Infection with foliar pathogenic fungi does not alter the receptivity of Norway spruce seedlings to ectomycorrhizal fungi

Velmala[1] SM, Rajala[1] T, Smolander[1] A, Petäistö[2] R-L, Lilja[1] A & Pennanen[1] T.

[1]Finnish Forest Research Institute – Metla, PO Box 18, 01301 Vantaa, Finland;
sannakajsa.velmala@metla.fi, tiina.rajala@metla.fi, aino.smolander@metla.fi, arja.lilja@metla.fi,
taina.pennanen@metla.fi; [2]Finnish Forest Research Institute – Metla, Suonenjoki, Finland,
raij.petaisto@gmail.com.

Author for correspondence:

Sannakajsa M. Velmala, Finnish Forest Research Institute, PO Box 18, 01301 Vantaa, Tel: +35829 5322580, Email: sannakajsa.velmala@metla.fi, Fax: +35829 5322103

Abstract

Aims: We studied whether the induction of defence against foliar pathogens affects the interaction of Norway spruce (*Picea abies*) with ectomycorrhizal fungi (EMF) and whether the response differs between seedlings originating from families showing variable growth performance in long-term trials.

Methods: The shoots were inoculated with *Botrytis cinerea* and *Gibberella avenacea.* The roots were simultaneously inoculated with sieved humus to provide the EMF inoculum. The severity of the pathogenic infection was based on the amount of damage and induced production of condensed tannins in the needles.

Results: EMF richness and colonisation were not affected by the pathogens and were also identical between the fast- and slow-growing seedlings. The fast-growing seedlings were more vulnerable to the pathogens; however, the constitutive level of condensed tannins in the needles did not correlate with their susceptibility to either the pathogenic or symbiotic fungi. *G. avenacea* induced a marginally greater production of condensed tannins in the slow-growing seedlings, which was linked to a slight reduction in EMF richness and less needle damage after wintering.

Conclusions: Our results suggest that there are differences in resource allocation strategies between the fast- and slow-growing spruce families, which may indicate the presence of underlying host effects that regulate interactions with associated fungi.

Introduction

As sessile organisms, coniferous trees rely on both constitutive and induced defence mechanisms against phytopathogenic microbes (Bonello et al. 2006). Many necrotising fungal pathogens infect container seedlings of Norway spruce (*Picea abies* (L.) Karst.), and a primary risk both during the growing period and after winter storage in frost is the common spruce pathogen *Botrytis cinerea* Pers. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel), which is the cause of grey mould disease (Petäistö et al. 2004; Petäistö 2006). *Fusarium* (anamorphic *Gibberella*) species are also widely distributed and cause damping-off and root rot diseases in nurseries (Hansen and Hamm 1988; Lilja et al. 1992) and infect the foliage of spruce saplings, resulting in needle death (Petäistö et al. 2012).

Damage by necrotising microbial attackers can lead to unspecific and comprehensive systemic responses distant to the actual location of damage and further primes defence reactions upon a second challenge (Becker and Conrath 2007). Systemic signals may be bidirectional as reported for the Austrian pine (*Pinus nigra* Arnold) (Blodgett et al. 2007) and asymmetric in time (Eyles et al. 2010). Moreover, the microbe induced systemic resistance may interfere with the mutualistic plant-microbe interactions in distal organs (Román et al. 2011; van Dam and Heil 2011) and may render the host even more vulnerable to other enemies (Heil and Baldwin 2002).

Both local and induced resistance have been reported in Norway spruce after infection with pathogenic fungi (Christiansen et al. 1999; Krokene et al. 2001; Swedjemark et al. 2007; Fossdal et al. 2012b; Nagy and Fossdal 2013) and beneficial microbes, such as ectomycorrhizal fungi (EMF) and mycorrhiza helper bacteria (Sampangi et al. 1986; Lehr et al. 2007; Likar and Regvar 2008; Nagy and Fossdal 2013) since also infection with mutualistic fungi suppresses the immune system of the host (Adomas et al. 2008; Heller et al. 2008; Nagy and Fossdal 2013). The resistance activation caused by pathogens may cause conflicting effects on fungal symbiosis with plants (van Dam and Heil 2011) depending on the symbiotic tissue (Blodgett et al. 2007) and the defence pathway it activates (de Román et al. 2011; Pfabel et al. 2012 etc.). Infecting fungi can produce phytohormones or induce the hormone production of the host plant (Robert-Seilaniantz et al. 2011). Infection with a necrotising fungus may activate the jasmonic acid and ethylene-based broad-spectrum nonspecific resistance, which may have positive effects on biotrophic associations such as the mycorrhizal colonization because jasmonic acid may suppress the endogenous salicylic acid - mediated biotroph-specific defence (van Dam and Heil 2011). Moreover, salicylic acid seems to support the production of cytokinins and gibberellins, and to antagonize auxin biosynthesis in

angiosperms , and this crosstalk of plant hormones shapes the physiological outcome of the pathogenic attack (Robert-Seilaniantz et al. 2011).

One important group of defensive phenolic substances are condensed tannins (proanthocyanidins), which are abundant in Norway spruce. These compounds are formed during the last step of flavonoid biosynthesis and function both in constitutive and induced defence. Condensed tannins form the major component of phenolics in needle mesophyll cells of Norway spruce (Soukupová et al. 2000) and different types of tannins inhibit the growth of several fungi and bacteria in pure culture (Kraus et al. 2003). They also appear to function as regulators of the fungal interactions of foliage, as reported from Fremont cottonwood (*Populus fremontii* S. Wats.) (Bailey et al. 2005) and Norway spruce (Rajala et al. 2014). The basal content of condensed tannins in trees is heritable; therefore, the genotype explains much of the intraspecific variation in the concentration of condensed tannins and other phenolic compounds, such as flavonoids (Mansfield et al. 1999; Lamhamedi et al. 2000; Evensen et al. 2000; Schweitzer et al. 2008; Henery 2008). Furthermore, the concentration of condensed tannins also varies depending on the organ, and the concentration is more adaptive in shoots and leaves compared to roots, where it is relatively stable (Kosola et al. 2006). Upon infection with fungal pathogens the phenolic biosynthesis is induced (Likar and Regvar 2008) and the accumulation of e.g. catechins, the building blocks of condensed tannins, has been reported in Norway spruce (Evensen et al. 2000). These simple phenolics are gradually converted to tannins and other insoluble polymers during an induced phenolic response (Brignolas et al.1995; Evensen et al. 2000).

Plant-mediated interactions between the above- and below-ground communities of microbes have been identified with angiosperms (de Román et al. 2011) and grasses (Mack and Rudgers 2008). Recently, we found a slight negative correlation between the abundance of fungal needle endophytes and the EMF richness of Norway spruce roots (Rajala et al. 2013). Also previously we have observed opposite relationships between the growth rates of 14-yr-old Norway spruce clones and the root and shoot associated fungal communities, such that the fast-growing spruces had higher EMF diversity (Korkama et al. 2006) but possessed significantly less saprotrophic needle endophytes (Korkama-Rajala et al. 2008) than the slow-growing spruces of equal age. However, based on our most recent studies, the ability to form ectomycorrhizas does not differ between the equally sized young spruce seedlings showing fast and slow growth performance later during their life span (Velmala et al. 2014). The associated fungi may cause trade-offs between growth, differentiation processes, and transferred resources as they bind host photosynthesised carbon and nutrients in the environment of limited resources (Herms and Mattson 1992). We have observed

some differences in resource allocation between seedlings of fast- and slow-growing Norway spruce origins (Velmala et al. 2014), and there might be differing needs of resource allocation between growth and defence of these contrasting seed families. The positive relationship observed between growth rate and the EMF richness of Norway spruce (Korkama et al. 2006) may be a result of genetically different use of resources or varying responses to fungal infection. The cause of differing EMF communities could also lie in the genetic susceptibility to pathogens, as it was shown that the genetic resistance/susceptibility to herbivory affected the EMF community structures of pinyon pines (*Pinus edulis* Engelm) (Sthultz et al. 2009).

To the best of our knowledge, no previous studies have addressed whether the induction of foliar defences in conifers extends to the roots and affects root-associated EMF symbiosis. Host-fungal relations may alter defence metabolism and may thereby affect tree resource allocation and further interfere with other plant-microbe associations. Furthermore, the observed differences in the associated fungal communities of seedlings with fast and slow growth during the later developmental stage could reflect differences in the interactions between distant fungal communities, including pathogens.

