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

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ABSTRACT

In greenhouse experiments, the ectomycorrhizal fungus *Laccaria laccata* was evaluated for biological control of pre-emergence, post-emergence and late damping-off of *Pinus pinea* caused by *Fusarium verticillioides* and *F. oxysporum*. In pre-emergence damping-off assays, preinoculation with *Laccaria laccata* did not significantly improve germination of seeds and no statistical significant differences were found in *Fusarium* treatments when compared with controls. At 18 weeks after sowing, inoculation with *L. laccata* reduced the incidence of post-emergence damping-off but differences were significant only in *F. oxysporum* treatments. *Pinus pinea* transplanted plants were used in late damping off assays, and only *F. oxysporum* produced significant damage. Inoculation with *L. laccata* did not attenuate significantly the virulence of *F. oxysporum*. However, mycorrhization percentage did not reached significant level, so the mycorrhizal fungus level could be not enough for an effective protection. Although very low percentages of mycorrhization were recorded in all mycorrhized treatments, and *Fusarium* occurrence significantly reduced mycorrhization, those levels have been efficient to reduce damage in *F. oxysporum* post-emergence damping-off assays.

In short, pre-emergence damping-off was not found; only *F. oxysporum* produced significant damage on *P. pinea* seedlings and *L. laccata* reduced damage when the percentage of mycorrhization reached significant levels. These results have been compared with previous work on *P. sylvestris* inoculated with the same mycorrhizae isolate and *Fusarium* pathogens.

Keywords: forest nursery, *Fusarium* damping-off, *Laccaria laccata*, mycorrhization, *Pinus pinea*, plant protection

INTRODUCTION

Forest nurseries are known to be affected by damping-off (Nef and Perrin, 1999). Damping-off is caused by different fungal and oomycetes species like *Pythium* spp., *Fusarium* spp. and *Rhizoctonia solani*, which occur very frequently. Another species as *Botrytis cinerea*, *Phytophthora* spp., *Alternaria* spp., *Phoma* spp. and *Phomopsis* spp. can also be present. *Fusarium* and *Pythium* are the most important genera that cause damage to containerized plants in nurseries (Jones and Benson, 2000) and *Fusarium verticillioides* Sheld. (teleomorph *Gibberella fujikuroi*) and *Fusarium oxysporum* Schltdl. are the most aggressive species within *Fusarium* pathogens involved in damping-off (Bloomberg, 1985; Chakravarty et al., 1999; Dick and Dobbie, 2002). Those pathogens have already been detected in Spanish nurseries and *Fusarium oxysporum, F. verticillioides* and the saprophytic fungus *Trichoderma viride*, have been very often isolated from rhizosphere of *Pinus* and *Quercus* in forest nurseries of Castilla y León region (West-central Spain) where those pathogens are responsible for considerable losses (Martin-Pinto et al. 2006a).

Primary inoculum of the pathogenic fungi can be present on seed, contaminated substrate or in water. Several fungicides are used to control this disease. However, many of them are not effective and do not protect the seedling (Williams, 1989; Dumroese et al., 1996) and most fungicides have only a temporary effect and therefore require repeated applications that increase their negative impact, since surface recolonization by *Fusarium* via blowing soil, surface water flow, and infested seed is observed (Bloomberg, 1985).

In recent decades, holistic integrated strategies for nurseries protection have been considered as the best approach to mitigate losses from this disease (Dumroese and

James, 2005). Those strategies have take into account the fungal resistance to chemicals and its residual toxicity and have considered the biological control as an important tool available (Dumroese et al., 1996; 1998). In order to develop integrated control procedures according to environmental principles, several microorganisms have been tested (Le Tacon and Bouchard, 1986; Pedersen et al., 1999; Mandeel, 2006 and 2007).

