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Influence of the ectomycorrhizal fungus *Laccaria laccata* on pre-emergence, post-emergence and late damping-off by *Fusarium moniliforme* and *F. oxysporum* on Scots pine seedlings

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Abstract

The role of the ectomycorrhizal (ECM) fungus *Laccaria laccata* a biological control agent against pre-emergence, post-emergence and late damping-off caused by *Fusarium moniliforme* (Fo) and *F. oxysporum* (Fm) on Scots pine was studied in greenhouse experiments. The vegetative mycelium of *L. laccata* in a vermiculite/peat carrier was added to potting substrate before inoculation with Fo or Fm spores. Seedlings were also inoculated only with ECM, Fo, Fm or water in order to compare treatments. *F. oxysporum* did not reduce germination when *L. laccata* was previously inoculated. Eighteen weeks after sowing, inoculation with *L. laccata* significantly reduced the damage caused by *F. moniliforme*, as seen in post-emergence assays, but no reduction of disease symptoms was observed for *F. oxysporum*. Significant late damping-off damage on Scots pine seedlings was only caused by *F. oxysporum*. *L. laccata* did not reduce disease expression in *F. oxysporum* treatments. Root colonization of seedlings by *L. laccata* was 51.8% and 26.8% for seed and seedling treatments, respectively. Mycorrhizal formation was significantly lower in plants treated with *F. moniliforme* or *F. oxysporum*. It is shown that the protective effect of *L. laccata* against the damping-off caused by *Fusarium* spp. in Scots pine seedlings varied among pre-emergence, post-emergence and late damping-off stages.

Keywords: Forest nursery, ectomycorrhiza, *Laccaria laccata*, damping-off, *Fusarium oxysporum*, *Fusarium moniliforme*, biological control

1. Introduction

Damping-off is one of the most common and feared diseases in forest nurseries around the world, occurring on germinating seeds and first-year seedlings of conifers and broadleaves, the former being generally more susceptible (Dick and Dobbie, 2002). Damping-off is a general term describing symptoms which may occur at three stages in the plant development, i.e., pre-emergence, post-emergence, and late seedling (Nef and Perrin, 1999; Butin, 1995). Pre-emergence damping-off kills seeds of germinants before they emerge and the resulting infections lead to a rapid decay of the affected tissues before the seedlings can reach the soil surface. Post-emergence damping-off occurs in the first months after seedlings have emerged and it is characterised by the collapse of the seedlings at ground level and the subsequent toppling of the above-ground parts. Late seedling mortality takes place after plant lignification and it occurs as a result of partial or complete destruction of the root system. About 30 different fungal species are known to cause damping-off to seedlings of forest plants around the world (Butin, 1995). In Spain, *Fusarium oxysporum* Schlecht. Emend. Snyd. & Hans. and *F. moniliforme* Sheldon are the main causal agents of damping-off in forest nurseries (Martín-Pinto et al., 2004, 2006a,b) and they are responsible for considerable losses, particularly in conifer species.

Several fungicides are used to control this disease; however, many of them are not very effective and do not protect the seedlings in the nurseries (Sinclair et al., 1975). Moreover, reducing residual toxicity from chemicals in the

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soil is also required for environmentally acceptable management (Hwang et al., 1995).

Mycorrhizae have a positive influence on the performance of seedlings (Guerin-Laguette et al., 2004) due to the mutual beneficial relationship between plants and mycorrhizal fungi. Furthermore, mycorrhizae are effective against various plant root rots (Duchesne, 2000; Linderman, 1994). Several studies have documented the protective role of mycorrhizae not only against fungal pathogens (Marx, 1972; Sylvia and Sinclair, 1983a; Morin et al., 1999), but also against nematodes (Diedhiou et al., 2003) and insects (Halldorsson et al., 2000).

Laccaria laccata (Scop. Ex Fr.) Berk. & Br. is a ubiquitous fungus which forms ectomycorrhizae with many tree species. The broad host range and the readiness of *L. laccata* for mycorrhizal formation make this fungus a candidate for artificial inoculation of tree seedlings aimed to provide biological control (Sinclair et al., 1982). Although *L. laccata* has been shown to be capable of protecting roots of young Douglas-fir (Sinclair et al., 1975; Stack and Sinclair, 1975; Sylvia and Sinclair, 1983b; Strobel and Sinclair, 1991) and Jack pine seedlings (Chakravarty and Hwang, 1991) against *F. oxysporum*, its effect at other different damping-off stages is unknown. The purpose of the current study was to analyse the protective effect of *L. laccata* against the damage caused by *F. oxysporum* and *F. moniliforme* to highly susceptible Scots pine seeds and seedlings (Kacprzak et al., 2001) at the pre-emergence, post-emergence and late seedling stages.