In our glasshouse experiment, we investigated whether the induction of foliar defences affect the ectomycorrhizal colonization and richness of Norway spruce. Therefore, two types of seedlings differing in long-term growth performance were infected with two necrotising fungal pathogens, *B. cinerea* and *Gibberella avenacea* R.J. Cook (Synonym: *Fusarium avenaceum* (Fr.) Sacc.). The experiment was designed to measure the responses of three well growing and three poorly growing Norway spruce seed families before any differences in growth rates are visible. We hypothesise that pathogen-induced stress will affect the ectomycorrhizal colonization and the EMF richness of Norway spruce and that the EMF richness is lower in seedlings that are either more resistant to fungal infection and thus foliar pathogens or show stronger induced responses to pathogenic infection. Moreover, we postulate that genetically different Norway spruce seedlings vary in their susceptibility to foliar pathogens and that the susceptibility to all fungal infections is higher in the fast-growing seedling. We propose that there will be differences in the carbon allocation as a response to pathogens and that the accumulation of condensed tannins varies in the needles after foliar infection in genetically different seedlings.

Materials and methods

Plant and fungal materials

The study was performed in the Suonenjoki nursery (62.625N, 27.122E) in eastern Finland with seven Norway spruce seed families from which three were classified as fast-growing (good and excellent growth) and three as slow-growing (stunted) families in long-term field trials (seed origins as in Velmala et al. 2014). The seventh spruce family represented seed-orchard-seeds used in forest regeneration in southern Finland (Online resource1 Table ESM1). In April 2011, the spruce seeds were germinated in nursery containers on unfertilised light sphagnum peat PP03 (Kekkilä, Vantaa, Finland) in a glasshouse. After six weeks, these seedlings were inoculated by transplantation into Plantek-81F containers (cell vol 85 cm^3) (BCC, Säkylä, Finland) filled with sieved forest humus layer, which acted as a natural source of EMF inoculum. The humus was excavated from the uppermost layer of fine sandy till of a Norway spruce stand out planted in 1993 at the nearby Ruotsinkylä research area located in southern Finland.

Inoculation with the two fungal pathogens and with tap water only were performed in June when the seedlings were indoors and were replicated in July and late August after the seedlings were moved outside in mid-June. Eighteen seedlings from each of the seven seed families were randomly placed in the three infection treatments. Before inoculation, the shoots were sprayed with tap water to moisten the foliage. The seedlings were inoculated with a spore suspension of either *Botrytis cinerea* isolate BcSjk1.1 (Petäistö et al. 2004) containing ca. 200 000 spores or the anamorphic spores of *Gibberella avenacea* [Pielavesi nursery isolate (*Fusarium avenaceum-G. avenacea*)] (Petäistö et al. 2012) containing ca. 90 000 spores. The inoculation occurred in June, July, and in August. In August the spore suspension of *G. avenacea* contained a fourfold amount of spores. The water only controls were treated with tap water by pipetting 200 µl of water terminally onto the seedlings. After each inoculation, the seedlings were maintained indoors in the glasshouse, and shoots were repeatedly sprayed with water to keep the foliage humid. Three days after the inoculation, the seedlings were moved outdoors into a nursery field. An EMF re-inoculation of roots was performed in August simultaneously with the foliar inoculations by adding 30 ml sieved forest humus to the base of each seedling.

The *B. cinerea* spores were liberated from two-week-old cultures grown at 17 °C (in darkness for 3 days and then moved to ambient light) on potato dextrose agar medium with sterile water rubbed with a glass rod. The *G. avenacea* spores were produced on autoclaved barley-corn with spruce and pine needle homogenate (Petäistö and Kurkela 1993; Winder 1999) and grown for three weeks at 17

°C in light, after which an orange spore mass was collected in sterile water by rubbing the plate with a glass rod. The number of spores in the filtered suspensions was estimated with a haemocytometer (Fuchs-Rosenthal, Paul Marienfeld GmbH & Co. KG, Germany). The vitality of each spore suspension was monitored with cultivation.

In general, the growth conditions of the seedlings during the summer adhered to the common seedling production practises in Finland. The seedlings were fertilised with approximately 10 mg N per seedling according to Kekkilä Forest-Superex (NPK 22-5-16) fertilisation program (Kekkilä, Vantaa, Finland). Wintering of the seedlings was performed in an open nursery field under natural snow cover.

Sampling

Seven replicate seedlings were sampled in late October after seven months of growth. The severity of infection was determined by counting the number of damaged needles per seedling. Thereafter, the shoots and roots were separated and the shoot heights measured. The level of EMF colonisation (%) in the roots and the root tip densities (tips/mm) and the numbers were assessed under a stereomicroscope. The following May, after wintering outdoors, the shoot length and number of damaged needles of the remaining seedlings (seven families, three treatments, seven replicate seedlings) were measured. Finally, after all sampling the roots and shoots were dried overnight at 60 ºC and weighed.

DGGE and sequence analysis

A randomised bulk sample (50 mg f.w.) of EMF fine root fragments from five replicate seedling was freeze dried and homogenised in quartz sand with a FastPrep® (FP120; Qbiogene, Cedex, France) and subjected to DNA extraction with NucleoSpin Plant II (Macherey-Nagel, Düren, Germany) columns according to the manufacturer's instructions. The roots were lysed using the CTAB lysis method based PL1 buffer and incubated for 30 min. Finally, the DNA was eluted with 50 µl and 100 µl PE buffer, precipitated with 0.6 vol of PEG-NaCl solution [20% PEG (w/v), 2.4 M NaCl] on ice for 20 min, centrifuged (16 000 *g* for 20 min) and washed with 70 % ethanol. The dry pellets were resuspended in diluted 30 µl TE buffer (1.5 mM Tris/HCl, 0.25 mM EDTA) and stored at -20 °C.

To verify that the infection with the foliar pathogens was successful, we randomly selected eight seedlings from both disease treatments and isolated the DNA from 80 mg (f.w.) needles. The freeze dried needles were homogenised with two nuts and a screw using a FastPrep® (FP120; Qbiogene,

Cedex, France), and then subjected to the NucleoSpin Plant II (Macherey-Nagel, Düren, Germany) DNA extraction protocol followed by PEG precipitation.

We used denaturing gradient gel electrophoresis (DGGE) (Muyzer et al. 1993) to analyse the fungal communities, which is based on the amplification of the internal transcribed spacer 1 (ITS1) region of rDNA (Andersson et al. 2003). The DGGE PCR products were generated with the primers ITS1F (Gardes and Bruns 1993) with a 40 bp GC-clamp (Muyzer et al. 1993) and ITS2 (White et al. 1990). The cycling was performed with DreamTaq™ Green polymerase (Thermo Scientific, Waltham, MA, USA) in a reaction volume of 50 µl according to the manufacturer's instructions. The thermal cycling conditions were as follows: an initial denaturation of 3 min at 95 °C followed by 34 cycles of 30 s at 95 °C, 30 s at 57 °C, 60 s at 72 °C, and a final extension of 72 °C for 5 min. The PCR products were electrophoresed with the DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, CA, USA) in an acrylamide denaturing 18–58 % gradient gel (75 V, 60 °C, 16 h), stained with SYBR® Gold (Molecular Probes®; Life Technologies, Carlsbad, CA, USA) and read under a SafeImager™ transilluminator (Invitrogen™; Life Technologies, Carlsbad, CA, USA) blue light. Image analysis and a binary matrix of band motility classes (occurrence of operational taxonomic units, OTUs) were performed with the GelCompar II software (Applied Maths NV, Sint-Martens-Latem, Belgium), version 5.1 with band matching optimisation of 0% and position tolerance of 1%.

The identification of various OTUs representing the fungal community was based on Sanger sequencing of at least two excised bands per OTU. Each DGGE band of interest was repeatedly excised two to four times until the contaminant sequences could not be detected in the DGGE gel. The pure bands were eluted with water and used as a template for PCR with the ITS1F and ITS2 primers, with 25 cycles as described above. The PCR fragments were purified and sequenced by the Macrogen Sequencing Service (Macrogen Europe, The Netherlands) using the ITS1F-primer. The sequences were revised in Geneious R6 (Biomatters, Auckland, New Zealand, available from http://www.geneious.com/). The OTUs were identified by comparing the sequences (Online resource1 Table ESM2; GenBank accession numbers KJ909938–KJ909957) against sequences in UNITE (http://unite.ut.ee/) and the INSD database.