Mycorrhizal symbiosis is an important factor in the establishment of seedlings in semiarid or degraded areas (Helm and Carling, 1993a and 1993b) and the effectiveness of mycorrhizal inoculation in producing an increase in the growth of *Pinus halepensis* Mill. has been demonstrated (Roldan and Albadalejo, 1994). Mycorrhizae have a positive influence on the performance of seedlings planted in reforestation (Krop and langlois, 1990), owing to the mutual beneficial relationship between plants and mycorrhizal fungi. Besides other beneficial effects, mycorrhizal fungi increase nutrient uptake, facilitate the transport of water to plant roots (Parke et al. 1983) and act as a defense mechanism against pathogenic organisms.

In 1942 Davis *et al*. suggested that ectomycorrhizae could protect feeder roots of nursery seedling against pathogens and since then several studies have noted this protective capability of mycorrhizae (Davis et al., 1942; Chakravarty and Hwang, 1991; Chakravarty et al., 1991; Duchesne, 1994; Hwang et al. 1995; Morin et al. 1999;).

Several mechanisms may be involved in plant protection. Mycorrhizal fungi can create physical barrier between roots and pathogens, exude antimicrobial metabolites, and use surplus carbohydrates, thereby reducing the attractiveness of roots to pathogenic organisms (Duchesne et al., 1987). Several studies of conifer seedling protection by mycorrhizal fungi against fungal pathogens such as *Phytophthora* (Marx and Davie, 1969), *Pythium* (Perrin and Garbaye, 1983) (both them now considered as fungal-like organisms), *Fusarium* (Machon et al., 2006), *Cylindrocladium*, and *Cylindrocarpon*

(Buscot et al, 1992; Chakravarty et al., 1999; Morin et al., 1999) have been recorded. Protective effect of mycorrhizae could be extended to other pathogens such as nematodes (Diedhiou, 2003). The effectiveness of root protection varies with the species of mycorrhizal fungi, host species, and soil conditions (Chakravarty and Unestam, 1987a and 1987b).

Laccaria laccata (Scop.:Fr) Berk. & Broome can be easily isolated from fruiting bodies and grown in laboratory conditions. The ability of this fungus to form mycorrhizae and its wide host range makes it very interesting organism for artificial inoculation of nursery plants (Molina, 1983; Molina and Chamard, 1983; Hung and Molina, 1986; Perrin and Soulas, 1996). This fungus has been recorded to give protection against several pathogenic fungi when associated with different plant species as *Pseudotsuga menziesi* (Mirb.) Franco, *Picea abies* (L.) Karst, *Pinus banksiana* Lamb, *Pinus nigra* Arnold or *Pinus sylvestris* L. (Chakravarty and Hwang, 1991; Chakravarty and Unestam, 1987a and 1987b; Sinclair et al. 1982; Sylvia and Sinclair, 1983a and 1983b, Martin-Pinto et al. 2006b). In our lab the effective protection of *P. sylvestris* seedlings by *L. laccata* have been recorded (Machon et al. 2006).

The aim of this study was to evaluate the protective effect of *L. laccata* against the damage caused by *Fusarium* spp in *Pinus pinea* seedlings. In this study we have take into account the three different stages on damping-off disease: pre-emergence damping-off that reduces the germination percentage; post-emergence damping-off affecting the early stages of seedlings and late damping-off when plants are older than four-months-old.

MATERIALS AND METHODS

Organisms

Pinus pinea seeds used in this study were provided by the forest nursery "Viveros Fuenteamarga" in Cabezón de Pisuerga (Castilla y Leon region in west central Spain). Seeds were disinfected by using 30% H_2O_2 for 30 min, and then washed 10 times with sterile distilled water to eliminate disinfectant before sowing

The root pathogens *Fusarium verticillioides* (Fm6) and *F. oxysporum* (Fo4), isolated from diseased seedlings in a greenhouse at the Imave Nursery (León) were used through the experiment. They were previously tested against *Pinus* spp (Martín-Pinto *et al*., 2006b) and both of them behaved as pathogens and produced damping-off symptoms. The monosporic cultures of *Fusarium* spp. were maintained on solid Komada medium (K) and inoculum of *Fusarium* spp was produced by culturing the fungus in liquid PDB (Potato dextrose broth) medium for 7 days in the dark. Spores were separated from the medium and resuspended at a concentration of 10^6 spores/ml.

