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SHORT COMMUNICATION

Mycelial inoculation of containerized Norway spruce seedlings with ectomycorrhizal fungi

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Abstract
An inoculation technique to create ectomycorrhizal symbiosis in 1.5-year-old Norway spruce (*Picea abies* (L.) Karst.) container seedlings was tested. The mycelia of ectomycorrhizal fungi (EMF) – *Tylospora asterophora*, *Piloderma olivaceum*, and *Cenococcum geophilum* – each grown in a silica dioxide powder carrier, was mixed with a conventional low-humified *Sphagnum* peat at the time of sowing. Seedlings were grown in four growth media: (1) conventional peat; (2) conventional peat mixed with sterile carrier; (3) conventional peat mixed with carrier containing *T. asterophora* and *C. geophilum*, (4) conventional peat mixed with carrier containing *P. olivaceum* and *C. geophilum*. The seedling development and EMF colonization was followed during the nursery production. Further, seedlings grown in the four media were planted on a former nursery field, and their development was observed for three years. At the end of the nursery production phase, there were no differences in the seedling height or stem diameter between the growing media. The colonization degree by the introduced EMF was low. The height growth of the seedlings inoculated with *T. asterophora* + *C. geophilum* was 16% higher during the first growing season after out-planting compared to seedlings grown in the conventional peat medium, but the effect was transient. At the end of the out-planting experiment, the seedlings grown in conventional peat had the highest proportion of healthy and lowest proportion of dead seedlings. The results emphasize the importance of the growing media for seedling quality and out-planting success. The tested inoculation technique was ineffective in creating substantial levels of EMF colonization.

Keywords Ectomycorrhiza · Growth media · Out-planting success · *Picea abies* seedling morphology

Introduction

Mycorrhizal symbiosis is important for ensuring plants' growth and survival as it provides nutrients and water in exchange for photosynthetic products. Mycorrhizal fungi have been linked to the increased drought tolerance of conifers (Parke et al. 1983; Yin et al. 2018).

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A plant typically holds symbiosis with several fungal species at a time, and in long living plants such as trees, the symbiotic species change over time and along succession of the stand (Palfner et al. 2004; Wallander et al. 2010).

Norway spruce (*Picea abies* (L.) Karst.) is the dominant tree species in artificial forest regeneration in northern Europe, including Finland. More than 99% of the produced Norway spruce seedlings in Finland are grown in containers (Natural Resources Institute 2022). The most common seedling type is 1.5-year-old seedlings sown in early to mid-June and grown in low-humified *Sphagnum* peat in hard plastic containers throughout the first and second growing season. During seedling production, ectomycorrhizal colonization may form from naturally occurring spores at the nursery. However, these fungal species may notbe optimal for the seedlings' out-planting success (Flykt et al. 2008).

Mycorrhizal fungi may be introduced to tree seedlings during their production by various methods of soil inoculum, spores, fungal slurries, or fungi cultured in carriers (Parladé et al. 2004; Repáč 2011; Lazarević et al. 2012; Velmala et al. 2018). Newly germinated and emerged Norway spruce seedlings are not disposed to form ectomycorrhizas. On the other hand, vegetative mycelia of ectomycorrhizal fungi (EMF) may be unable to survive in the growth media for extended periods without symbiosis. However, adding the mycelia inoculum to the growth media at the susceptible time for the symbiosis to form would require the transplanting of the seedlings or other measures, increasing the production costs. The traditional EMF vegetative mycelia production systems use peat-vermiculite -mixture as carrier (Marx et al. 1982; Villeneuve et al. 1991). However, this type of carrier may contain competing fungi to which many EMF of coniferous trees are sensitive to. Despite autoclaving, the carrier can contain contaminants appearing during long incubations (Vuorinen et al. 2015). Inoculation with persistent spores of EMF would be more straightforward, but spore production is not feasible from the EMF species compatible with Norway spruce seedlings in boreal forests (Huusko et al. 2015). Better techniques to introduce ectomycorrhizal fungi to container seedlings are therefore sought after.