Measurement of condensed tannins

The dry needles were homogenised with a FastPrep® in 2-ml polypropylene tubes with FastPrep®matrix A ceramic spheres and subjected to condensed tannin analysis using a spectrophotometric, modified acid-butanol assay (proanthocyanidin assay, Waterman & Mole 1994)

as described by Kanerva et al. (2008). Briefly, the assay consists of a 70% acetone extraction followed by HCl-catalysed depolymerisation of the condensed tannins in butanol to yield a pink-red anthocyanidin product. However, in the present study, the extraction (3x5min, 40 ºC) of 0.1 g homogenised needle material was performed with the Accelerated Solvent Extraction equipment (ASE-350, Dionex, USA). As a calibrator, we used the condensed tannins extracted and purified from Norway spruce needles according to the method of Kanerva et al. (2006).

Statistical analyses

To determine the differences in the growth parameters, the number of damaged needles, the condensed tannin content in the needles, the levels of EMF colonisation and the EMF OTU richness of the fast- and slow-growing seedlings in different treatments, we used linear and generalised linear mixed models {lmer, glmer; **lme4**} (Bates et al. 2013). In the first model, the effect of the growth group (fast or slow) on various parameters was studied separately in all treatments, and the growth performance group was set as the fixed effect and seed origin was set as the random effect. The second model solely evaluated the effect of treatment and did not distinguish between the fast and slow growing seedlings; therefore, the treatment was set as the only fixed effect. The third model included the treatment, growth performance group and their interaction as the fixed factors. By including the interaction of the growth type and treatment, we determined whether the response to treatment varied between the growth types. The interaction term was omitted from the model when it was not at least marginally significant (P-value > 0.1). Seedling growth and needle damage between autumn and spring were calculated with the growth performance group and year as the fixed effects. The seed family was set as random in all of the models described above. The P-values for the lmer-objects were calculated with the Satterthwaite approximation (Satterthwaite 1946) for degrees of freedom {**lmerTest}** (Kuznetsova et al. 2012). For the counts, such as the OTU richness and root tip number, we used a Poisson error distribution, and for the percentage, the binomial error distribution. Wald chi-square likelihood-ratios were generated with {Anova; **car**} (Fox and Weisberg 2011).

To assess the EMF community composition, a Bray-Curtis distance matrix (Bray and Curtis 1957) was generated and exposed to 2-dimensional non-metric multidimensional scaling (NMDS) {metaMDS: vegan} (Oksanen et al. 2013). The lowest stress was achieved through replicating the loop 20 times with a maximum number of 40 starts. We also addressed the effect of seed origin and other measured traits on the multivariate community data of the EMF with permutational

MANOVA with 4999 permutations and the seed origin as the stratum {adonis: vegan} (Oksanen et al. 2013).

The linear relationships between the different traits were analysed using Pearson's correlation statistics. All statistical analyses were performed in R 2.15.3 (R Development Core Team 2013), and the graphs were created with packages ggplot2 (Wickham 2009) and vegan (Oksanen et al. 2013).

Results

Ectomycorrhizal fungal colonization and community composition

The inoculation with natural humus was successful, and the level of EMF colonisation (%) in the roots of Norway spruce seedlings was greater than 80% (Table 1). In the slow-growing seedlings, the condensed tannin content in the needles positively correlated with the root EMF colonisation % in all treatments (*G. avenacea*: t_{19}=3.40, r =0.61, P =0.003), (water only control t_{19}=1.70, r =0.36, P =0.106), (*B. cinerea*: t_{19}=2.57, r =0.51, P =0.019) and the induced production of condensed tannins for the *G. avenacea* (t_{19}=3.87, r =0.66, P =0.001) and *B. cinerea* (t_{19}=2.18, r =0.45, P =0.04) foliar treatments. However, in the fast-growing seedlings, EMF colonisation (%) positively correlated with the condensed tannin content (t_{19}=2.85, r =0.55, P =0.010) and the induced level of condensed tannins (t_{19}=2.38, r =0.48, P =0.03) only when the seedlings were infected with *B. cinerea*.

The EMF OTU richness was not affected by the constitutive level of condensed tannins or the fungal pathogen induced resistance in the foliage and was similar in all treatments and for both growth performance groups (Table 1). Of the slow-growing families, the EMF OTU richness positively correlated with the constitutive level of condensed tannins in the needles (water only control: t_{13}=2.76, r =0.60, P =0.02). The EMF OTU richness was lowest in the *G. avenacea* treatment, but the reduction was not statistically significant (Table 1). Furthermore, there was a marginally significant negative correlation between the *G. avenacea* induced production of condensed tannins in the needles and the EMF OTU richness in the roots of the slow-growing seedlings (*G. avenacea*: t_{13}=-1.89, r =-0.46, P =0.08). Of the fast-growing seedlings, the EMF OTU richness negatively correlated with the root biomass (*G. avenacea*: t_{13}=-2.24, r =-0.53, P =0.04), but the other root growth traits did not have any linear relationships with the EMF OTU richness.

The EMF community comprised Atheliaceaes, such as *Amphinema byssoides*, *Tylospora asterophora* and *Piloderma sphaerosporum*, as well as *Thelephora terrestris*, *Lactarius rufus*, and

Cenococcum geophilum (Fig. 2). Two of the sequences corresponded to uncultured environmental EMF root tip sequences in the UNITE database, and two other OTUs corresponded to ectomycorrhiza associated fungi (Archaeorhizomyces (Rosling et al. 2011) and fungi inhabiting *Cenococcum sp.* ectomycorrhizae). The other fungal OTUs corresponded to ascomycoteous moulds and saprotropic soil fungi (*Mortierella, Cryptococcus, Ilyonectria, Trichosporon*, and *Fomitopsis* species). All non-EMF OTUs were omitted from the EMF OTU count, but the total DGGE-based fungal OTU richness is reported in Table 1.

The EMF community structures of the fast- and slow-growing Norway spruce seedlings greatly overlapped and were independent of treatment (Fig. 2). There was a weak linear correlation between the shoot:root ratio and the 2-dimensional ordination of the EMF community (r^2=0.077, P =0.03) (Fig. 2). Based on the permutational multivariate ANOVA, over 95% of the variation in the EMF community remained unexplained with the measured traits.

Growth

As expected (Velmala et al. 2014), there were no significant differences in the growth between seedlings of fast and slow origins during their first year of growth (Table 1, Online resource1 Table ESM3). The shoot and root biomass had a strong and systematic positive relationship in the entire dataset (t_{145}=18.96, r=0.84, P <0.001). The foliar infection with *Botrytis cinerea* and *Gibberella avenacea* increased the root (F_2=6.186, P=0.002) and shoot (F_2=4.378, P=0.015) biomass of the seedlings compared to the uninfected water only control treatment in autumn (Table 1). Seedling growth was significantly affected by the necrotising treatment with *B. cinerea* (Table 1), thereby decreasing the shoot:root ratio (F_2=7.867, P<0.001). The shoot height was differentially affected by the necrotising pathogens for both the fast- and slow-growing seedlings. The fast-growing seedlings were taller both in the water only control and *B. cinerea* treatments, but by contrast, for the *G. avenacea* treatment, the slow-growing seedlings were taller (significant interaction of treatment and growth type F_2=3.251, P=0.042). The seedlings that were left overwintering did not show any differences in shoot height the following spring either between the treatments (F_2=0.487, P=0.616) or growth performance groups (F_1=1.947, P=0.235). Additionally, the shoot height did not differ significantly between the autumn and spring samplings (F_1=0.63, P =0.434, Table 1).

The root tip number of fast- and slow-growing seedlings was differentially affected by the foliar treatment (significant interaction of treatment and growth type X^2_2=419.0, P<0.001, Table 1). The slow-growing seedlings had higher root tip number than the fast-growing seedlings for both the water only control (F_1=6.86, P=0.012, Table 1) and *B. cinerea* treatments (F_1=3.17, P=0.082, Table

1), whereas for the *G. avenacea* treatment, no differences in the short root densities were observed. There were no linear correlations between the root biomass and short root density for either growth performance group in any of the treatments. Regardless of the treatment, the root tip density of the seed-orchard seedlings (s1002) was similar to that of the fast-growing seedlings (Table 1).

