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Mycorrhization, Establishment and Growth of Outplanted *Picea abies* Seedlings Produced under Different Cultivation Systems

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In forest nurseries seedlings are commonly produced using different cultivation systems which may have a profound effect on the extent of root colonisation by ectomycorrhizal (ECM) fungi and, consequently, on subsequent performance of the outplanted seedlings under field conditions. In this study, we compare effects of bare-root and containerised cultivation systems on mycorrhization, establishment and growth of nursery-produced *Picea abies*. One hectare experimental plantation was established on poor fertility sandy site. In total, 1250 seedlings of each treatment were planted in rows as ten individual replicates. The results of this study showed that production of *P. abies* seedlings using containerised system can yield abundant ECM colonisation of seedling roots and significantly improve seedling survival in the field. Some reduction in height increment may occur during the first years as a possible cost for support of ECMs. Study demonstrated that selection of proper cultivation system might result in similar or higher mycorrhization and survival rates of outplanted seedlings than achieved by expensive and laborious artificial mycorrhization.

Keywords forest nursery, cultivation system, ectomycorrhizal fungi, *Picea abies* seedlings
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1 Introduction

In forest nurseries seedlings are commonly produced using standardised cultivation systems which can be divided into two major types: i) bare-root systems – seedlings are grown in an open field beds; ii) containerised systems – seedlings are grown (in the greenhouse or outdoor) in different types of interconnected plastic pots or other specialised vessels filled with the growth substrate. It has been demonstrated that cultivation system may have a significant effect on the extent of root colonisation by beneficial ectomycorrhizal (ECM) fungi (Menkis et al. 2005). ECM are known to provide nutritional benefits for their hosts and protection against unfavourable abiotic and biotic stress factors (Smith and Read 1997), and therefore they may be of considerable importance for seedling performance in the field. A number of studies has reported that colonisation of roots by particular ECM fungi may significantly promote survival, establishment and growth of tree seedlings in the forest plantations (Garbaye and Churin 1997, Pera et al. 1999, Ortega et al. 2004, Menkis et al. 2007). The main mechanisms behind those effects are thought to be enhanced uptake of water and nutrients through a greatly increased root-absorbing surface (Smith and Read, 1997), increased longevity and growth of roots (Chilvers and Gust 1982, Wilcox 1996), and protection of roots against drought, pathogens and heavy metal pollution (Morin et al. 1999, Van Tichelen et al. 2001, Ortega et al. 2004).

Intensive nursery cultivation (use of fertilisation, irrigation, mechanical and chemical weed, and pest control) may adversely affect ECM colonisation of seedling roots (Väre 1990, O'Neill and Mitchell 2000, Laatikainen and Heinonen-Tanski 2002). To overcome deficiency of ECM fungi in roots of nursery cultivated seedlings, artificial ECM inoculation was often suggested (Trappe 1977, Dunabeitia et al. 2004, Menkis et al. 2007) but this is associated with a large investment of resources. An alternative to this would be to explore an existing natural mycorrhization in forest nurseries by selecting a cultivation system that may yield seedling material with a high extent of ECMs.

The main aim of this study was to evaluate whether production of *Picea abies* (L.) H. Karst. seedlings using bare-root and containerised cultivation systems has any impact on mycorrhization, survival and growth of the seedlings following their outplanting in the field. Consequently, we assessed the possibility to avoid expensive and laborious artificial ECM inoculation, studying specifically field performance of the seedlings produced under two different cultivation systems with respectively lowest and highest ECM colonisation of seedling roots, showing that selection of seedlings produced under particular cultivation system which provides conditions for abundant natural ECM colonisation of seedling roots in the nursery may result in better performance of those seedlings after their transfer to the field, which is of considerable practical importance to nursery managers and foresters.

2 Materials and Methods

2.1 Study Site and Experimental Design

The study site comprised one hectare of perennial meadow situated in the vicinity of the Baltic Sea coast in western Lithuania (N 55°57´, E 21°06´). The site is characterised by relatively poor fertility sandy soil. Within the area, mean annual precipitation is ca. 720 mm and the length of the growing season is ca. 195 days. Temperatures average ca. 14 °C during the growth season.