The ectomycorrhizal fungus, *Laccaria laccata* (isolated from fruiting bodies) was provided by Dr. M. Fernandez from the Valonsadero Forestry Centre (Soria) on modified Melin Norkrans´(MMN) medium (Marx and Davie 1969). Inoculum of *L. laccata* consisted of mycelium growing at 25ºC for two months in 2-l Flasks containing 1000 ml vermiculite, 100 ml peat and 500 ml MMN liquid medium (pH adjusted to 5.0). The vermiculite and peat were previously sterilised twice at 120ºC during 60 min. The resulting mixture was autoclaved for 90 min at 121ºC prior to inoculation with the mycorrhizal fungus. Uninoculated flasks were prepared for control treatments.

Pre-emergence and post-emergence Damping-off

The experiment consisted of 6 treatments: 1 control (Not inoculated), 2 *Laccaria laccata* (Myc), 3 *Fusarium verticillioides* (Fm), 4 *Fusarium oxysporum* (Fo), 5

Fm+Myc, and 6 Fo+Myc. Each treatment consisted of 3 replicates of 48 seeds. The experiment was carried out in a completely randomised design.

Pine seeds were sown in multipots (250 ml) in a greenhouse (ETSIIA-Palencia) in early February 2004. All seedlings were grown in a mixture of Sphagnum (Finn peat) and vermiculite (1:1). The pot substrate was autoclaved twice at 121ºC for 90 min before sowing. The Myc treatment was inoculated with 50 ml of media containing *L. laccata* inoculum. A 5 ml spore suspension (10^6 spores ml-1) of *Fusarium verticillioides* or *Fusarium oxysporum* was added to each pot of Fm, Fm+Myc and Fo, Fo+Myc treatments. Control seedlings were inoculated with 5 ml of MMN medium.

Seedlings were grown in a greenhouse until mid-July. Watering and other procedures were common nursery practice; no fungicides applied. Fifteen seedlings of each treatment were randomly taken at the end of July.

Thirteen weeks after sowing, Pre-emergence damping-off was estimated by counting the number of germinated seeds in each treatment.

Eighteen weeks after sowing, Post-emergence damping-off was analysed and classified into four damage classes: (0) no damage; (1) slight damage, (2) moderate damage; (3) dead seedling (Halldorsson *et al*. 2000).

The shoot dry weight, diameter, root length, root dry weight and number of mycorrhizal short roots were measured 18 weeks after planting. Soil was washed off the root, which was subsequently cut off the seedlings and the mycorrhizae were examined by a binocular magnifier (Nikon SMZ2T). The intensity of root colonization was expressed as percentage of mycorrhized apexes within 250 observed apexed plants.

Late damping-off

Simultaneously, in order to analyze late damping-off, Stone pine seeds were sown in multipots (50 ml) as previously described. Two-month-old seedlings were transferred to multipots (250 ml) for assay and six treatments were applied: 1 control (Not inoculated), 2 *Laccaria laccata* (Myc), 3 *Fusarium verticillioides* (Fm), 4 *Fusarium oxysporum* (Fo), 5 Fm+Myc, and 6 Fo+Myc. Each treatment consisted of 3 replicates of 36 seeds. The experiment was carried out in a completely randomised design.

Inoculum of *Fusarium* spp. and *L. laccata* was prepared as described previously. The Myc treatment was inoculated with 50 ml of media contained *L. laccata* inoculum. Fifteen days after sowing, a 5-ml spore suspension (10^6 spores ml-1) of *Fusarium verticillioides* and *Fusarium oxysporum* was added to each pot of Fm, Fm+Myc and Fo, Fo+Myc treatments respectively.

Eighteen weeks after inoculation, Late damping-off was estimated by recording seedling damage. All sample seedlings were evaluated into four damage classes: (0) no damage; (1) slight damage, (2) moderate damage; (3) dead seedling (Halldorsson et al. 2000).