Susceptibility to foliar pathogens: Constitutive and induced defence

Foliar pathogens, *B. cinerea* and *G. avenacea*, led to a successful infection and were identified in the necrotic needles by visual necrosis and sequence analysis (Online resource1 Table ESM2). The necrotising pathogens caused only moderate foliar damage, resulting in an increased number of dead needles (X^2_2=81.299, P<0.001, Table 1). The necrotic infections proceeded slowly throughout late summer and autumn, and during the winter the number of dead needles had increased significantly (X^2_1=61.32, P<0.001, Table 1) for all treatments. During the autumn, the only difference in the number of damaged needles was between the water only control treatment and necrotising fungal treatments, and between the spruce families (Fig. 1). Overwintering revealed visible differences in the number of dead needles between the growth performance groups. The fast-growing seedlings had significantly higher number of necrotic needles in the *G. avenacea* treatment group than the slow-growing seedlings (X^2_1=10.173, P=0.001, Table 1).

The amount of condensed tannins in the needles did not vary statistically significantly between the seedlings of different origin or between the treatments (Fig. 1, Table 1). The condensed tannin content in the fast-growing seedlings correlated negatively with the needle damage during the autumn for the *G. avenacea* infection (t_{19}=-2.53, r=-0.5, P =0.02), and marginally significantly for the infection with *B. cinerea* (t_{19}=-1.77, r=-0.38, P =0.09). The foliar treatment did not significantly induce the production of condensed tannins in needles in the entire dataset, partially due to high within family variation for the level of condensed tannins. On average, the increase in the condensed tannin content for the *B. cinerea* infection was 12% for the fast-growing seedlings and 21% for the slow-growing seedlings. These values were 4% and 22%, respectively, for the *G. avenacea* infection (Fig. 1, Table 1). This induced level of condensed tannins was assessed by dividing the concentration of condensed tannins under foliar treatment with the average concentration of condensed tannins in the water only control treatment separately for each seed origin.

For the *G. avenacea* infection, the root biomass of the slow-growing seedlings positively correlated with the concentration of condensed tannins in the needles (t_{19}= 2.31, r = 0.61, P <0.01) and the induced level of tannins (t_{19}= 3.01, r = 0.57, P <0.01). The condensed tannins did not have an

unambiguous linear correlation with the short root density; however, the fast-growing seedlings infected with *G. avenacea* showed a positive relationship between the concentration of condensed tannins and the total number of root tips (t_{19}= 2.31, r = 0.46, P =0.03).

Discussion

Contrary to our hypothesis, the Norway spruce seedlings were similarly receptive to EMF for all treatments. The EMF richness of the fast- and slow-growing seedlings did not differ, as in our previous study with pure culture EMF inoculation of spruce seedlings with identical origins (Velmala et al. 2014). The induced defences against the common grey mould *Botrytis cinerea* and *Gibberella avenacea* did neither significantly alter the EMF associations in the roots, even though induced defences triggered by foliar pathogenic fungi have repetitiously been reported in Norway spruce (Krokene et al. 2001; Nagy et al. 2004; Swedjemark et al. 2007). It is good to keep in mind that the DGGE based EMF OTU richness is an estimate of the true EMF richness, and it was not possible to partition ectomycorrhizal abundances. Moreover we might have missed rare EMF species, since pooling and PCR based techniques do not necessarily recover real species richness and abundance of the fungal communities (Avis et al. 2010).

As hypothesised, the susceptibility to pathogenic fungal infection varied between the spruce families and between the fast-and slow-growing seedlings when the follow-up period was extended over winter. The fast-growing seedlings were slightly more susceptible to foliar damage than the slow-growing seedlings, particularly for the *G. avenacea* infection. During defence activation, resources that could be used for growth are redirected to defence, thereby creating a trade-off of resources between growth and defence (Eyles et al. 2010; Schultz et al. 2013). Nevertheless, in the present study, the foliar infection and consequent production of defence compounds did not have any relevant cost for seedling growth during the first year. This may be explained by the rapid sink-source transitions between the carbon sinks created by various fungal points of infection (both ectomycorrhizal and pathogenic fungi) along the plant (Schultz et al. 2013). In fact we observed a positive relationship between growth and infection with foliar pathogenic fungi, and when exposed to foliar stress by *B. cinerea,* the seedlings invested more resources belowground compared with the uninfected water only control treatment. Some of these growth effects may result from changes in hormone concentrations or ratios as phytohormones are suggested to be important players in carbohydrate pathways and in dry-mass partitioning between shoots and roots (Rook and Bevan 2003). Changes in hormone balance, stimulated or produced by the fungal pathogen, may have

drastic effects; Exogenous gibberellin treatment of angiosperms have shown to increase growth and also salicylic acid production, which in turn reduces jasmonic acid content and leads to increased susceptibility to necrotizing fungi (Robert-Seilaniantz et al. 2011). Furthermore, reductions of cytokinins, involved in the regulation of shoot and root growth, increase the ratio of auxin and cytokinins which have been observed to stimulate root growth (Thomas et al. 1995). Plants also have the tendency to respond to pathogenic attack by moving valuable resources away from the diseased tissue (Schultz et al. 2013), as is a commonly outcome reported for foliar stresses (Barto and Rilling 2010). In Velmala et al. (2014) we showed that after an intensive EMF inoculation, the fast-growing seedlings invested slightly more carbon in the belowground biomass compared to the shoot biomass than the slow-growing seedlings. In the present study the slow-growing seedlings responded more robustly to foliar infection and were less damaged by the foliar pathogens after winter. They also showed a marginal decrease in EMF richness after *G. avenacea* infection. It is unlikely that *G. avenacea* reduced the ability of the seedling to support root symbiotic fungi via a reduction in the photosynthetic capacity because the degree of foliar damage caused was very moderate (approximately 5%) and because removal of the photosynthetic tissue will not reduce but rather boost the carbon flow belowground (Barto and Rillig 2010). Therefore, the pathogen-triggered defence mechanisms in the shoots of slow-growing Norway spruce seedlings may have been stronger than the defence mechanisms of the fast-growing seedlings.

The pathogen-triggered defence measured by the production of condensed tannins in the needles was relatively weak. The synthesis of phenols, such as condensed tannins, is very energy intensive and plants may prefer the production of other defence signalling molecules, such as salicylic acid (more in Pfabel et al. 2012). Our study supports the view that conifers show very little response to foliar induced defences compared to broadleaf trees, because they primarily rely on constitutive defence and contain high levels of defensive secondary metabolites which decrease the need of induced resistance (Wagner 1988; Mattson et al. 1988; Henery 2008). Notably, we measured the constitutive and induced defence only in the needles because they are adaptive organs (Kosola et al. 2006) and we do not know whether the foliar treatments affected the phenol content of the roots. The induced systemic resistance has shown to move from the roots to the shoots in Norway spruce as e.g. root inoculation with the mycorrhiza helper bacterium *Streptomyces* sp. increased the needle resistance of Norway spruce against *B. cinerea* (Lehr et al. 2007). However the systemic signal is not necessarily bidirectional because in our study, the *B. cinerea* infection had no effect on the EMF richness in the roots, and exposure to *G. avenacea* only marginally reduced the EMF richness in the slow-growing seedlings. Similar results from a field experiment were obtained for *Phytophthora*-

408 infected chestnut (*Castanea sativa* Mill.), in which no significant differences were observed for the
409 EMF communities between healthy and infected trees (Blom et al. 2009). Moreover, the negative
410 effects of systemic resistance of soy (*Glycine max* (L.) Merr.) on its mycorrhizal colonization has
411 shown to be temporary and last only a short period. Additionally, the established mycorrhizas show
412 no response to the induction of resistance (de Román et al. 2011). We performed the EMF
413 inoculation and foliar infections simultaneously, which may explain why we observed no long-term
414 effects of induced defence on the associated EMF community of Norway spruce seedling roots.