Planting was carried out in spring 2006 with 4-year-old *P. abies* seedlings produced in the forest nurseries Kulautuva and Varena (Menkis et al. 2005) using two cultivation systems: i) bare-root in open field beds (BR); ii) containerised in polyethylene rolls (CR) (Fig. 1). The soil in which BR seedlings were grown was derived from a sandy loam podzol. The substrate in which CR seedlings were grown was low-humified *Sphagnum* peat. In nurseries, fertilizers were applied routinely at annual levels of 34–56 N kg/ha for BR seedlings and 0.11–0.15 N kg/m^3 of substrate for CR seedlings (Juska et al. 1982), which were estimated to be the optimal N levels for seedling growth in each respective cultivation systems. Both BR and CR seedlings used in this study represented standard planting material of each respective cultivation system and at outplant-

Fig. 1. *Picea abies* seedlings containerised in the polyethylene rolls (unwrapped). Arrows show abundant colonisation of seedling roots and substrate by ECM basidiomycete *Amphinema byssoides.*

ing were visually similar in their morphological parameters.

In previous study, *P. abies* yielded highest ECM colonisation in CR and the lowest in BR cultivation system, and the detailed description of ECM species composition in seedling roots of each respective nursery and cultivation system is in Menkis et al. (2005). Before outplanting of seedlings, the site was ploughed with a forestry plough in 20 rows at 2 m intervals. CR seedlings were prepared using standard procedure as follows: polyethylene was removed and root systems of individual plants were gently separated from each other to preserve fine roots and attached substrate. Seedlings of both treatments were established in the bottom of prepared rows in ten replicates, arranging different treatments in every second row. In order to avoid human factor in planting quality, standardised planting was done using an automated seedling planter. In total, 1250 *P. abies* seedlings were planted in each treatment at an initial density of 2500 seedlings per hectare.

2.2 Assessment of Root Mycorrhization

To assess the extent of root mycorrhization at outplanting, 40 seedlings were randomly sampled from the stock of each treatment. Assessment of roots was carried out using mycorrhizal morphotyping as described by Menkis et al. (2005). Briefly, each root system was washed in the tap water to remove the soil and 20 single root tips from each plant were randomly collected from different parts of the root system using forceps. Sampled roots were assessed for ECM colonisation using dissection microscope. Mycorrhizal tips were identified by the presence of a mantle, external hyphae or rhizomorphs, and the absence of root hairs.

2.3 Field Measurements

Survival of plants was determined three times, at the end of first, second and third growing seasons (2006, 2007, and 2008), by counting all living trees. The height increment was measured for all living trees at the end of the second and third growing seasons (2007 and 2008).

2.4 Statistical Analyses

The impact of the treatment on seedling mycorrhization and survival was analysed using chi-square (χ^2) tests (Mead and Curnow 1983). The survival of the seedlings was evaluated separately for each respective year 2006, 2007 and 2008. Height increment of the seedlings was analysed separately in 2007 and 2008 by one-way analysis of variance (ANOVA) (Chalmers and Parker 1989). The statistics were computed using Minitab® Statistical Software v. 15.1 (Minitab Inc., Pennsylvania).

3 Results

At outplanting, ECM fungi colonised 36.25% ± 0.48 SE of roots in BR and 100% ± 0.00 SE in CR ($p<0.0001$) in which basidiomycete *Amphinema byssoides* (Pers.) J. Erikss. was the most distinct ECM species (Fig. 1).

After the first growing season, seedling survival in the plantation was high in both treatments (96.7% ± 0.91 SE in CR and 97.2% ± 0.96 SE in BR) and the differences were statistically insignificant (χ^2 test; $p>0.05$) (Fig. 2). After the second season, seedling survival decreased in both treatments, but CR seedlings showed significantly better survival than BR seedlings (88.5% ± 1.13 SE and 83.8% ± 1.04 SE, respectively; $p<0.0001$) (Fig. 2). After the third season, seedlings from CR showed even higher survival than seedlings from BR (88.2% ± 0.94 SE and 81.7% ± 1.07 SE, respectively; $p<0.0001$).

Certain differences in height increment were observed between CR and BR treatments after second and third growing seasons (Fig. 3). After the second season, BR seedlings showed significantly higher growth increment than CR seedlings (21.6 cm ± 0.26 SE and 18.0 cm ± 0.23 SE, respectively; $p<0.0001$). However, already during the third season CR seedlings demonstrated better growth, thus the gap in height increment between CR and BR seedlings after three years has decreased, yet the difference still remained significant (31.5 cm ± 0.32 SE in BR and 29.5 cm ± 0.37 SE in CR, $p<0.0001$) (Fig. 3).

