oxalic acid. Canadian Journal of Botany 67: 2726–2730.

Duñabeitia, M., Rodríguez, N., Salcedo, I. & Sarrionandia, E. 2004. Field mycorrhization and its influence on the establishment and development of the seedlings in a broadleaf plantation in the Basque Country. Forest Ecology and Management 195: 129–139.

Finnish Statistical Yearbook of Forestry. 2006. Finnish Forest Research Institute, Vantaa Research Unit.

Fransson, P.M.A., Taylor, A.F.S. & Finlay, R.D. 2000. Effects of continuous optimal fertilization on belowground ectomycorrhizal community structure in a Norway spruce forest. Tree Physiology 20: 599–606.

Garbaye, J. & Churin, J.L. 1996. Effect of ectomycorrhizal inoculation at planting on growth and foliage quality of Tilia tomentosa. Journal of Arboriculture 22: 29–34.

— 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.

Gardes, M. & Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Jany, J.-L., Martin, F. & Garbaye, J. 2003. Respiration activity of ectomycorrhizas from Cenococcum geophilum and Lactarius sp. in relation to soil water potential in five beech forests. Plant and Soil 255: 487–494.

Juntunen, M.-L. 2001. Use of pesticides in Finnish forest nurseries in 1996. Silva Fennica 35: 147–157.

— & Rikala, R. 2001. Fertilization practice in Finnish forest nurseries from the standpoint of environmental impact. New Forests 21: 14–158.

Kårén, O. & Nylund, J.-E. 1997. Effects of ammonium sulphate on the community structure and biomass of ectomycorrhizal fungi in a Norway spruce stand in South West Sweden. Canadian Journal of Botany 75: 1628–1643.

Kernaghan, G., Sigler, L. & Khasa, D. 2003. Mycorrhizal and root endophytic fungi of containerized Picea glauca seedlings assessed by rDNA sequence analysis. Microbial Ecology 45: 128–136.

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

Korkama, T., Pakkanen, A. & Pennanen, T. 2006. Ectomycorrhizal community structure varies among Norway spruce (Picea abies L.) clones. New Phytologist 171: 815–824.

— , Fritze, H., Pakkanen, A. & Pennanen, T. 2007. Interactions between extraradical ectomycorrhizal mycelia, microbes associated with the mycelia and growth rate of Norway spruce (Picea abies) clones. New Phytologist 173: 798–807.

Laatikainen, T. 2002. Mycorrhizal growth in pure cultures in the presence of pesticides. Microbiological Research 157: 127–137.

Landis, T.D. 1989. Mineral nutrients and fertilization. In: Landis, T.D., Tinus, R.W., McDonald, S.E. & Barnett, J.P. (eds.). The container tree nursery manual. 4. Department of Agriculture, Forest Service, Washington DC. p. 1–67.

Le Tacon, F., Alvarez, I.F., Bouchard, D., Henrion, B., Jackson, R.M., Luff, S., Parlade, J.I., Pera, J., Stenström, E., Villeneuve, N. & Walker, C. 1992. Variations in field response of forest trees to nursery ectomycorrhizal inoculation in Europe. In: Read, D.J., Lewis, D.H., Fitter, A. & Alexander, I. (eds.). Mycorrhizas in ecosystems. CAB International, Wallingford, UK. p. 119–134.

Lehto, T. 1989. Mycorrhizal status of Scots pine nursery stock in Finland. Folia Forestalia 762. 15 p.

— 1992. Effect of drought on Picea sitchensis seedlings inoculated with mycorrhizal fungi. Scandinavian Journal of Forest Research 7: 177–182.

Marx, D.H., Ruehle, J.L., Kenney, D.S., Cordell, C.E., Riffle, J.W., Molina, R.J., Pawuk, W.H., Navratil, S., Tinus, R.W. & Goodwin, O.C. 1982. Commercial vegetative inoculum of Pisolithus tinctorius and inoculation techniques for development of ectomycorrhizae on container-grown tree seedlings. Forest Science 28: 373–400.

Menkis, A., Vasiliauskas, R., Taylor, A.F.S., Stenlid, J. & Finlay, R. 2005. Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. Mycorrhiza 16: 33–41.

— 2007. Afforestation of abandonned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi – impact on plant performance and mycorrhizal community. Mycorrhiza 17: 337–348.

Mikola, P. 1970. Mycorrhizal inoculation in afforestation. In: Romberger, J.A. (ed.). International review of forestry research. 3. Academic Press, London. p. 123–196.