2. Materials and Methods

Organisms

The plant material used in the assays consisted of Scots pine seeds (from "Montaña Soriano-Burgalesa" provenance) provided by the Junta de Castilla y León forest nursery. The root pathogens *F. moniliforme* (Fm-6P) and *F. oxysporum* (Fo-4P) used throughout the experiment were isolated from diseased seedlings growing in commercial nurseries located in León (Imave nursery) and Soria (Indesfor nursery), in Spain. The ECM fungi *Laccaria laccata* (Ll-1, isolated from a fruiting body) was provided by the Valonsadero Forestry Research Centre (Soria, Spain). The cultures of *Fusarium* spp. and *L. laccata* were maintained on Komada (K) medium (Komada, 1975) and modified Melin Norkrans (MMN) medium (Marx, 1969), respectively.

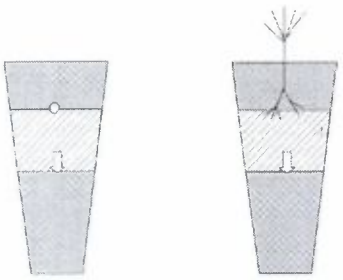
Damping-off assays

Inoculum of *Fusarium* spp. was produced by culturing the fungus in liquid Potato Dextrose Agar (PDA) medium for 7 days in the dark. Spores (macro and microconidia) were obtained by filtration and resuspended in sterile distilled water at a concentration of 10^6 spores/ml. The inoculum of *L. laccata* was prepared by culturing the fungus in a mixture of 1000 ml vermiculite and 100 ml peat moss (Pindstrup Mosebrug S.A.E., Burgos), twice-sterilised at 120°C for 60 min and moistened with 500 ml of MMN liquid medium (pH adjusted to 5.0) in 2 l flasks. Once the culture medium was added, the flasks were autoclaved for 20 min at 121°C; after cooling, the mycorrhizal fungus was inoculated by adding 20 bits (5 mm Ø) of solid cultures. All the flasks were maintained at 25°C in the dark for two months. Uninoculated flasks were also prepared for control treatments.

In early December 2003, Scots pine seeds were surface-sterilized in 30% H_2O_2 for 30 min, then washed 10 times with sterile distilled water to eliminate disinfectant before sowing in multipot trays (50 cm^3 per pot) containing a mixture of peat moss and vermiculite (1:1) previously sterilized twice at 121°C for 90 min. The resulting seedlings were maintained in a greenhouse until early February 2004. Afterwards, Scots pine seedlings were transferred to multipot trays (250 cm^3 per pot), and assayed simultaneously with seeds, sterilized as described above, sown in the same kind of containers. Seed and seedlings were grown under 6 treatments: (1) control (Not Inoculated), (2) *Laccaria laccata* (ECM), (3) *Fusarium moniliforme* (Fm), (4) *Fusarium oxysporum* (Fo), (5) Fm+ECM and (6) Fo+ECM. The treatments contained a sterilized peat moss basal layer (165 cm^3) where a 5 ml spore suspension (10^6 spores ml^{-1}) of *F. moniliforme* or *F. oxysporum* was pipetted to the Fm and Fm+ECM or to the Fo and Fo+ECM treatments, respectively (Fig. 1). Control seedlings were inoculated with 5 ml of distilled water. Fifty ml of *L. laccata* inoculum were transferred to the pots in the ECM treatments and 50 ml of the sterilized MMN-vermiculite-peat moss mixture to the pots without the ectomycorrhizal fungi. After seeding or transplanting, the seeds or the seedling collars were covered with 15 cm^3 of sterile peat moss and irrigated with 20 ml of sterile distilled water. Seeds and seedlings were irrigated daily during the first two weeks. After that, watering and other procedures were conducted as per routine nursery practice, except that no fertilizers or fungicides were applied.

Every treatment consisted of 3 replicates (trays) of 48 seeds or seedlings each (one seed per pot), resulting in a total of 864 seeds or seedlings (=6 treatments × 3 replicates × 48 seeds or seedlings) assayed. The trays were randomly assigned to different locations on the bench. Multipot trays were maintained in a greenhouse without light or temperature control until early July.

Pre-emergence damping-off was estimated by counting the number of germinated seeds (%) in each treatment 12 weeks after sowing. Post-emergence and late damping-off was analysed by recording the damage to the seedlings 18 weeks after sowing or transplant, respectively.