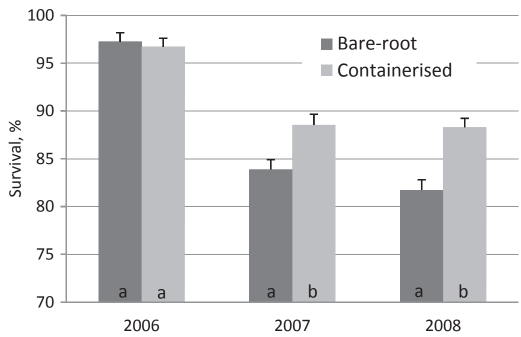

Fig. 2. Survival of *Picea abies* seedlings during three consecutive growing seasons. Within the same year, statistically significant differences between the treatments (bare-root and containerised) in chi-square tests are designated by different letters. Error bars indicate standard error of the mean.

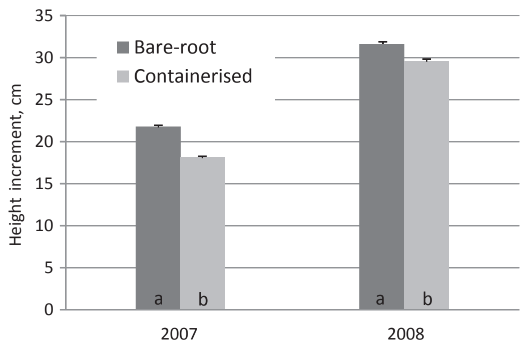

Fig. 3. Height increment of *Picea abies* seedlings after the second (2007) and third (2008) growing seasons. Within the same year, statistically significant differences between the treatments (bare-root and containerised) in one-way ANOVA are designated by different letters. Error bars indicate standard error of the mean.

4 Discussion

The results of the present study on ECM colonisation of seedling roots appeared to be largely consistent with those from earlier study, where containerised *P. abies* seedlings showed 82.7% mycorrhization as compared to 44.1% observed in bare-root cultivation system (Menkis et al. 2005), confirming Rincón et al. (2005) that the growth environment may strongly influence intensity of ECM colonisation of seedling roots in the nursery.

We observed that in both treatments there was certain pattern in seedling survival rate during three consecutive years: i) equally high survival rates in both CR and BR during the first year, ii) decreased in both but significantly higher survival in CR after the second year, and iii) even larger difference in survival after the third year. This could have been a consequence of annual fluctuations of environmental conditions in the plantation site, but seedling survival in CR was better, probably because ECMs are known to mitigate environmental stress (Rodriguez et al. 2004). Moreover, beneficial effects of ECM fungi are likely to be most pronounced on poor fertility planting sites under harsh environmental conditions (Smith and Read 1997).

Vaario et al. (2009) suggested that ECMs on roots of containerised *P. abies* nursery seedlings was one of the best indicators of seedling performance after outplanting. In their study, *A. byssoides* has undergone a relatively rapid expansion from marginal levels of occurrence in the nursery seedlings to moderate levels in the third year seedlings after outplanting. Even more rapid, only during one growing season, change in ECM community structure towards dominance of *A. byssoides* in the root systems of outplanted seedlings was reported by Menkis et al. (2007). Results of those studies suggest that *A. byssoides* is an efficient root coloniser of *P. abies* seedlings. Its common occurrence in newly established forest plantations (Kranabetter J 2004, Menkis et al. 2007, Vaario et al. 2009), and better survival of abundantly colonised CR seedlings of the present study further suggest that *A. byssoides* may play an important role in seedlings survival and establishment following their outplanting. In support to this, Jones et al. (2009) showed that ^{15}N accumulation in shoots and roots of spruce seedlings colonised by *A. byssoides* was respectively moderate and high compared to the seedlings colonised by other ECMs.

Furthermore, we hypothesise that in addition to the benefits provided by ECMs, organic substrate attached to the root systems of outplanted CR seedlings has likely contributed to better protection of the roots against unfavourable environmental conditions of the site as e.g. frosts and droughts. In addition, this could also improve nutrient availability to the seedlings at early stage of their establishment. Such improved seedling survival during first plantation years is important for practical silviculture.

The observed reduction in height increment in CR seedlings is likely to be temporal, but to confirm this more continuous observations are needed. In a related Finnish study, Vaario et al. (2009) reported that containerised *P. abies* seedlings grown in a similar substrate and with similar mycorrhization levels as CR seedlings, although produced satisfactory shoot growth in the nursery showed reduced growth rates after transfer to the field. Similar growth patterns, when *P. abies* seedlings with most abundant ECM colonisation showed reduced rates of height increment were observed in the study conducted on replanted forest clear-cut (Menkis et al. 2010). As ECM fungi are exclusively dependent on their hosts for carbohydrates, we hypothesise that support of ECMs in CR seedlings was probably the reason for reduced height increment observed during the first two years of this study. In support to this, it was shown that under the field conditions up to 22% of plant-produced carbohydrates can be allocated to ECMs and that growth reduction in plants may be equivalent to such allocations (Hobbie 2006).