— 1973. Application of mycorrhizal symbiosis in forestry practice. In: Marks, G. & Koslowski, T. (eds.). Ectomycorrhizae – their ecology and physiology. p. 383–411.

Nikkanen, T., Kariven, K., Koski, V., Rusanen, M. & Yrjänä-Ketola L. 1999. Kuusen ja männyn siemenviljelykset ja niiden käyttöalueet. [Seed cultivation of spruce and pine and their range of usage]. Finnish Forest Research Institute, Research Papers 730. 203 p.

Parlade, J. 1993. Coinoculation of aseptically grown Douglas-fir with pairs of ectomycorrhizal fungi. Mycorrhiza 3: 93–96.

Pennanen, T., Heiskanen, J. & Korkama, T. 2005. Dynamics of ectomycorrhizal fungi and growth of Norway spruce seedlings after planting on a mounded forest clearcut. Forest Ecology and Management 213: 243–252.

Perry, D.A. 1987. Mycorrhizae, mycorrhizospheres, and reforestation – current knowledge and research needs. Canadian Journal of Forest Research 17: 929–940.

Phillips, J.M. & Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158–160.

Pigott, C.D. 1982. Survival of mycorrhiza formed by Cenococcum geophilum Fr. in dry soils. New Phytologist 92: 513–517.

Quoreshi, A.M. & Timmer, V.R. 1998. Exponential fertilization increases nutrient uptake and ectomycorrhizal development of black spruce seedlings. Canadian Journal of Forest Research 28: 674–682.

Quoreshi, M. & Timmer, V.R. 2000. Growth, nutrient dynamics, and ectomycorrhizal development of container-grown Picea mariana seedlings in response to exponential nutrient loading. Canadian Journal of Forest Research 30: 191–201.

Rikala, R. 1994. Miksi taimet kuolevat – tarvitaanko tutkimusta? Metsäntutkimuslaitoksen tiedonantoja 496: 11–26.

Rudawska, M., Leski, T., Trocha, L.K. & Gornowicz, R. 2006. Ectomycorrhizal status of Norway spruce seedlings from bare-root forest nurseries. Forest Ecology and Management 236: 375–384.

Rytter, L., Ericsson, T. & Rytter, R.-M. 2003. Effects of demand-driven fertilization on nutrient use, root:plant ratio and field performance od Betula pendula and Picea abies. Scandinavian Journal of Forest Research 18: 401–415.

Schelkle, M. & Peterson, R.L. 1996. Suppression of common root pathogens by helper bacteria and ectomycorrhizal fungi in vitro. Mycorrhiza 6: 481–485.

Selosse, M.A., Bouchard, D., Martin, F. & Le Tacon, F. 2000. Effect of Laccaria bicolor strains inoculated on Douglas-fir (Pseudotsuga menziesii) several years after nursery inoculation. Canadian Journal of Forest Research 30: 360–371.

Stenstrom, E. 1991. The effects of flooding on the formation of ectomycorrhizae in Pinus sylvestris seedlings. Plant and Soil 131: 247–250.

— & Ek, M. 1990. Field growth of Pinus sylvestris following nursery inoculation with mycorrhizal fungi. Canadian Journal of Forest Research 20: 914–918.

— , Ek, M. & Unestam, U. 1990. Variation in field response of Pinus sylvestris to nursery inoculation with 4 different ectomycorrhizal fungi. Canadian Journal of Forest Research 20: 1796–1803.

Tammi, H., Timonen, S. & Sen, R. 2001. Ectomycorrhizal fungi and their root colonisation dynamics in rhizospheres of Scots pine seedlings grown in natural humus or nursery peat. Canadian Journal of Forest Research 31: 746–756.

Trappe, J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annual Review in Phytopathology 15: 203–222.

Trocha, L.K., Rudawska, M., Leski, T. & Dabert, M. 2006. Genetic diversity of naturally established ectomycorrhizal fungi on Norway spruce seedlings under nursery conditions. Microbial Ecology 52: 418–425.

Vainio, E.J., Korhonen, K. & Hantula, J. 1998. Genetic variation in Phlebia gigantea as detected with random amplified microsatellite (RAMS) markers. Mycological Research 102: 187–192.

Werner, A. & Zadworny, M. 2003. In vitro evidence of mycoparasitism of the ectomycorrhizal fungus Laccaria laccata against Mucor hiemalis in the rhizosphere of Pinus sylvestris. Mycorrhiza 13: 41–47.

White, T., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfaud, D.H., Sninsky, J.J. & White, T. (eds.). PCR protocols. A guide to methods and applications. Academic Press, San Diego. p. 315–322.

Total of 57 references