A B

Figure 1. Schematic representation of the inoculation procedure. The inoculum of *Laccaria* (cross hatching) was disposed over the down layer of peat moss in the pot (grey colour) after the inoculation of the spores of *Fusarium* spp on that layer (arrow). The seeds (picture A) or the seedlings (picture B) were disposed over the *Laccaria* layer in direct contact with it and covered with peat moss.

Table 1. ANOVA table for *Pinus sylvestris* germination in the pre-emergence assay.

Source	d.f.	MS	F-value	p-value
Fusarium	2	614.4	6.038	0.015*
ECM	1	40.8	0.400	0.538
Fusarium × ECM	2	238.2	2.341	0.138

d.f., degrees of freedom; MS, means squares; ECM, ecto-mycorrhizal fungus *Laccaria laccata*.

Table 2. ANOVA table for *Pinus sylvestris* mortality in the post-emergence assay.

Source	d.f.	MS	F-value	p-value
Fusarium	2	1.12930	26.394	0.000*
ECM	1	0.20753	4.851	0.047*
Fusarium × ECM	2	0.20724	4.844	0.028*

d.f., degrees of freedom; MS, means squares; ECM, ecto-mycorrhizal fungus *Laccaria laccata*.

Three damage classes were established: (1) none to light damage (less than 10% shoot affected); (2) moderate to severe damage (over 10% of shoot affected); (3) dead seedling. A Seedling Disease Index (SDI) was calculated for each treatment as the mean value of seedling damage in the three replications.

Fifteen randomly selected seedlings for each treatment were taken to the laboratory at the beginning of July (18 weeks after sowing), and shoot height, shoot dry weight, root collar diameter, root length, root dry weight and number of mycorrhizal short roots were measured. Soil-washed excised roots were examined for mycorrhizae and placed in vials containing a FAA preserving solution (5 ml of formaldehyde, 5 ml of acetic acid, and 90 ml of ethyl alcohol). Intensity of root colonization was expressed as the percentage of mycorrhized apexes within 250 observed apexes per plant.

Statistical analysis

Data were subjected to standard ANOVA procedures ($p<0.05$) using Statistica 7.0 (StatSoft Inc. 1984–2005, Tulsa, OK) software. LSD Fisher test ($P<0.05$) was applied to compare mean values when significant differences were found.

3. Results

Pre-emergence damping-off assay

Germination of Scots pine seeds was significantly reduced ($p=0.002$) in the pots treated with *F. oxysporum* at 12 weeks after sowing. Plant stand in *F. oxysporum* treatment was 66.7% compared to 97.2% in the non-inoculated control (Fig. 2). On the contrary, germination of Scots pine seeds was not significantly impaired in the pots treated with *F. moniliforme* spores ($p=0.566$).

Table 3. Effect of *Laccaria laccata* (ECM), *Fusarium oxysporum* (Fo) and/or *F. moniliforme* (Fm) on shoot height, shoot dry weight, root collar diameter, root length, root dry weight and number of mycorrhizal short roots of Scots pine seedlings 18 weeks after sowing.

Treatment	Shoot height (cm)	Shoot dry weight (g)	Diameter of root collar (mm)	Root length (cm)	Root dry weight (g)	Number of mycorrhizal short roots (%)
Not inoculated	6.047 ab	0.047 a	0.560 a	12.71 ab	0.030 a	0 c
Fm	6.087 b	0.055 a	0.607 a	14.52 c	0.037 ab	0 c
Fo	5.700 ab	0.048 a	0.560 a	12.09 a	0.028 a	0 c
ECM	6.180 b	0.071 b	0.737 b	14.81 c	0.042 b	51.89 a
Fm+ECM	5.493 a	0.052 a	0.577 a	13.64 bc	0.036 ab	30.63 b
Fo+ECM	5.840 ab	0.055 a	0.607 a	13.15 ab	0.035 ab	24.53 b

Fm, *Fusarium moniliforme*; Fo, *F. oxysporum*; ECM, ectomycorrhizal fungus *Laccaria laccata*. Means followed by different letters within each column are significantly different (LSD Fisher test, $P=0.05$, $n=15$).