415 Norway spruce has a multifaceted defence system and single phenolic markers for resistance are
416 difficult to assess (Fossdal et al. 2012a). Similar to studies with strawberry (*Fragaria* x *ananassa*
417 Duch.) (Hébert et al. 2002) and grapevine (*Vitis vinifera* L.) (Iriti et al. 2005), the increase of
418 condensed tannins in the needles seemed to be a suitable marker for resistance activation against *B.*
419 *cinerea* and *G. avenacea*. However, the condensed tannins had contradictory relationships with the
420 different fungal species. Rajala et al. (2014) suggested that the condensed tannins reduced the
421 fungal richness in the needles most likely by excluding some species more than others.
422 Correspondingly, resistant tree clones do not always contain higher concentrations of phenols, such
423 as condensed tannins or flavonoids than the susceptible clones (Henery 2008; Evensen et al. 2000).
424 In poplar (*Populus trichocarpa x deltoides*) an increase in the proportion of condensed tannins in
425 leaves has been reported after EMF infection (Pfabel et al. 2012). In our study, the constitutive
426 condensed tannin content in the needles of the fast-growing seedlings did not prevent the *G.*
427 *avenacea* infection, and the condensed tannin concentration had even a positive relationship with
428 the EMF colonisation % in the unstressed seedlings. Therefore, rather than acting as broad-
429 spectrum antifungal components, an adequate level of condensed tannins may indicate good health
430 and vitality of the seedling. Healthy plants can remain in a stressful environment longer but will
431 eventually succumb to the pathogen if they are not resistant.

432 Fisher et al. (2006) suggested that there might be a genetically based relationship between fine root
433 production and the content of foliar condensed tannins in poplar (*Populus angustifolia* James and *P.*
434 *fremontii* Watson) that may have adaptive significance. In this study, the fast-growing seedlings
435 showed this type of connection under *G. avenacea* stress, in which the seedlings with high levels of
436 condensed tannins in their shoots grew more fine roots. This trait may have adaptive significance in
437 nature because increased fine root production has been suggested to secure nutrient gain under
438 stressed conditions (e.g., Nadelhoffer et al. 1985, Hendricks et al. 2000). In general, the fine root
439 architecture of the seedlings in the uninfected water only control treatment was as expected based
440 on our previous study Velmala et al. (2014), and the slow-growing seedlings had denser root

systems and higher root tip density than the fast-growing seedlings. The necrotising pathogen attacks levelled this difference such that the root tip density of the slow-growing seedlings decreased to that of the fast-growing and seed-orchard (s1002) seedlings.

To conclude, we demonstrate that the induction of foliar defence does not affect the root-associated EMF community of Norway spruce seedlings. There was also no direct and unambiguous relationship between the susceptibility to foliar fungal infection and the belowground EMF species richness of the seedlings. However, certain differences between the spruce groups were observed: The fast-growing seedlings were more damaged by the necrotising foliar pathogens, and the high concentration of condensed tannins in the needles of fast-growing seedlings did not provide resistance against *B. cinerea* and *G. avenacea*. The slow-growing seedlings were less damaged by the pathogens, and they showed a somewhat stronger response to pathogenic fungi in the needles. Moreover, the *G. avenacea* triggered defence appeared to be harmful to the root associated EMF symbionts of slow-growing seedlings because the defence response marginally significantly reduced the EMF richness. These trends indicate differing strategies of resource allocation between fast- and slow-growing spruce families which may indicate underlying host effects for the selection of symbionts for the spruce.

Acknowledgments

This work was funded by the Academy of Finland (project 128229) and Otto A. Malm foundation. We are grateful to the staff of the Suonenjoki nursery, M-L Napola, M-L Jalkanen, R Kattainen, M Oksanen, A Rautiainen, H Ruhanen, S Ullah, and R Vesala.

References

Adomas A, Heller G, Olson Å, Osborne J, Karlsson M, Nahalikova J, Zyl LV, Sederoff R, Stenlind J, Finlay R, Asiegbu FA (2008) Comparative analysis of transcript abundance in *Pinus sylvestris* after challenge with a saprotrophic, pathogenic, or mutualistic fungus. Tree Physiol 28:885–897

Andersson IC, Campbell CD, Prosser JI (2003) Diversity of fungi in organic soils under a moorland – Scots pine (*Pinus sylvestris* L.) gradient. Environ Microbiol 5:1121–1132

Avis PG, Branco S, Tang Y, Mueller GM. 2010. Pooled samples bias fungal community descriptions. Mol Ecol Resour 10: 135 141

Bailey JK, Deckert R, Schweitzer JA, Rehill BJ, Lindroth RL, Gehring C, Witham TG (2005) Host plant genetics affect hidden ecological players: links among Populus, condensed tannins, and fungal endophyte infection. Can J Bot 83: 356–361

Barto EK, Rillig MC (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. J Ecol 98:745–753

Bates D, Maechler M, Bolker B, Walker S (2013) Linear mixed-effects models using Eigen and S4. Version 1.0-5 http://cran.r-project.org/web/packages/lme4/lme4.pdf Accessed 2 December 2013

Beckers GJM, Conrath U (2007) Priming for stress resistance: from the lab to the field. Curr Opin Plant Biol 10:425–431

Blodgett JT, Eyles A, Bonello P (2007) Organ-dependent induction of systemic resistance and systemic susceptibility in *Pinus nigra* inoculated with *Sphaeropsis sapinea* and *Diploidia scrobiculata*. Tree Physiol 27:511–517

Blom JM, Vannini A, Vettraino AM, Hale MD, Godbold DL (2009) Ectomycorrhizal community structure in a healthy and a Phytophthora-infected chestnut (*Castanea sativa* Mill.) stand in central Italy. Mycorrhiza 20:25–38

Bonello P, Gordon TR, Herms DA, Wood DL, Erbilgin N (2006) Nature and ecological implications of pathogen-induced systemic resistance in conifers: A novel hypothesis. Physiol Mol Plant P 68:95–104

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 27: 325–349

Brignolas F, Lacroix B, Lieutier F, Saucars D, Drouet A, Claudot A-C, Yart A, Berryman AA, Christiansen E.1995. Induced responces in phenolic metabolism in two Norway spruce clones after wounding and inoculation with *Ophiostoma polonicum*, a bark beetle-associated fungus. Plant Physiol 109:821-827

Christiansen E, Krokene P, Berryman AA, Franceschi VR, Krekling T, Lieutier F, Lönneborg A, Solheim H (1999) Mechanical injury and fungal infection induce acquired resistance in Norway spruce. Tree Physiol 19:399–403

van Dam NM, Heil M (2011) Multitrophic interactions below and above ground: en route to the next level. J Ecol 99:77–88

Evensen PC, Solheim H, Høiland K, Stenersen J (2000) Induced resistance of Norway spruce, variation of phenolic compounds and their effects on fungal pathogens. For Path 30:97–108

Eyles A, Bonello P, Ganley R, Mohammed C (2010) Induced resistance to pests and pathogens in trees. New Phytol 185:893–908

Fisher DG, Hart SC, Rehill BJ, Lindroth RL, Keim P, Whitham TG (2006) Do high-tannin leaves require more roots? Oecologia 149:668–675

503 Fossdal CG, Nagy NE, Hietala AM, Kvaalen H, Slimestad R, Woodward S, Solheim H (2012a) Indications
504 if heightened constitutive or primed host response affecting the lignin pathway transcripts and
505 phenolics in mature Norway spruce clones. Tree Physiol 32:1137–1147

506 Fossdal CG, Yaqoob N, Krokene P, Solheim H, Yakolev A (2012b) Local and systemic changes in
507 expression of resistance genes, nb-lrr genes and their putative microRNAs in Norway spruce after
508 wounding and inoculation with the pathogen Ceratocystis polonica. BMC Plant Biol 12:105–115

509 Fox J, Weisberg S (2011) Companion to Applied Regression 2nd edition, Sage,Thousand Oaks, CA,
510 http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

511 Gardes M, Burns TD (1993) ITS primers with enchanced specificity for basidiomycetes —application to the
512 identification of mycorrhizae and rusts. Mol Ecol 2:113–118

513 Hansen EM, Hamm PB (1988) Canker diseases of Douglas-fir seedlings in Oregon and Washington bareroot
514 nurseries. Can J Forest Res 35:432–439

515 Hébert C, Charles MT, Gauthier L, Willemot C, Khanizadeh S, Cousineau J (2002) Strawberry
516 proanthocyanidins: biochemical markers for *Botrytis cinerea* resistance and shelf-life predictability.
517 ISHS Acta horticulare 567: IV International Strawberry Symposium.