A growing system in which microbes are grown in a silica dioxide -carrier is used in the commercial production of several bio-controlling microbial cultures in both a submerged liquid system (Hamberg et al. 2011) and in a solid-state bioreactor (Virtanen et al. 2008). Solid cultivation offers several advantages over submerged cultivation; reduced contamination risk, lower energy consumption and an environmentally friendlier process since less wastewater is produced (Virtanen et al. 2008). However, it has not been tested as a means to introduce EMF in container seedling production. We studied if an inoculation technique using vegetative mycelia of EMF grown in fine particle silica dioxide powder carrier (Sipernat) mixed with the standard growth media, i.e., low-humified *Sphagnum* peat, prior to sowing would be effective in creating EMF symbiosis in 1.5-year-old container seedlings and improving the seedlings' performance after out-planting.

Three ectomycorrhizal fungal species of Norway spruce were tested in two mixtures. Our aim was to test the co-inoculation of two very different but common mycorrhizal fungi of Norway spruce; basidiomycetous (*Tylospora asterophora* and *Piloderma olivaceum)* and ascomycetous (*Cenococcum geophilum*) as these fungal species are known to co-exist in the roots of spruce seedlings in the field (e.g. Huusko et al. 2015). These fungi are also known to have different growing habits, thus potentially providing an optimal colonization for the seedling.

Material & methods

Nursery experiment

Vegetative mycelia of the three fungal strains were grown separately in an aseptic solid matrix system developed by Vuorinen et al. (2015). The strains were *Tylospora asterophora* R-SP01, *Piloderma olivaceum* R-SP02, and *Cenococcum geophilum* R-FC03, all isolated from wild seedlings of Norway spruce (Vuorinen et al. 2015). The fungal isolates were first grown for two weeks in liquid culture, then homogenized and pipetted into sterile and aerated plastic bags filled with solid silica carrier (Sipernat 22 S, silicon dioxide, SiO_2 Evonic industries, Essen, Germany). Forty ml of homogenized liquid culture were added per kg of carrier medium. The nutrients in the carrier medium consisted of malt extract and a humic acid product. Moreover, the pH was adjusted to 5.8, using 0.4 g dolomite lime/l. The fungi were grown in the dark at 20 °C ca. one month, or until the silica medium was thoroughly occupied by fungal vegetative mycelia. For the formulation of the growth media equal quantities of carriers including *T. asterophora* and *C. geophilum* were mixed, as well as carriers including *P. olivaceum* and *C. geophilum*.

Four different nursery growth media were formulated in the experiment:

1. Conventional growing medium: Base-fertilized, low-humified *Sphagnum* peat (Kekkilä W F6. Data given by the manufacturer: Average particle distribution: 0–1 mm 13%, 1–4 mm 40%, 4–7 mm 16%, 7–16 mm 30%, > 16 mm 1%, base fertilized with Kekkilä Starter 6 NPK 16 − 4–17 kg m-3, pH of water suspension 4.3, conductivity of water suspension 1.4 mS/cm).
2. Sterile control: Conventional growing medium with 10% (volume) sterile Sipernat 22 S-carrier.
3. Conventional growing medium with 10% (volume) Sipernat 22 S -carrier including *T. asterophora* + *C. geophilum* -inoculum.
4. Conventional growing medium with 10% (volume) Sipernat 22 S -carrier including *P. olivaceum* + *C. geophilum* -inoculum.

Four hard plastic Plantek 81 F nursery containers (BCC, Landskrona, Sweden, 81 cells per container, 546 cells m^2, cell volume 85 cm^3) with each media were filled on June 15, 2015. Media 2–4 were prepared by mixing the peat and Sipernat -carriers in the desired proportions prior to filling the containers.

A 0.5 cm dent was pressed into the media in each cell and one seed of a commercial first-generation seed orchard (Sairila, Sv177) seed lot EY/FIN T03-14-0401 was then sown in each cell. The surface of the media was then covered with a thin layer of gravel (grain size 2–4 mm) used as mulch. The containers were taken the same day to a greenhouse at the Suonenjoki Research Nursery at Natural Resources Institute Finland (62°39'N, 27°03'E, 142 m above sea level) and placed on raised metal growing pallets (1.2 m × 2.4 m), each with room for 18 containers.

The experiment comprised of four blocks, with one pallet equaling block. Each block contained one container of each growth media in a random order. The experimental containers were surrounded on the pallets by containers of a commercial seedling crop with the same container type grown in the conventional growing medium and, sown at the same time

with the same seed lot. The pallets holding the experimental containers were further grown among a commercial seedling crop. The sown area was irrigated thoroughly on the day of the sowing.