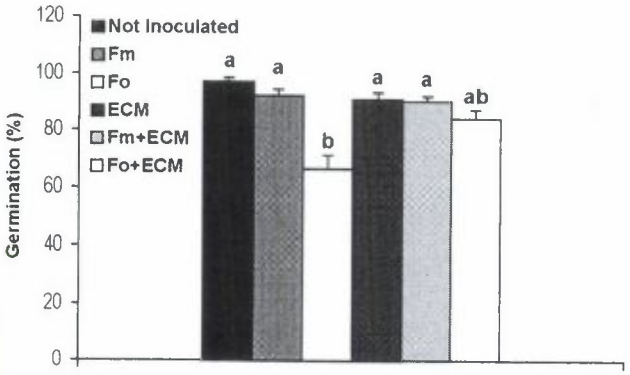


Figure 2. Effect of *Laccaria laccata* (ECM), *Fusarium oxysporum* (Fo) and/or *F. moniliforme* (Fm) on germination of Scots pine seeds 12 weeks after sowing. Vertical bars followed by different letters are significantly different (LSD Fisher test, P=0.05).

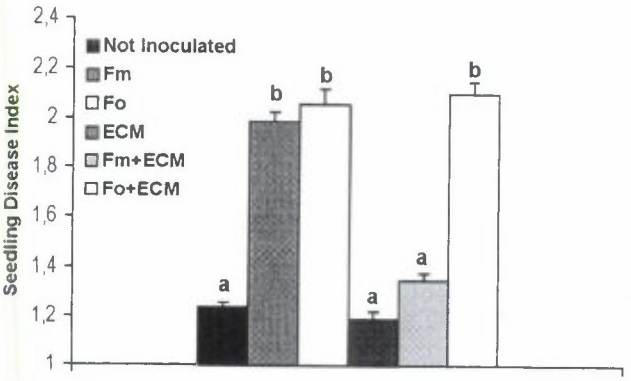


Figure 3. Effect of *Laccaria laccata* (ECM), *Fusarium oxysporum* (Fo) and/or *F. moniliforme* (Fm) on post-emergence damping-off index (SDI) of Scots pine seedlings 18 weeks after sowing. Vertical bars followed by different letters are significantly different (LSD Fisher test, P=0.05).

Two-way ANOVA confirmed significant differences among *Fusarium* species (Table 1), the average seed germination of plants inoculated with *F. moniliforme* isolate (91.3%) being greater than that of *F. oxysporum* (75.3%; p=0.015), when all the treatments containing the same *Fusarium* species were considered together.

Inoculation with *Laccaria laccata* did not improve germination of seeds (88.4% average in ECM, ECM+Fm, and ECM+Fo treatments) compared to treatments without the mycorrhizal fungus (85.4% average in non-inoculated control, Fm and Fo treatments) (p=0.538, Table 1). In particular, there was no significant difference (p=0.056) between pots treated with *F. oxysporum* preinoculated with *L. laccata* and pots treated with *F. oxysporum* that were not preinoculated with *L. laccata*. Moreover, the differences found between non-inoculated and Fo treatments disappeared when these treatments contained *L. laccata* (p=0.135, Fig. 2).

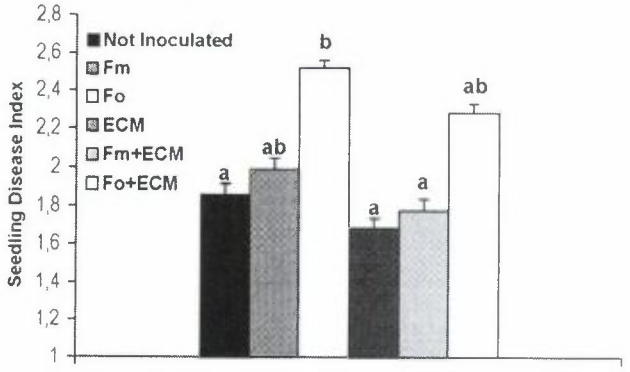


Figure 4. Effect of *Laccaria laccata* (ECM), *Fusarium oxysporum* (Fo) and/or *F. moniliforme* (Fm) on late damping-off index (SDI) of Scots pine seedlings 18 weeks after transplanting. Vertical bars accompanied by different letters are significantly different (LSD Fisher test, P=0.05).

Post-emergence damping-off assay

The Seedling Disease Index (SDI) was significantly higher for treatments containing *F. oxysporum* or *F. moniliforme* (p=0.000). Eighteen weeks after sowing, the disease index for *F. oxysporum* and *F. moniliforme* treatments was 2.06 and 1.99, respectively, versus 1.24 for the non-inoculated control (Fig. 3). Inoculation with *L. laccata* significantly reduced post-emergence damping-off in pots treated with *F. moniliforme* (SDI: 1.35, p=0.002). On the contrary, no differences were observed between Fo and Fo+ECM treatments (p=0.817). Similar low values of damage where found in non-inoculated controls and pots treated only with the ECM fungi (p=0.804). After pooling the data, the two-way ANOVA (Table 2) showed a significant effect of the *Fusarium* (p=0.000) and ECM (p=0.047) variables and interaction among them (p=0.028).