Pine seedling measures and mycorrhizal colonization were evaluated as previously described in post-emergence assays.

Statistical analysis

All the data were processed by one-way analysis of variance (ANOVA), and repeated measures test ANOVA ($p<0.05$) using STATISTICA Software. The differences between means were considered significant ($p<0.05$) according to a least significant difference (LSD) multiple range test. For each treatment (Control, Myc, Fm, Fo, Fm+Myc, Fo+Myc), data were obtained by calculating the mean of three different

measures of 48 seedlings. Differences of weight, length, damping-off damage and mycorrhization were evaluated.

RESULTS

Pre-emergence damping-off

Thirteen weeks after sowing, germination percentage was around forty percent in almost all cases (Table 1). Those values were almost reached six-seven weeks after inoculation (Figure 1) and minimal variation was found on following weeks. Significant differences in plants inoculated with *F. oxysporum* or *F verticillioides* were not found. *F. verticillioides* seems to be more aggressive than *F. oxysporum*; however, no differences were found when compared with control. An increase of 5 to 7 percent of the germination percentage in mycorrhizae treatments was recorded; however, differences were statistically significant only when data were pooled (Myc vs. No Myc).

Post-emergence damping-off

In post-emergence damping-off assays, significant damping-off levels were recorded in all *F. oxysporum* treatments. The presence of mycorrhizae (Fo+Myc) significantly reduces damping-off (Figure 2) but damage level remained higher than control. *Fusarium verticillioides* was no different from control, and presence of mycorrhizae slightly reduces severity, but statistical differences were absent. The mycorrhization percentage (Table 4) ranged from 2.6 percent (Fo+Myc) to 6.8 percent (Myc). Although

the percentages were very poor, those levels were statistically significant and the reduction of *F. oxysporum* damping-off damage was also significant.

Significant increasing of damage was found in the early weeks buts differences on the last weeks (12^{th} to 18^{th}) were minimal (Figure 3).

Eighteen weeks after sowing, measurements of seedlings dimensions showed no significant differences in the shoot height (Table 2). Above ground biomass of inoculated seedlings was no different to their respective controls. However, seedlings inoculated with *F. verticillioides* and mycorrhizae had greater biomass than uninfected-non mycorrhized control.

Root length was shorter in all treated cases compared with untreated control, but differences were only significant in *F. oxysporum* inoculated treatments and those inoculated with *F. verticillioides* and *L. laccata*. No significant differences were found when compared with the mycorrhized control. Root weight was significantly lower in seedlings inoculated with *F. oxysporum* and mycorrhizae than untreated control and mycorrhized control.

Late damping-off

Fusarium oxysporum was found to be pathogenic in all cases but in this instance mycorrhizae showed no effect (Figure 4). The damage values reached the final values at about 16 weeks after inoculation (Figure 5). *Fusarium verticillioides* damage was so scarce that statistical differences could not be established. Mycorrhization (Table 4) was low in both control and *F. verticillioides* inoculated seedlings (9.3% and 3.4%, respectively), and in *F. oxysporum* inoculated seedlings no significant levels (2.0 %) were found when those were compared with non mycorrhized controls (0%).

Eighteen weeks after inoculation, significant differences in shoot height were found. In *Fusarium oxysporum* and *F. verticillioides* treatments (Fo4, Fm6, Fo4+Myc, Fm6+Myc) shoot height was shorter than the mycorrhized control (Myc) and the untreated plants (Control) (Table 3). Furthermore, the mycorrhizae showed a negative effect on shoot height. Above ground biomass was significantly reduced in all fungal treatments with or without the mycorrhyzae. The exception was *F. verticillioides* treatment, in which significant differences were absent when compared with the control and the other fungal treatments. Mycorrhizae inoculation did not produce positive effect in above ground biomass.

The root length of inoculated and uninoculated seedlings showed no difference between treatments and controls. Root weight was significantly lower in mycorrhized seedlings, but in *F. oxysporum* and *F. verticillioides* inoculated seedlings no significant effect was found when compared with their respective controls.