The growing measures typical for growing a 1.5-year-old seedling crop were applied throughout the cultivation and the experimental seedlings were treated similarly to the commercial seedling crop. During the first growing season (2015) no fertilization was used in addition to the base-fertilization existing in the peat. During the second summer (2016), the seedlings received fertilization (Forest Superex, Kekkilä; Total N 21.9%, nitrate 5.8%, ammonium 2.1%, urea 13.8%, water soluble P 5.0%, water soluble K 16.0%, Mg 1.2%, B 0.06%, Cu 0.02%, Fe 0.17%, Mn 0.08%, Mo 0.005%, and Zn 0.025%) with irrigation water on 12 occasions. The need for fertilization in the crop was assessed by measuring the water conductivity of the growth media. The amount of total nitrogen provided in 2016 was approximately 22 g m-2, which equates to 38 mg/seedling. No fungicide or pesticide spray was applied to the experimental seedlings.

After the first growing season in late September, the height and stem diameter (1 cm above the growth media surface) of 10 randomly selected seedlings chosen with a randomizer list (created with Research Randomizer; Urbaniak G. C. & Plous, S. www.randomizer.org) from each container were measured. The health and survival of all the seedlings in the experiment were recorded. From each container, three seedlings (12 seedlings from each growth media, 48 seedlings altogether) were lifted, and freezer stored for root microscopy. However, ectomycorrhizal development was at a very early stage in the young seedlings, and it was therefore impossible to observe signs of the inoculated EMF. The 1st year results are therefore not shown. The containers overwintered under natural snow cover in an outdoor area on the site, fenced to protect against mammal herbivory.

In May 2016, the seedling containers were moved to an irrigated field outdoors in the nursery, again on raised pallets and among the commercial seedling crop. The seedlings lifted for microscopy were replaced with seedlings from the commercial crop to keep the growing density the same as in the first summer. A short-day treatment (12 h) was applied between July 4 and 26 to end shoot elongation. In early October 2016, the quality of all the seedlings was assessed to determine the proportion of cull seedlings. The assessment was based on the standard nursery production criteria presented in Rikala (2012) and Himanen and Nygren (2015). The height and stem diameter of five randomly selected seedlings was measured, and the number of healthy and living seedlings counted. Additionally, four randomly selected seedlings were lifted from each container (16 seedlings from each growth media, 64 seedlings altogether) and stored for root microscopy. The remaining seedlings were packed in cardboard boxes, with seedlings from each growing container in an individual box and stored at -3 °C in a nursery freezer until the following spring.

The mycorrhizal status of the sample seedlings was examined for the color and texture of their root tips (morphotype) under a stereomicroscope to identify the fungal species and their colonization degree in the roots (Agerer 1987–1998). The roots of the seedlings were washed and cut into 1–2 cm pieces, which were placed in water-filled Petri dishes. The root pieces were randomly selected until about 200 short roots were analyzed under a dissecting microscope (Zeiss Stemi 2000-C). The degree of colonization of the potential inoculated fungus per total number of short roots was determined, as well as that of other fungi and the proportion of bare root tips (Table 1). Additionally, the shoot and root biomasses were weighed after drying at 40 ºC.

Table 1 The mean root tip densities, EMF colonization degree and morphological characteristics (±SE) of Norway spruce container seedlings (16 seedlings per treatment) at the end of the second growing season in the nursery. The EMF % describes the EMF colonization degree of the seedlings. The seedlings were grown in four growth media: conventional *Sphagnum* peat; sterile control (peat with 10% sterile Sipernat-carrier); peat with 10% Sipernat -carrier including *Tylospora asterophora*+*Cenococcum geophilum* inoculum; and peat with 10% Sipernat -carrier including *Piloderma olivaceum*+*C. geophilum* -inoculum

	Root tips	EMF	Stem height	Stem diameter	Stem biomass	Root biomass	Root/shoot
	Number/cm root	%	cm	mm	g d.m.	g d.m.	ratio
Conventional	6.70±0.278	93.0±11.5	27.4±1.4	3.57±0.170	2.56±0.225	0.79±0.065	0.32±0.038
Sterile control	6.51±0.071	96.4±24.5	28.9±0.8	3.21±0.152	2.30±0.264	0.67±0.112	0.30±0.44
TC inoculum	7.12±0.323	95.9±26.8	28.5±1.0	3.42±0.235	2.50±0.278	0.62±0.061	0.26±0.043
PC inoculum	7.00±0.703	95.9±23.1	29.8±2.0	3.46±0.355	2.38±0.567	0.68±0.152	0.29±0.041