The ECM treatment offered the highest values for all the other plant variables analysed, but only shoot dry weight, root collar diameter, root length, root dry weight and number of mycorrhizal short roots were significantly enhanced in Scots pine seedlings inoculated with *L. laccata* (Table 3). Presence of ECM did not promote shoot height, unexpectedly it even decreased in the Fm+ECM treatments compared to the Fm treatment (p=0.020). No significant differences in any of the studied variables, except for mycorrhized roots, were observed between Fo and Fo+ECM treatments, even though all of them showed higher values in presence of the ECM fungus.

Root colonization by *Laccaria laccata* was 51.8% in the ECM treatment and it was significantly reduced (p=0.000) when the pots were co-inoculated with *Fusarium moniliforme* (30.6%) or *F. oxysporum* (24.6%, Table 1). As expected, no mycorrhizal formations were found in seedlings untreated with *L. laccata*.

Late damping-off assay

The SDI was significantly higher in transplanted Scots pine seedlings inoculated with *F. oxysporum* than in controls (2.5 vs. 1.8, respectively; p=0.031) (Fig. 4). *F. moniliforme* treatment resulted in a SDI value between that of the *F. oxysporum* and the control treatments, but not significantly different from them.

However, two-way ANOVA showed significant differences between *Fusarium* species (Table 4). When all treatments containing the same *Fusarium* species were considered together, the SDI for *F. oxysporum* reached higher values than those for *F. moniliforme* (2.40 vs. 1.88; p=0.015).

Inoculation with *L. laccata* did not improve the average SDI in Scots pine seedlings (1.91 average in treatments containing ECM compared to 2.12 average in treatments without mycorrhiza; p=0.208) (Table 4). However, as it occurred in the pre-emergence damping-off assay, the significant differences found between non-inoculated and Fo treatments disappeared when both of them contained *L. laccata* (p=0.147).

Plants treated with *L. laccata* (ECM, Fo+ECM and Fm+ECM) apparently showed higher values for all the plant growth-related variables compared with the plants without the mycorrhizal fungus (Table 5). Shoot height, shoot dry weight and root collar diameter were significantly enhanced in two-month-old Scots pine plants inoculated with *L. laccata*. The presence of ECM stimulated root length (11.88 vs. 9.91, respectively; p=0.026) in *F. oxysporum* treated plants, shoot dry weight (0.040 vs. 0.029; p=0.023) and root dry weight (0.030 vs. 0.015; p=0.002) in plants treated with *F. moniliforme* (Table 5).

Root colonization by *L. laccata* was 26.8% for the ECM treatment. Mycorrhizal formation was significantly reduced when two-month-old Scots pine plants were co-inoculated with *F. moniliforme* (13.6%, p=0.002) or *F. oxysporum* (2.0%, p=0.000) (Table 5). Root colonization was absent in the treatments without the ECM inoculum.

Table 4. ANOVA table for *Pinus sylvestris* mortality in the late damping-off assay.

Source	d.f.	MS	F-value	p-value
Fusarium	2	0.66028	6.07	0.015*
ECM	1	0.19243	1.76	0.208
Fusarium × ECM	2	0.00210	0.01	0.980

d.f., degrees of freedom; MS, means squares; ECM, ectomycorrhizal fungus *Laccaria laccata*.

4. Discussion

The present study suggests that inoculation of Scots pine seedlings with the ECM fungus *L. laccata* may reduce the damage caused by damping-off and may have a positive effect on seedling growth. The disease index for *F. moniliforme* in post-emergence damping-off was significantly reduced when the Scots pine plantlets were grown with *L. laccata*, and its value decreased to the same levels as those for the non-inoculated control. Furthermore, inoculation of *L. laccata* significantly reduced disease levels caused by *F. oxysporum* to levels seen on control seedlings in the pre-emergence and late DO assays. A similar protective effect of *L. laccata* was also obtained in studies on Douglas-fir (Sinclair et al., 1982; Sylvia and Sinclair, 1983b) and Jack pine (Chakravarty and Hwang, 1991). Antagonism against *F. moniliforme* has also been shown in jack pine seedlings mycorrhized with *Paxillus involutus* (Hwang et al., 1995). However, the protection offered by *L. laccata* against damping-off in our study was low and did not yield complete protection against *F. oxysporum* in Scots pine seedlings. In this sense, the protective effect may be related to pathogen virulence, since *F. moniliforme* resulted in less damage to the seedlings than *F. oxysporum*.