518 Heil M, Baldwin IT (2002) Fitness cost of induced resistance: emerging experimental support for a slippery
519 concept. Trends Plant Sci 7:61–67

520 Heller G, Adomas A, Li G, Osborne J, van Zyl L, Sederoff R, Finlay RD, Stenlid J, Asiegbu FO (2008)
521 Transcriptional analysis of *Pinus sylvestris* roots challenged with the ectomycorrhizal fungus *Laccaria*
522 *bicolor*. BMC Plant Biol 8:19

523 Hendricks JJ, Aber JD, Nadelhoffer KJ, Hallett RD (2000) Nitrogen Controls on Fine Root Substrate Quality
524 in Temperate Forest Ecosystems. Ecosystems 3:57–69

525 Henery ML, Wallis IR, Stone C, Foley WJ (2008) Methyl jasmonate does not induce changes in *Eucalyptus*
526 *grandis* leaves that alter the effect of constitutive defences on larvae of specialist herbivore. Oecologia
527 156: 847–859

528 Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335

529 Iriti M, Rossoni M, Borgo M, Ferrara L, Faoro F (2005) Induction of Resistance to Gray Mold with
530 Benzothiadiazole Modifies Amino Acid Profile and Increases Proanthocyanidins in Grape: Primary
531 versus Secondary Metabolism. J Agr Food Chem 53: 9133–9139

532 Kanerva S, Kitunen V, Kiikkilä O, Loponen J, Smolander A (2006) Response of soil C and N
533 transformations to tannin fractions originating from Scots pine and Norway spruce needles. Soil Biol
534 Biochem 38: 1364–1374

535 Kanerva S, Smolander A (2008) How do coniferous needle tannins influence C and N transformations in
536 birch humus layer? Eur J Soil Biol 44:1–9

537 Korkama T, Pakkanen A, Pennanen T (2006) Ectomycorrhizal community structure varies among Norway
538 spruce (*Picea abies*) clones. New Phytol 171:815–824

539 Korkama-Rajala T, Müller M, Pennanen T (2008) Decomposition and fungi of needle litter from slow- and
540 fast-growing Norway spruce (*Picea abies*) clones. Microb Ecol 56:76–89

541 Kosola KR, Parry D, Workmaster BAA (2006) Responces to condensed tannins in poplar roots to
542 fertilization and gypsy moth defoliation. Tree Physiol 26: 1607–1611

543 Kraus TEC, Dahlgren RA, Zasoski RJ (2003) Tannins in nutrient dynamics of forest ecosystems - a review.
544 Plant Soil 256:41–66

545 Krokene P, Solheim H, Christiansen E (2001) Induction of disease resistance in Norway spruce (*Picea abies*)
546 by necrotizing fungi. Plant Pathol 50:230–233

547 Kuznetsova A, Brockhoff PB, Christensen RHB (2012) Tests for random and fixed effects for linear mixed
548 effects models (lmer objects of lme4 package). Version 2.0-3 http://cran.r-
549 project.org/web/packages/lmerTest/lmerTest.pdf Accessed 12.12.2013.

550 Lamhamedi MS, Chamberland H, Bernier PY, Tremblay FM (2000) Clonal variation in morphology,
551 growth, physiology, anatomy and ultrastructure of container-grown white spruce somatic plants Tree
552 Physiol 20:869-880

553 Lehr NA, Schrey SD, Bauer R, Hampp R, Tarkka MT (2007) Suppression of plant defence response by a
554 mycorrhiza helper bacterium. New Phytol 174:892–903

555 Likar M, Regvar M (2008) Early defence reactions in Norway spruce seedlings inoculated with the
556 mycorrhizal fungus *Pisolitus tinctorus* (Persoon) Coker & Couch and the pathogen *Heterobasidion*
557 *annosum* (Fr.) Bref. Trees 22: 861–868

558 Lilja A, Lilja S, Poteri M, Ziren L. 1992. Conifer seedling root fungi and root dieback in Finnish nurseries.
559 Scan J Forest Res 7: 547-556.

560 Mack KML, Rudgers JA (2008) Balancing multiple mutualists: asymmetric interactions among plants,
561 arbuscular mycorrhizal fungi, and fungal endophytes. Oikos 117: 310–320.

562 Mansfield JL, Curtis PS, Zak DR, Pregitzer KS (1999) Genotypic variation for condensed tannin production
563 in trembling aspen (*Populus tremuloides*, salicaceae) under elevated CO2 and in high- and low-
564 fertility soil. Am J Bot 86:1154–1159

565 Mattson WJ, Lawrence RK, Haack RA, Herms DA, Charles PJ (1988) Defensive strategies of woody plants
566 against different insect-feeding guildsin relation to plant ecological strategies and intimacy of
567 association with insects. In: Mechanisms of woody plant defences against insects: Search for pattern
568 pp. 3–38, Springer-Verlag, NY, US

569 Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturating
570 gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S
571 rRNA. Appl Environ Microb 59:695–700

572 Nagy NE, Fosdahl CG (2013) Host responses in Norway spruce roots induced to the pathogen *Ceratocystis*
573 *polonica* are evaded or suppressed by the ectomycorrhizal fungus *Laccaria bicolor*. Plant Biol 15: 99–
574 110

575 Nagy NE, Fossdahl CG, Krokene P, Kreckling T, Lönneborg A, Solheim H (2004) Induced responces to
576 pathogen infection in Norway spruce phloem: changes in polyphenolic parenchyma cells, chalcone
577 synthase transcript levels and peroxidase activity. Tree Physiol 24: 505–515

578 Nadelhoffer KJ, Aber JD Melillo JM (1985) Fine Roots, Net Primary Production, and Soil Nitrogen
579 Availability: A New Hypothesis. Ecology 66:1377–1390

580 Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens
581 MHH, Wagneret H (2013) Community Ecology Package. Version 2.0-10 http://cran.r-
582 project.org/web/packages/vegan/vegan.pdf Accessed 16.12.2013.

583 Petäistö RL (2006) *Botrytis cinerea* and Norway spruce seedlings in cold storage. Baltic Forestry 12:24–33

584 Petäistö RL (2012) Effects of light and winter storage conditions on Norway spruce seedlings with special
585 emphasis on the occurance of *Gibberella avenacea*. Baltic Forestry 18:73–82

586 Petäistö RL, Heiskanen J, Pulkkinen A (2004) Susceptibility of Norway spruce seedlings to grey mould in
587 the greenhouse during the first growing season. Scan J Forest Res 19:30–37

588 Petäistö RL, Kurkela T (1993) The susceptibility of Scots pine seedlings to *Gremmeniella abietina*: effect of
589 growth phase, cold and drought stress. Eur J Forest Pathol 23:385–399

590 Pfabel C, Eckhardt KU, Baum C, Struck C, Frey P, Weih M (2012) Impact of ectomycorrhizal colonization
591 and rust infection on the secondary metabolism of poplar (*Populurs trichocarpa* x *deltoides*). Tree
592 Physiol 32: 1357–1364

R Development Core team (2013) R: a language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria

Rajala T, Velmala SM, Smolander A, Pennanen T (2014) The community of needle endophytes reflects the current physiological state of Norway spruce. Fungal Biol 118:309–315

Rajala T, Velmala SM, Tuomivirta T, Haapanen M, Müller M, Pennanen T (2013) Endophyte communities vary in needles of Norway spruce clones. Fungal Biol 117:182–190

Robert-Seilaniantz A, Grant M, Jones JDG. 2011. Hormone crosstalk in plant disease and defense: More than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49:317–343

de Román M, Fernández I, Wyatt T, Sahrawy M, Heil M, Pozo MJ (2011) Elicitation of foliar resistance mechanisms transiently impairs root association with arbuscular mycorrhizal fungi. J Ecol 99: 36–45

Rook F, Bevan MW. 2003. Genetic approaches to understanding sugar-response pathways. J Exp Bot 54:495–501

Rosling A, Cox F, Cruz-Martinez K, Ihrmark K, Grelet GA, Lindahl BD, Menkis A, James TY (2011) Archaeorhizomycetes - a class of ancient, widespread soil fungi. Science 333:879–879

Sampangi R, Perrin R, Le-Tacon F (1986) Disease suppression and growth promotion of Norway spruce and Douglas-fir seedlings by the ectomycorrhizal *Laccaria laccarata* in forest nurseries. In: Mycorrhizae: physiology and genetics, Ed. Gianinazzi-Pearson V and Gianinazzi S, 1st ESM, Dijon, 1-5 July 1985, INRA, Paris, 799–806

Satterthwaite FE (1946) An Approximate Distribution of Estimates of Variance Components. Biometrics Bull 2: 110–114

Schultz JC, Appel HM, Ferrieri, AP, Arnold TM (2013) Flexible resource allocation during plant defense. Front Plant Sci 4:324.