Out-planting experiment

For the following three-year out-planting experiment, the seedling boxes were lifted from the storage and allowed to thaw in an outdoor shaded storage at ambient temperature between June 7 and 12, 2017. The seedlings were then planted in an out-door planting field (a former nursery field of fine sandy soil with a surface layer of a silt-clay-peat mixture) at Natural Resources Institute Finland's Suonenjoki Research Station.

The soil was harrowed prior to planting, and the ground vegetation mowed once each year of the experiment in July. The seedlings were planted in rows with a spacing of 50 cm, each row containing 10 seedlings originating from a single growing container during the nursery experiment. The order of the rows in the planting design was random. The same planting design was replicated in three rectangular planting blocks, each 4.5 of m × 8 m. The three blocks formed an L-shaped area, with the second block in the center. A total of 480 seedlings was planted, 120 seedlings grown in each growing media.

The air temperature (2 m above ground) and precipitation data for the planting site (10×10 km grid) was obtained for May–August 2017, 2018, and 2019 from the Finnish Meteorological Institute, as well as data for the previous 30 years to calculate monthly averages. The mean temperature during May–August was below the 30-year average during the first summer of the out-planting experiment (2017), and the precipitation was above average during June–August (SI 4). In the second summer (2018), the average May, July, and August temperatures were above the longtime average, with low precipitation particularly during July. In the final year of the experiment (2019) the largest deviation from the 30-year average was low precipitation during June–August.

The height and stem diameter of each seedling were measured after the planting, as well as in September 2017, 2018, and 2019 after the shoot growth had ceased. In the fall measurements, each seedling was determined as (1) healthy, (2) weakened (showing chlorosis, needle loss of < 50% or small cankers), (3) severely weakened or damaged (severe chlorosis, death of the leading shoot, needle loss of > 50%, and/or severe cankers) or (4) dead.

Statistical analysis

The effect of growing media on seedling height and stem diameter in the nursery was analyzed using ANOVA models with a pallet/container -block structure.

The effect of growing media on seedling morphology in the three-year out-planting experiment was analyzed using linear mixed models. For the analysis of annual height growth, final height, the annual growth of the stem diameter, and the final stem diameter, the analyzed data included all the seedlings that were alive (quality classes 1–3) at the time of the measurement. The analysis was performed assuming normal distribution and using an identity link function.

For the analysis of seedling health at the end of the out-planting experiment, individual generalized linear mixed models were created for each seedling quality class (healthy, weakened, severely weakened, and dead). In these models, binomial distribution and a logit link function were used.

In the linear and generalized linear mixed models, the model's form was:

$$y = b_0 + b_1 G + u_{planting\,block(pallet)}, \quad (1)$$

where y = response variable. The fixed term of all models was growing media (G), and planting block (pallet) -nested structure was used as a random term.

The models' overall fit was examined using graphical summaries of the residuals vs. fitted values and covariates.

The fixed model terms were considered statistically significant at $p \leq 0.05$ in all analyses. The analyses were conducted using Genstat 19 software (VSN International Ltd. 2018).

Results

Nursery experiment

The proportion of empty cells resulting from non-germinating seeds or early mortality varied from 3.1 to 5.2% between the growing media at the end of the nursery production (September 2016). The proportion of seedlings assessed as cull seedlings varied from 4.6 to 14.8% between the growing media, being lowest in the sterile control (Media 2) and highest in the conventional growing media (Media 1).

The growing media had no effect on the seedling height in the nursery (Fig. 1, SI 1). There was a statistically significant difference in the stem diameter of the seedlings after the first growing season in the nursery: the seedlings grown in the conventional substrate (Media 1) had the largest stem diameter. The differences in the stem diameter disappeared during the second year at the nursery.