The age of Scots pine seedlings influenced the damage caused by both *Fusarium* species, as recently emerged

Table 5. Effect of *Laccaria laccata* (ECM), *Fusarium oxysporum* (Fo) and/or *F. moniliforme* (Fm) on shoot height, shoot dry weight, root collar diameter, root length, root dry weight and number of mycorrhizal short roots of Scots pine seedlings 18 weeks after transplanting.

Treatment	Shoot height (cm)	Shoot dry weight (g)	Diameter of root collar (mm)	Root length (cm)	Root dry weight (g)	Number of mycorrhizal short roots (%)
Not inoculated	6.227 a	0.031 ab	0.530 bc	11.52 ab	0.015 ab	0 c
Fm	6.027 a	0.029 a	0.497 abc	12.33 b	0.015 ab	0 c
Fo	6.013 a	0.025 a	0.447 a	9.91 a	0.013 a	0 c
ECM	7.533 b	0.053 c	0.657 d	11.56 ab	0.022 bc	26.88 a
Fm+ECM	6.593 a	0.040 b	0.570 c	12.41 b	0.030 c	13.66 b
Fo+ECM	6.313 a	0.033 ab	0.473 ab	11.88 b	0.015 ab	2.06 c

Fm, *Fusarium moniliforme*; Fo, *F. oxysporum*; ECM, ectomycorrhizal fungus *Laccaria laccata*. Means followed by different letters within each column are significantly different (LSD Fisher test, P=0.05, n=15).

seedlings (post-emergence assay) were more susceptible to pathogens than seeds (pre-emergence assays) or two-month-old seedlings (late DO assay), where dead plants were rarely observed. Similar results were obtained in Douglas-fir studies, where seedlings were most often killed by *Fusarium* spp. if primary roots were attacked during the first few weeks after germination. When the older and larger root systems became infected, they seemed to be able to support and tolerate higher populations of pathogenic *Fusarium* spp. (Sinclair et al., 1982). In the same way, the antagonistic effect of *L. laccata* was also influenced by seedling age, as evidenced by the fact that different results were obtained in pre-emergence, post-emergence and late damping-off. On the other hand, the protective effect of *L. laccata* was different in both *Fusarium* species. The best results were obtained against *F. moniliforme* in post-emergence assays, where the SDI was significantly lower in the mycorrhized plants. This effect was not observed, however, in pre-emergence and late damping-off assays, probably due to the lack of significant damage caused by *F. moniliforme*. In the plants inoculated with *F. oxysporum*, *L. laccata* offered some protection against pre-emergence and late damping-off. Here, although there were no differences between Fo and Fo+ECM treatments in either of the assays, inoculation of *Laccaria* significantly reduced disease levels caused by *F. oxysporum* to levels seen on control seedlings. These results support the idea that the protective effect of *L. laccata* depends on the host's age.

The interaction of ECM fungi with root pathogens of pines is still not well understood. Marx (1972, 1973) and Zak (1964) hypothesized that root protection by the ECM may be the result of three effects: a protective barrier effect caused by the presence of a fungal mantle around the roots, the production of antimicrobial substances either by the mycosymbiont or by the host plant, and a competition for nutrients in the rhizosphere. In our study, the antagonism caused by *L. laccata* was not related to the percentage of mycorrhizal apexes formed in the host roots. Thus, in the post-emergence assays this value was similar in Fo+ECM and Fm+ECM treatments, but *L. laccata* antagonism was only effective against *F. moniliforme*. Similar results were obtained by Diedhiou et al. (2003) on *Meloidogyne incognita*, where a clear relationship between mycorrhization and nematode control could not be established.

Several authors have reported disease suppression by ECM fungi associated to fungal-produced antimicrobial substances (Duchesne et al., 1987; Chakravarty and Hwang, 1991). Toxic effects of mycorrhizal fungi have been described not only on plant pathogens, but also on insects (Halldorsson et al., 2000) and nematodes (Diedhiou et al., 2003). The antagonism of *L. laccata* on both *in vitro* growth and spore germination of *F. oxysporum* and of *F. moniliforme* by toxic-like compounds was confirmed in previous studies conducted in our lab (Martín-Pinto *et al.* 2006b). Suppression of *F. moniliforme* damping-off by *L. laccata* was likely caused in part by the production of antifungal compounds by this ECM. Production of antimicrobial compounds by the plant itself in presence of the ECM is yet another mechanism that should be taken in account, as well as the strength of nutrient competition in the rhizosphere.