Schweitzer JA, Madritch MD, Bailey JK, LeRoy CJ, Fischer DG, Rehill BJ, Lindroth RL, Haregman AE, Wooley SC, Hart SC, Whitham TG (2008) From genes to ecosystems: The genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. Ecosystems 11:1005–1020

Sthultz CM , Whitham TG, Kennedy K, Deckert R, Gehing CA (2009) Genetically based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a foundation tree species. New Phytol 184:657–667

Soukupová J, Cvikrova C, Albrechtová J, Rock BN, Eder J. 2000. Histochemical and biochemical approaches to the study of phenolic compounds and peroxidises in needles of Norway spruce (*Picea abies*). New Phytol 146:403–414

Swedjemark G, Karlsson B, Stenlid J (2007) Exclusion of *Heterobasidion parviporum* from inoculated clones of *Picea abies* and evidence of systemic induced resistance. Scan J Forest Res 22:110–117

Thomas JC, Smigocki AC, Bohnert HJ (1995) Light-induced expression of ipt from *Agrobacterium tumefaciens* results in cytokinin accumulation and osmotic-stress symptoms. Plant Mol Biol 27:225–35

Velmala SM, Rajala T, Heinonsalo J, Taylor AFS, Pennanen T (2014) Profiling functions of ectomycorrhizal diversity and root structuring in fast- and slow-growing Norway spruce seedlings. New Phytol 201:610–622

White TJ, Burns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gefland DH, Sninsky JJ, White TJ (eds) PCR protocols guide to methods and amplifications. Academic San Diego, CA, pp 315–322

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer, New York

Winder RS (1999) The influence of substrate and temperature on the sporulation of *Fusarium avenaceum* and its virulence on marsh reed grass. Mycol Res 103:1145–1151

Table 1 Traits of Norway spruce seedlings inoculated with forest humus and exposed to two necrotising foliar diseases Mean ± standard deviation for separate treatments and fast- and slow-growing seedlings. The letters in the right corner of the mean values indicate different mean values between the treatments (P<0.05). The underlined numbers emphasised with italics indicate statistically significant differences (P<0.05), and only underlined numbers (P<0.1) indicate marginally significant differences between fast- and slow-growing spruce seedlings within each treatment. The seed orchard seeds, s1002, are presented only as a reference; therefore, these seedlings are omitted from the mean values calculated for the fast- and slow-growing seedlings.

Figure captions

Figure 1 The number of damaged needles (a), the condensed tannin concentration in needles (b), the normalized condensed tannin content (c) and the EMF OTU richness (d) in three fast- and three slow-growing Norway spruce seed families exposed to two necrotising foliar diseases Light grey bars indicate the mean values of fast-growing seedlings and dark grey bars the mean values of slow-growing seedlings. Vertical error bars show the standard error based on nonparametric bootstrapping (n=7 in each bar). Statistically significant differences can be found between families with non-overlapping error bars.

Figure 2 Two-dimensional NMDS of the ectomycorrhizal fungal communities of fast- and slow-growing Norway spruce seedlings exposed to two necrotising foliar diseases Ellipses are drawn based on standard errors of the mean scores of fast- (light grey ellipses) and slow-growing (dark grey ellipses) seedlings. Treatments are separated by line type: dotted line illustrates the water only control treatment without necrotising fungal pathogens. The ectomycorrhizal fungal OTU centroids are marked with a triangle. The left-pointing arrow show the direction of increasing shoot:root ratio (r^2=0.08, *P*=0.03) on the NMDS plot.

Electronic supplementary material

Table ESM1 Growth and origin information of Norway spruce (*Picea abies* (L.) Karst.) seed families used in the study Families 612, 298, 1162 represent fast-growing seedlings and families 1183, 394 and 427 slow-growing seedlings. Seed orchard s1002, was used as an outer reference only, and was omitted from the calculations made for the fast- and slow-growing origins. The information of these spruce plus trees is archived in the forest genetic register maintained at the Finnish Forest Research Institute. Growth performance is assessed from 14 yr old trees from 7 to 10 experimental fields. The seedling information overlaps with Velmala *et al*. 2014, New Phytol 201: 610–622.

Table ESM2 Description and ISDN sequence accession numbers of fungal species inhabiting roots and needles of Norway spruce (*Picea abies* (L.) Karst.) seedlings Identification of OTUs is based on BLAST search from the UNITE and INSD databases.

Table ESM3 The effects of foliar treatment and growth performance group on growth, root characteristics and ectomycorrhizal fungal communities of Norway spruce seedlings. Results are based on general and generalised linear mixed models with foliar treatment and growth group and their interaction as explanatory variables and seed origin as a random. Statistically significant *P*-values are bolded.

able
Click here to download table: Table1.xls

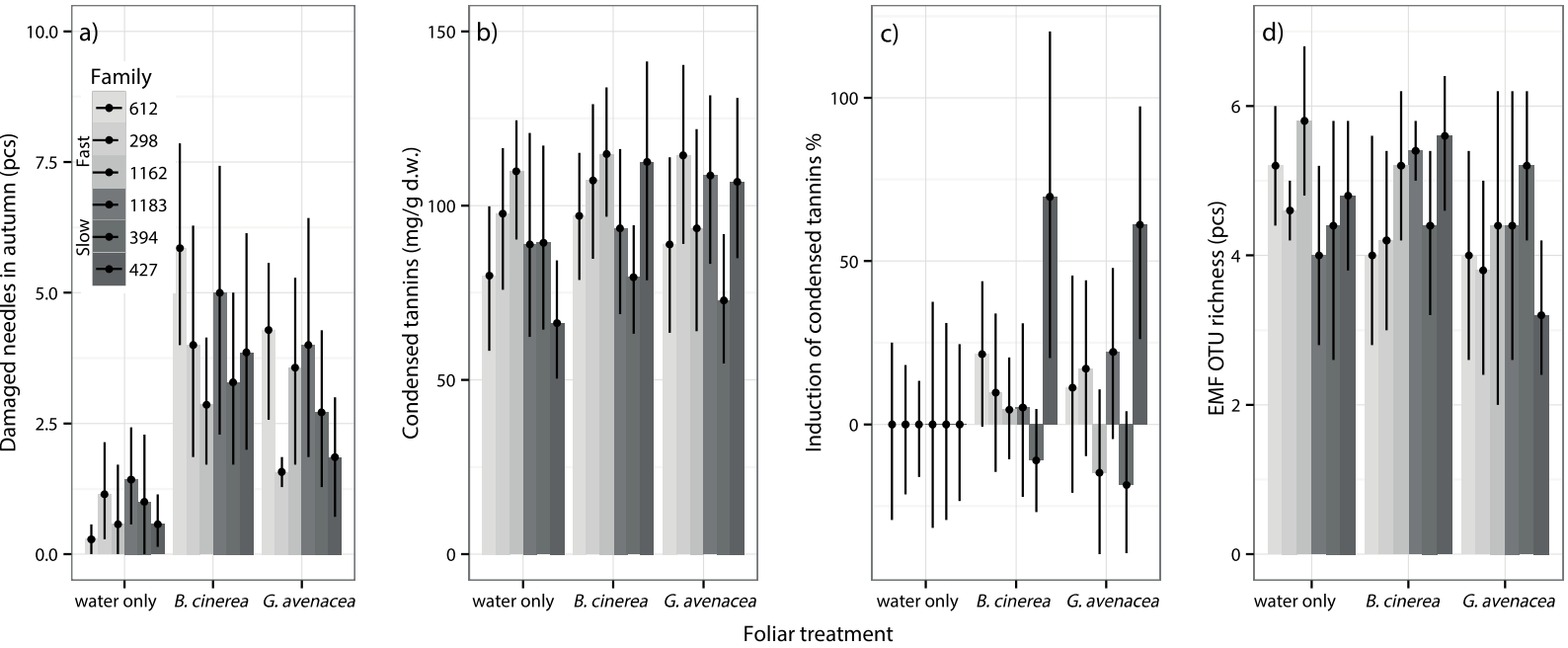
Table 1 Traits of Norway spruce seedlings inoculated with forest humus and exposed to two necrotising foliar diseases