The root tip densities were similar among the media (Table 1). The EMF colonization degree of the seedlings was high in all treatments, mostly due to the ectomycorrhizal fungi *Thelephora terrestris* and *Amphinema* sp. (Fig. 2). The colonization of the inoculated strains *T. asterophora*, *C. geophilum*, and *P. olivaceum* varied between 1% and 10%.

Out-planting experiment

The height growth of the seedlings was statistically significantly different between the growing media in the first growing season after out-planting (2017) (Fig. 3, SI 2). The height growth was 16% higher in seedlings grown in Media 3 containing *T. asterophora* + *C. geophilum* -inoculum, compared to the conventionally grown seedlings. The differences in height growth in the second and third growing seasons were not statistically significant, nor were the differences in the absolute height of the seedlings at the end of the three-year experiment.

The differences in the annual growth of the stem base diameter were not statistically significant during the first two growing seasons after out-planting (Fig. 3, SI 3). However, the annual stem growth during the third growing season and the final stem base diameter were the highest in the seedling grown in the conventional peat substrate, and the differences between the growing media were statistically significant.

At the end of the first growing season after out-planting, no seedlings had died or were classified being severely weakened. Of the seedlings inoculated with *P. olivaceum* + *C. geophilum*, 8.3% were classified as weakened, and 5.0% of seedlings in all other treat-

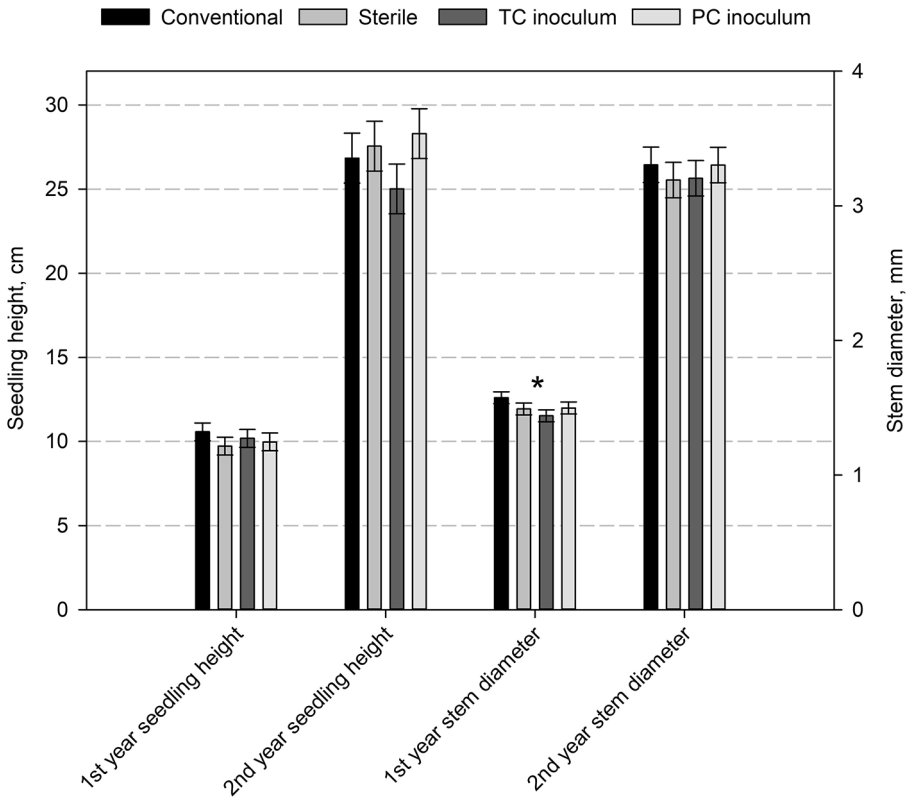

Fig. 1 The mean seedling height and stem diameter (±SE) at the end of the first and second growing season of 1.5-year-old Norway spruce container seedlings in the nursery grown in different media: conventional *Sphagnum* peat; sterile control = peat with 10% sterile Sipernat-carrier; TC inoculum = peat with 10% Sipernat -carrier including *Tylospora asterophora* + *Cenococcum geophilum* inoculum; and PC inoculum = peat with 10% Sipernat -carrier including *Piloderma olivaceum* + *C. geophilum* -inoculum. The statistically significant difference between the growth media is marked with an asterisk

ments. After the second growing season, 31.1% of the conventionally grown seedlings were classified as healthy, while the proportion of healthy seedlings varied between 16.7% and 19.2% in the other treatments. The proportion of dead seedlings was lowest (17.6%) in the conventionally grown seedlings and highest in the seedlings grown in sterile control media (43.3%).