Shoot dry weight, root collar diameter, root length and root dry weight were significantly higher in mycorrhizal seedlings of post-emergence assays compared to plants in the controls without *Laccaria*. This was also true for shoot height, shoot dry weight and root collar diameter of transplanted plants in late damping-off. Moreover, presence of *L. laccata* significantly increased shoot and root dry weights in plants treated with *F. moniliforme*, and root length in those inoculated with *F. oxysporum*. A similar enhancement of growth after mycorrhizal colonization has also been shown in other studies (Guerin-Laguette et al., 2004).

The percentage of mycorrhizal short roots obtained in the ECM treated seedlings was higher in the post-emergence assay than in the late damping-off assay (51% vs. 26%, respectively), both values lying within the range observed for other ECM fungi (Hwang et al., 1995; Pedersen et al., 1999). The greater value attained in the post-emergence plants could have been the result of a higher percentage of *L. laccata* inoculum per root surface in the pre-emergence assay. Taking into account the fact that the same inoculum dosage was used with different plant material (seeds in pre- and post-emergence assays, and seedlings in the late damping-off assay), chances for root colonization by the ECM would have probably been lower for Scots pine seedlings than for seeds that were entirely surrounded by the inoculum after seeding. This result may have practical implications for the production of mycorrhized plants in nurseries where 2 to 4-month-old seedlings are now being used. Thus, the mechanization of the mycorrhization process during sowing would be easier and more effective than the current plant transplanting method used to obtain inoculated plants. Having in mind that many nurseries have proper sowing equipment, inoculation at the seedling stage appears more suitable and cost effective than inoculation of transplanted seedlings.

The number of mycorrhizal short roots in *P. sylvestris* seedlings was significantly lower in presence of either *Fusarium oxysporum* or *F. moniliforme*, suggesting that *Fusarium* spp. inhibit ECM formation by *L. laccata*. These results are consistent with those on *Suillus tomentosus* and *F moniliforme* reported by Hwang et al. (1995), but contrast with results obtained in other studies where root colonization was significantly enhanced in presence of saprophytic *F. oxysporum* strains (García-Romera et al., 1998; Diedhiou et al., 2003). This points out the need to establish the pathogenicity of the isolates in order to compare different results obtained in mycorrhizal assays.

Results presented here are quite promising for biological control of damping-off in forest nurseries, but further field studies are required to establish the effectiveness of *L. laccata* against other soil pathogens and its potential interactions with naturally occurring mycorrhizal fungi.

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REFERENCES

Butin, H. 1995. *Tree Diseases and Disorders: Causes, Biology, and Control in Forest and Amenity Trees*. Oxford University Press, New York. pp. 252.

Chakravarty, P. and Hwang, S.F. 1991. Effect of an ectomycorrhizal fungus, *Laccaria laccata*, on *Fusarium* damping-off in *Pinus banksiana* seedlings. *European Journal of Forest Pathology* **21**: 97–106.

Dick, M.A. and Dobbie, K. 2002. Species of *Fusarium* on *Pinus radiata* in New Zealand. *New Zealand Journal of Plant Protection* **55**: 58–62.

Diedhiou, P.M., Hallmann, J., Oerke, E.C., and Dehne, H.W. 2003. Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation of tomato. *Mycorrhiza* **13**: 199–204.

Duchesne, L.C. 2000. Role of ectomycorrhizal fungi in biocontrol. In: *Mycorrhizae and Plant Health*. Pfleger, F.L. and Linderman, R.G., eds. APS Press, St. Paul, Minnesota. pp. 27–46.

Duchesne, L.C., Peterson, R.L., and Ellis, E.B. 1987. The accumulation of plant produced antimicrobial compounds in response to ectomycorrhizal fungi: a review. *Phytoprotection* **68**: 17–27.

Garcia-Romera, I., Garcia-Garrido, J.M., Martin, J., Fracchia, S., Mujica, M.T., Godeas, A., and Ocampo, J.A. 1998. Interactions between saprophytic *Fusarium* strains and arbuscular mycorrhizas of soybean plants. *Symbiosis* **24**: 235–246.

Guerin-Laguette, A., Shindo, K., Matsushita, N., Suzuki, K., and Lapeyrie, F. 2004. The mycorrhizal fungus *Tricholoma matsutake* stimulates *Pinus densiflora* seedling growth *in vitro*. *Mycorrhiza* **14**: 397–400.