In conclusion, present study demonstrated that selection of proper cultivation system in forest nursery might result in similar or higher mycorrhization and survival rates of outplanted seedlings than achieved by expensive and laborious artificial mycorrhization. In order to obtain more comprehensive picture on how different cultivation systems affect natural mycorrhization of nursery-grown tree seedlings and their subsequent field performance, more related studies are needed, encompassing wider range of tree species and field conditions.

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References

Chalmers, N. & Parker, P. 1989. Fieldwork and statistics for ecological projects, second edition. The Open University, Dorchester.

Chilvers, G.A. & Gust, W.L. 1982. Comparison between the growth rates of mycorrhizas, uninfected roots and a mycorrhizal fungus of Eucalyptus st. johnii R.T. Bak. New Phytologist 91: 453–466.

Dunabeitia, M., Rodriguez, 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.

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

Hobbie, E.A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology 87: 563–569.

Jones, M.D., Grenon, F., Peat, H., Fitzgerald, M., Holt, L., Philip, L.J. & Bradley, R. 2009. Differences in N-15 uptake amongst spruce seedlings colonized by three pioneer ectomycorrhizal fungi in the field. Fungal Ecology 2: 110–120.

Juska, V., Baltrusaitiene, V., Jasiukaitis, L., Kirklys, A. & Veisreideriene, E. 1982. Growth technology of plant material under controlled environment conditions. Raide, Kaunas (in Lithuanian).

Kranabetter, J. 2004. Ectomycorrhizal community effects on hybrid spruce seedling growth and nutrition in clearcuts. Canadian Journal of Botany 82: 983–991.

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

Mead, R. & Curnow, R.N. 1983. Statistical methods in agriculture and experimental biology. Chapman & Hall, London.

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.

— , Vasiliauskas, R., Taylor, A.F.S., Stenlid, J. & Finlay, R. 2007. Afforestation of abandoned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi – impact on plant performance and ectomycorrhizal community. Mycorrhiza 17: 337–348.

— , Uotila, A., Arhipova, N. & Vasaitis, R. 2010. Effects of stump and slash removal on growth and mycorrhization of Picea abies seedlings outplanted on a forest clear-cut. Mycorrhiza 20: 505–509.

Morin, C., Samson, J. & Dessureault, M. 1999. Protection of black spruce seedlings against Cylindrocladium root rot with ectomycorrhizal fungi. Canadian Journal of Botany 77: 169–174.

O'Neill, J.J.M. & Mitchell, D.T. 2000. Effects of benomyl and captan on growth and mycorrhizal colonization of Sitka-spruce (Picea sitchensis) and ash (Fraxinus excelsior) in Irish nursery soil. Forest Pathology 30: 165–174.

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

Pera, J., Alvarez, I.F., Rincon, A. & Parlade, J. 1999. Field performance in northern Spain of Douglas-fir seedlings inoculated with ectomycorrhizal fungi. Mycorrhiza 9: 77–84.

Rincón, A., Parladé, J. & Pera, J. 2005. Effects of ectomycorrhizal inoculation and the type of substrate on mycorrhization, growth and nutrition of containerised Pinus pinea L. seedlings produced in a commercial nursery. Annals of Forest Science 62: 817–822.

Rodriguez, R.J., Redman, R.S. & Henson, J.M. 2004. The role of fungal symbioses in the adaptation of plants to high stress environments. Mitigation and Adaption Strategies for Global Change 9: 261–272.

Smith, S.E. & Read, D.J. 1997. Mycorrhizal Symbiosis. Academic Press, London, UK.

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

Vaario, L.M., Tervonen, A., Haukioja, K., Haukioja, M., Pennanen, T. & Timonen, S. 2009. The effect of nursery substrate and fertilization on the growth and ectomycorrhizal status of containerized and outplanted seedlings of Picea abies. Canadian Journal of Forest Research 39: 64–75

Van Tichelen, K.K., Colpaert, J.V. & Vangronsveld,

J. 2001. Ectomycorrhizal protection of Pinus sylvestris against copper toxicity. New Phytologist 150: 203–213.
Väre, H. 1990. Effects of soil fertility on root colonization and plant growth of Pinus sylvestris nursery seedlings inoculated with different ectomycorrhizal fungi. Scandinavian Journal of Forest Research 5: 493–499.
Wilcox, H.E. 1996. Mycorrhizae. In: Waisel, Y., Eshel, A. & Kafkafi, U. (eds.). Plant roots: the hidden half. Marcel Dekker, New York. p. 689–721.

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