At the end of the three-year out-planting experiment, the seedlings grown in conventional peat substrate had the highest proportion of healthy and lowest proportion of dead seedlings (Table 2). The seedlings grown in the sterile control media had the lowest proportion of healthy seedlings and highest mortality. The growing media had a statistically significant effect on the seedling health in the models for healthy and dead seedlings ($p = 0.015$ and $p = 0.009$ respectively). In the models for weakened and severely weakened seedlings the growing media did not have a statistically significant effect ($p = 0.807$ and $p = 0.122$ respectively).

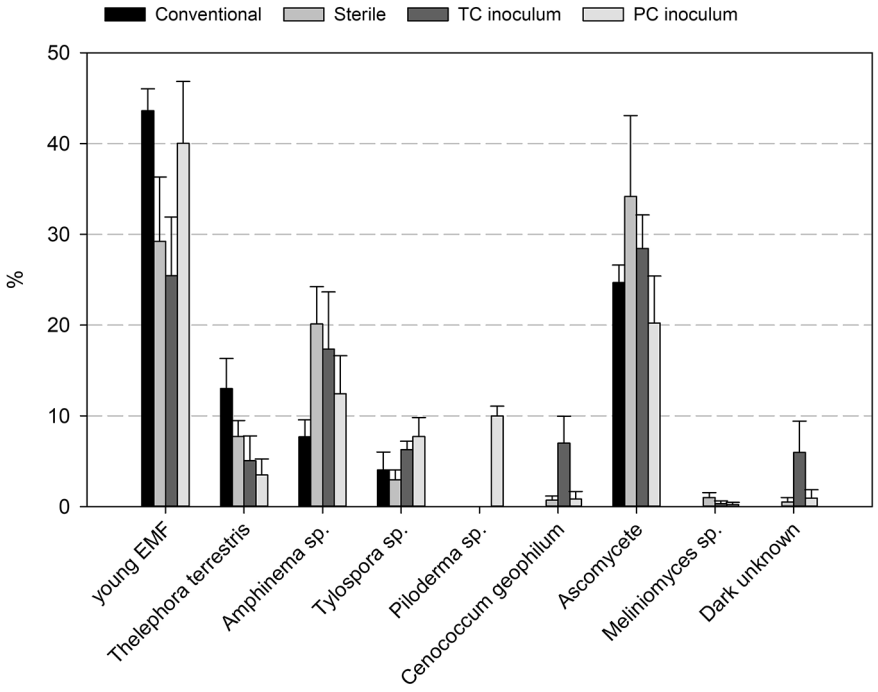

Fig. 2 Colonization degree (percentage of root tips colonized) of various ectomycorrhizal fungi or young stages of mycorrhiza development, where the fungal species is unidentified (young EMF), in 1.5-year-old Norway container seedlings after the second growing season at the nursery grown in four different growing media (see caption on Fig. 1). *Tylospora asterophora, Piloderma olivaceum, and Cenococcum geophilum* -strains were introduced to the seedlings at the time of sowing

Discussion and conclusions

The seedling height and stem diameter were similar at the end of the nursery production regardless of growth media (Fig. 1). The result is contrary to studies in which EMF inoculum increased seedling growth at the nursery in *Pinus pinaster* (Sanches-Zabala et al. 2013) for example or in *Picea sitchensis* (Thomas and Jackson 1983). The observed seedling morphology can be regarded as typical for 1.5-year-old Norway spruce seedlings (Rikala 2012; Luoranen et al. 2019). The proportion of cull seedlings (small or deformed) was highest in the conventional growing medium. No clear reason for this could be identified. As the irrigation and other growth measures were the same for all the seedlings in the experiment, it may be that they were more suitable for seedlings grown in the substrate containing fine particle silica powder (Media 2 to 4).