Halldórsson, G., Sverrisson, H., Eyjólfsdóttir, G., and Oddsdóttir, E. 2000. Ectomycorrhizae reduce damage to Russian larch by *Otiorhynchus* larvae. *Scandinavian Journal of Forest Research* **15**: 354–358.

Hwang, S., Chakravarty, P., and Chang, K. 1995. The effect of two ectomycorrhizal fungi *Paxillus involutus* and *Suillus tomentosus*, and of *Bacillus subtilis* on *Fusarium* damping-off in jack pine seedlings. *Phytoprotection* **76**: 57–66.

Kacprzak, M., Asiegbu, F.O., Daniel, G., Stenlid, J., Manka, and M., and Johansson, M. 2001. Resistance reaction of conifer species (European larch, Norway spruce, Scots pine) to infection by selected necrotrophic damping-off pathogens. *European Journal of Plant Pathology* **107**: 191–207.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review Plant Protection Research* **8**: 114–125.

Linderman, R.G. 1994. Role of VAM fungi in biocontrol. In: *Mycorrhizae and Plant Health*. Pfleger, F.L. and Linderman, R.G., eds. APS Press, St. Paul, Minnesota. pp. 1–26.

Martín-Pinto, P., Nanos, N., Pajares, J., and Diez, J.J. 2004. Site and seasonal influences on the fungal community on leaves and stems of *Pinus* and *Quercus* seedlings in forest nurseries. *Sydowia* **56**: 23–37.

Martín-Pinto, P., Pajares, J.A., Pando, V., and Diez, J.J. 2006a. Fungi isolated from diseased nursery seedlings in Spain. *New Forests* **31**: 41–56.

Martín-Pinto, P., Pajares, J.A., and Diez, J.J. 2006b. *In vitro* effects of four ectomycorrhizal fungi, *Boletus edulis*, *Rhizopogon roseolus*, *Laccaria laccata* and *Lactarius deliciosus* on *Fusarium* damping-off in *Pinus nigra* seedlings. *New Forests. In press.*

Marx, D.H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pathogenic infections. I Antagonism of mycorrhizal fungi to pathogenic fungi and soil bacteria. *Phytopathology* **59**: 153–163.

Marx, D.H. 1972. Ectomycorrhizae as biological deterrents to pathogenic root infections. *Annual Review of Phytopathology* **10**: 429–454.

Marx, D.H. 1973. Mycorrhizae and feeder root diseases. In: *Ectomycorrhizae – Their Ecology and Physiology*. Marks, G.C. and Kozlowski, T.T., eds. Academic Press, New York. pp. 351–382.

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

Nef, L. and Perrin, R. 1999. Practical Handbook on Damping-off Agents in the European Forest Nurseries. European Commission, Luxembourg. pp. 352.

Pedersen, E.A., Reddy, M.S., and Chakravarty, P. 1999. Effect of three species of bacteria on damping-off root rot development and ectomycorrhizal colonization of lodgepole pine and white spruce seedlings. *European Journal of Forest Pathology* **29**: 123–134.

Sinclair, W.A., Cowles, D.P., and Hee, S.M. 1975. *Fusarium* root rot of Douglas fir seedlings: Suppression by soil fumigation, fertility management and inoculation with spores of the fungal symbiont *Laccaria laccata*. *Forest Science* **21**: 390–399.

Sinclair, W.A., Sylvia, D.M., and Larsen, A.O. 1982. Disease suppression and growth promotion in Douglas-fir seedlings by the ectomycorrhizal fungus *Laccaria laccata*. *Forest Science* **2**: 191–201.

Stak, R.W. and Sinclair, W.A. 1975. Protection of Douglas fir seedlings against *Fusarium* root rot by ectomycorrhizal fungus *Laccaria laccata*. *Forest Science* **28**: 191–201.

Strobel, N.E. and Sinclair, W.A. 1991. Role of flavanolic wall infusions in the resistance induced by *Laccaria bicolor* to *Fusarium oxysporum* in primary roots of Douglas fir. *Phytopathology* **81**: 420–425.

Sylvia, D.M. and Sinclair, W.A. 1983a. Phenolic compounds and resistance to fungal pathogens induced in primary roots of Douglas-fir seedlings by the ectomycorrhizal fungus *Laccaria laccata*. *Phytopathology* **73**: 390–397.

Sylvia, D.M. and Sinclair, W.A. 1983b. Suppressive influence of *Laccaria laccata* on *Fusarium oxysporum* and Douglas-fir seedlings. *Phytopathology* **73**: 384-389.

Zak, B. 1964. Role of mycorrhizae in root disease. *Annual Review of Phytopathology* **2**: 377–392.