Foliar treatment	Water only control				*Botrytis cinerea*	
Mean ± sd	n=49	Slow, n=21	Fast, n=21	s1002, n=7	n=49	Slow, n=21
Shoot biomass (g)	**0.62±0.2**	0.59±0.18	0.67±0.2	0.56±0.27	**0.70±0.28**	0.7±0.29
Root biomass (g)	**0.20±0.09**[a]	0.19±0.07	0.22±0.1	0.18±0.11	**0.27±0.14**[b]	0.27±0.14
Shoot height autumn (mm)	**135.4±31.1**	132.7±27.7	142.6±29.9	121.9±42.0	**142.0±42.8**	137.9±41.1
Short root density (root tips/mm)	**0.83±0.21**	*0.92±0.17*	*0.76±0.22*	0.74±0.22	**0.81±0.23**	0.87±0.24
Shoot height following spring (mm)*	**142.5±38.1**	146.4±25.0	156.3±34.5	123.5±46.4	**132.0±45.7**	134.6±34.3
Shoot:root ratio	**3.27±0.73**[b]	3.22±0.77	3.22±0.67	3.55±0.83	**2.79±0.68**[a]	2.81±0.7
Number of root tips (pcs)	**1675±902**[a]	1808±825	1632±832	1405±1334	**2253±1490**[c]	2391±1409
Damaged needles summer (pcs)	**0.35±0.72**[a]	0.52±0.81	0.19±0.68	0.29±0.49	**3.16±2.82**[b]	3.52±3.03
Damaged needles autumn (pcs)	**0.82±1.24**[a]	1±1.38	0.67±1.24	0.71±0.76	**3.92±2.83**[b]	4.05±3.01
Damaged needles following spring (pcs)*	**5.39±2.87**[a]	5.04±2.26	5.93±2.54	5.2±3.7	**10.31±6.39**[b]	10.52±6.54
Condensed tannins (mg/g d. w.)	**88.7±33.7**	81.5±36.7	95.8±29.7	-	**100.8±33.5**	95.2±37.0
EMF OTU richness (pcs)**	**4.63±1.46**	4.4±1.59	5.2±1.08	3.6±1.52	**4.69±1.45**	5.13±1.19
ECM colonisation (%)	**88.8±11.5**	88.1±13.8	91.9±7.2	81.4±12.4	**85.6±14.1**	82.7±14.5
Total fungal OTUs	**9.69±3.04**	9.40±3.40	9.87±2.50	10±3.94	**10.23±3.12**	9.73±3.03

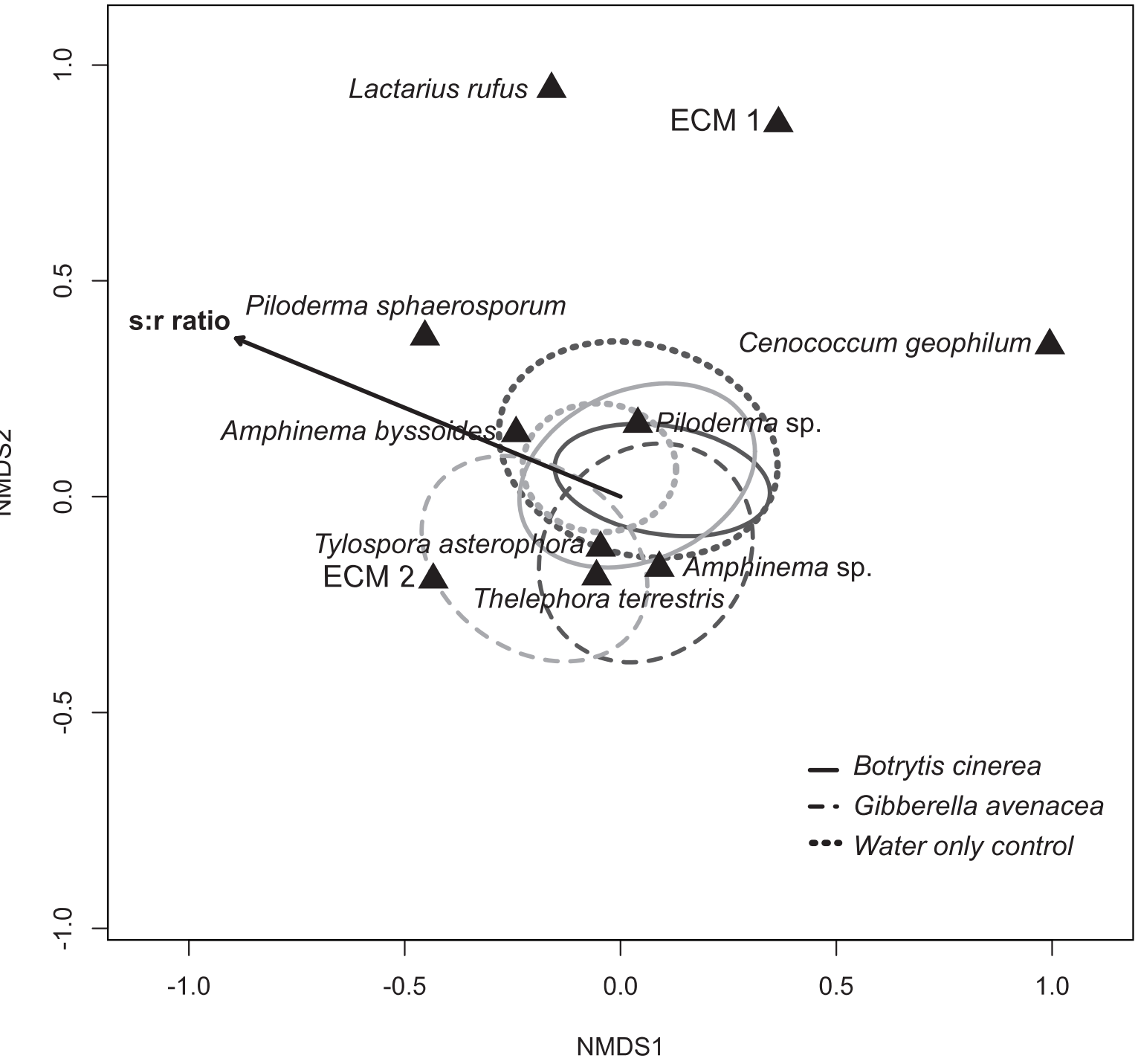
* Due to the destructive sampling in autumn, these traits are measured from a set of overwintering seedlings the following spring
** EMF richness was assessed from 5 replicate seedlings

		Gibberella avenacea			
Fast, n=21	s1002, n=7	**n=49**	Slow, n=21	Fast, n=21	s1002, n=7
0.81±0.18	0.33±0.21	**0.71±0.22**	0.71±0.2	0.75±0.2	0.6±0.29
0.33±0.11	0.1±0.06	**0.23±0.08**[ab]	0.23±0.08	0.24±0.08	0.18±0.08
166.3±18.5	81.1±37.7	**148.27±30.4**	151.5±28.7	148.2±31.1	138.7±35.8
0.75±0.22	0.79±0.21	**0.83±0.22**	0.83±0.2	0.82±0.26	0.87±0.23
157.6±36.7	102.7±48.7	**136.6±44.5**	139.5±41.3	162.9±29.8	106.1±42.9
2.58±0.61	3.35±0.56	**3.21±0.52**[b]	3.19±0.59	3.23±0.41	3.19±0.64
2625±1526	724±300	**1829±751**[b]	1827±693	1903±764	1616±943
3.48±2.77	1.14±1.46	**2.59±2.35**[b]	2.38±2.6	2.57±2.23	3.29±2.14
4.24±2.88	2.57±1.9	**3.04±2.33**[c]	2.86±2.59	3.14±2.2	3.29±2.14
11.63±7.09	8.73±5.28	**9.33±5.5**[b]	*8.7±4.79*	*11.89±4.37*	7.31±6.32
106.4±29.4	-	**97.5±37.0**	96.1±35.3	98.9±39.5	-
4.47±1.60	4.0±1.58	**4.23±1.88**	4.27±1.79	4.07±2.07	4.6±2.07
88.2±13.4	86.0±15.5	**89.3±8.7**	91.9±7.3	85.6±9.3	93.1±6.5
11.00±2.83	9.4±4.28	**9.03±3.11**	8.07±2.79	10.00±3.37	9.2±2.95

ine figure
Click here to download line figure: Figure1_Velmala_final_R.eps



ine figure
Click here to download line figure: Figure2_Velmala_final_R.eps



Electronic supplementary material

Click here to download Electronic supplementary material: Velmala_TablesESM1_3.pdf