Our results of the small impact of the inoculation to the EMF colonization degree, and the presence of naturally occurring fungi, are similar to a study by Repáč (2007) where three EMF were introduced to Norway spruce bare-root seedlings with vegetative alginate-bead

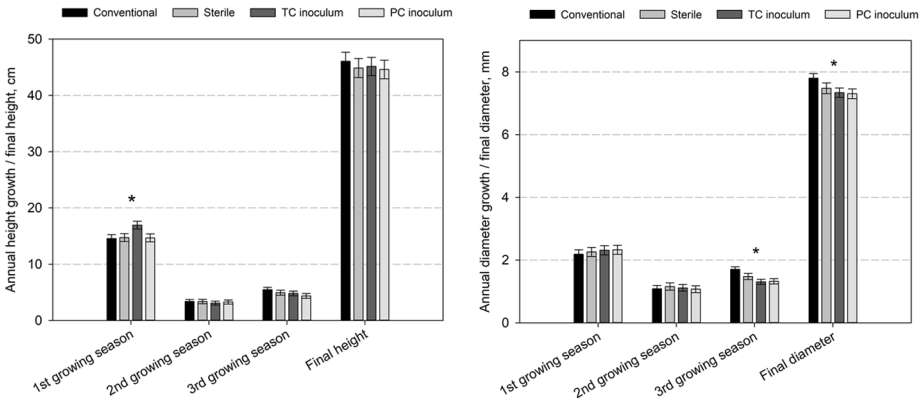

Fig. 3 The predicted mean annual height growth (±SE) (left figure) and stem diameter growth (right figure) of planted Norway spruce seedlings during three growing seasons, and the final seedling height and final stem diameter after the tree growing seasons. In the experiment, the container seedlings were grown in four different growth media (see caption on Fig. 1) during the nursery production and subsequently planted in an out-door field. Statistically significant differences between the growth media are marked with an asterisk

inoculum. The overall EMF colonization degree in the current study is higher than observed for instance by Repáč et al. (2014).

The positive impact of *T. asterophora* + *C. geophilum* on seedling height growth (+16%) in the first year after out-planting is surprisingly large compared to the low level of colonization by these isolates observed in the nursery. Marx et al. (1982) showed that >20% colonization degree is needed to show the positive effect of *Pisolithus tinctorius* on the performance of pine (*Pinus* sp.) seedlings on reforestation sites. However, both *T. asterophora* and *C. geophilum* are very slowly growing fungus (Vuorinen et al. 2015), so many of these morphotypes may have been classified in the large group of young emerging mycorrhizas, which could not yet be identified at the end of the nursery production. Furthermore, stimulating effect of the EMF inoculation on the Norway spruce seedling growth have been reported even though the fungi applied did not form distinctive treatment-specific EMF (Repáč 2007; Repáč and Sendecký 2018). Better seedling growth was associated to higher abundance of *Tylospora fibrillose* -like morphotype in the inoculation treatment, but an improved growth might also have resulted in inoculation independent factors as such as plant growth hormones produced by EMF, or decomposed mycelium in substrate as source of nutrients and activities of other microbes in the rhizosphere (Repáč and Sendecký 2018). Positive effects of the EMF inoculum to growth in out-planting experiments have been reported in *P. pinaster* (Sanches-Zabala et al. 2013), *Pinus radiata* (Ortega et al. 2004) and *Quercus suber* (Sebastiana et al. 2013), for example.

The disappearance of this positive effect in the following years of this inoculation may be because of the poor persistence of the inoculated EMF symbionts after out-planting. In a study by Hortal et al. (2009) a very high initial EMF colonization level (>50%) was related to higher persistence of the EMF after out-planting, but with initial colonization around 30% a rapid decrease in the colonization levels of most inoculated EMF *Lactarius delicious* strains was observed. The different EMF inoculation systems are known to affect the colonization success and persistence. For example in *Pinus* seedlings, Parladé et al. (2004)

Table 2 The estimated mean proportions and asymmetric standard errors of mean (SE) of Norway spruce seedlings in different quality categories at the end of a three-year out-planting experiment. The container seedlings were grown in different growing media prior to out-planting (see caption in Table 1). The seedlings were visually assessed as (1) healthy, (2) weakened (showing chlorosis, needle loss of < 50% or small cankers), (3) severely weakened or damaged (severe chlorosis, death of the leading shoot, needle loss of > 50%, and/or severe cankers) or (4) dead

	Healthy		Weakened		Severely weakened		Dead	
Growing media	Mean, %	SE lower-upper limit	Mean, %	SE lower-upper limit	Mean, %	SE lower-upper limit	Mean, %	SE lower-upper limit
Conventional	39.2	28.4–51.1	30.0	25.5–34.9	11.6	8.8–15.2	17.0	11.0–25.4
Sterile control	18.3	12.1–26.6	28.3	24.0–33.1	7.5	5.6–9.9	43.6	31.8–56.2
TC inoculum	24.2	16.4–34.0	34.2	29.3–39.4	19.1	14.7–24.4	19.5	12.7–28.6
PC inocolum	23.3	15.8–33.0	33.3	28.6–38.5	13.3	10.1–17.3	27.0	18.2–38.1

found that vegetative inoculum grown in peat:vermiculite showed the highest colonization degrees while alginate bead -entrapped mycelium inoculation resulted in the lowest level of colonization. Furthermore, varying abiotic conditions in the nursery, intraspecific EMF–plant relationships and competing indigenous EMF fungi hamper the practical testing of EMF inoculation (Repáč and Sendecký 2018).

The persistence of the mycorrhiza depends on the EMF strain, but also strongly on physical, chemical, and biotic characteristics of the plantation site (Menkis et al. 2007, Hortal et al. 2009). The site – a former nursery field – only partially mimics boreal forest soil. The benefit of the site from the experiment's perspective is its homogeneity and the lack of competing tree or shrub vegetation that may hold the same or competing EMF species. However, the nutrient levels and their relative proportions are probably atypical of forest soil. It is generally well established that EMF fungi are sensitive to high environmental nutrient loads, especially of N and P (review by Lilleskov et al. 2019), and also the used EMF fungal inoculants are known to be negatively affected by a large amount of nutrients (Flykt et al. 2008).

The overall good health of the seedlings after the first summer after the out-planting can be explained by the humid low-stress conditions that season (SI 4). Seedling growth and health deteriorated, and mortality increased during the second summer after out-planting irrespectively of the growth media, in comparison to the first summer. This can be contributed to the harsh weather conditions that summer, especially in July and August when monthly temperatures were above and precipitation below the 30-year average.

The varying performance of the seedlings in years of different environmental stress in the out-planting experiment indicates the need to follow the effects of the inoculation and growing media for multiple growing seasons. Our results resemble those of Kipfer et al. (2012), who found inoculation with mycorrhizas more beneficial for the growth of *Pinus sylvestris* seedlings in moist conditions opposed to drought stress. The current results are, however, surprising when compared to Nikolova et al. (2020) who found Norway spruce to rely heavily on EMF during intense drought stress. The effect of EMF colonization for drought tolerance, photosynthetic activity, and nutrient and water uptake has been shown to be complex for instance in the seedlings of *P. sitchensis* (Lehto 1992).

The harmfulness of the carrier media addition became evident during the drought stress. At end of the out-planting experiment, the seedling health was best, and the final stem diameter highest, in the seedlings grown in the conventional peat substrate. The negative effect on the seedlings may have been due to the altered physical structure in the silicon dioxide powder containing growing media; the decreasing porosity of the substrate may cause water logging at the nursery (Wall and Heiskanen 2003) harming the root development, despite the similarity of the shoot morphology among the growth media.

We conclude that the tested inoculation technique was ineffective in creating substantial ectomycorrhizal connections in the Norway spruce container seedlings. The *T. asterophora* + *C. geophilum* -inoculum improved the height growth of the seedlings during the first growing season after out-planting, but the positive effect was transient. At the end of the three-year out-planting experiment, seedling health was best in seedlings grown at the nursery in the conventional peat substrate. The result emphasizes the importance of the growing media for seedling quality and further out-planting success. The utilization of EMF fungi in the forest tree nursery production seeks more seedling-friendly inoculation techniques and techniques enabling a higher degree of colonization by the inoculated fungi.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11056-023-09964-y.

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Author contributions All authors contributed to the study conception. The nursery and out-planting experiments were designed and conducted by Katri Himanen and Markku Nygren. The preparation of the inoculates and the microscopy of the EFM were conducted by Taina Pennanen. The statistical analyses were performed, and the first draft of the manuscript written by Katri Himanen. Markku Nygren assisted in statistical analysis. The manuscript was supplemented and commented by Markku Nygren and Taina Pennanen.

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Ethics declaration

Conflict of Interest The authors declare no conflict of interest.

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