Mycorrhization

It must be noticed that the percentage of mycorrhization was very low in all cases and did not reach ten percent (Table 4). The lowest values were found in *F. oxysporum* treatments but *F. verticillioides* also reduced significantly mycorrhization percentage. In mycorrhized controls, the results ranged from 6.8 percent in post-emergence assays to 9.3 percent in late damping-off assays. It must be noticed that in *F. oxysporum* treatment (Fo4+Myc) at the late damping-off assays, the percentage of mycorrhization did not reached significant levels when compared to non-mycorrhized control.

DISCUSSION

Fusarium oxysporum and *F. verticillioides* are two important members of the fungal complex that produces damping-off on several conifer species. The *Fusarium* isolates (*F. oxysporum* Fo4 and *F. verticillioides* Fm6) used in this study have been previously described as pathogens against other pine species (Martin-Pinto et al., 2006a; Machon et al. 2006). Furthermore, *in vitro* tests (Martin-Pinto et al., 2006b) and *in vivo* assays (Machon et al., 2006) of *L. laccata* isolate against *Fusarium* isolates have been efficient. In the present study, only *F. oxysporum* showed a pathogenic capability against *P. pinea* seedlings. *Fusarium verticillioides* damages were scarce and no significant levels were found.

The percentage of germination was recorded as measure of pre-emergence damping-off caused by *Fusarium* isolates. Although *Fusarium* isolates did not produce statistically significant damage, the mycorrhizae influence was positive and the germination percentage was higher in mycorrhizae treatments (average 43.3%) than in non mycorrhizae treatment (38%). Previous results on *P. sylvestris* showed that the pathogen isolate Fm6 (*F. verticillioides*) did not produce significant damage on *P. sylvestris*, however, isolate Fo4 (*F. oxysporum*) produced significant damage that was reduced by the presence of mycorrhizae (Machon et al. 2006).

In post-emergence damping-off assays, only *F. oxysporum* damage reached significant values different from controls. Both height and weight of the seedlings were affected by *Fusarium* and mycorrhizae inoculation, but no statistical differences could be established between each treatment and their respective controls. On *P. sylvestris* seedlings both pathogens reached significant levels of post-emergence damage and only *F. verticillioides* damping-off was controlled by *L. laccata* (Machon et al. 2006).

Mycorrhizae significantly reduced severity of *F. oxysporum* damping-off but the damage value was still statistically higher than control. The mycorrhization percentage was very low (2.6%), but our results showed that these levels of mycorrhization could be enough for plant protection, and thus, the mantle barrier hypothesis (Marx, 1972) could not explain the mechanism of protection. Simultaneous studies carried out in our lab (Machon et al., 2006) with *P. silvestris* showed higher mycorrhization percentage. Those mycorrhization percentages were more than 50% at mycorrhized control and about 25-30% when inoculated with *Fusarium* spp. In this case the barrier effect or the competition capabilities could be taking into account. In further experiments it would be very interesting to evaluate if the level of mycorrhization could be correlated with different levels of seedling protection as registered with other species (Morin et al., 1999).

The late damping-off results showed that *F. verticillioides* isolate behaved as a weak pathogen against *P. pinea*, and damage level was apparently higher than controls but differences were not statistically significant. Similar result were found on *P. sylvestris* seedlings assay (Machon et al., 2006) in which *F. verticillioides* damage values were slightly higher than control. On the other hand, *Fusarium oxysporum* reached significant damage level, but the presence of mycorrhizae did not reduce severity of lesions in the late damping-off assays. However, the mycorrhization percentage in the *F. oxysporum* treatment was no significantly different from control. Those results agreed with the *P. sylvestris* assay (Machon et al., 2006) in which *F. oxysporum* produce significant damage and mycorrhization percentage was not different from control but in this case a damage reduction was observed.

The mycorrhization percentage in *F. oxysporum* late damping-off assays was very low (2%) and inefficient for protection, but it was only a bit lower than in post-emergence

damping-off assays (2.6%) in which the mycorrhizae provided a protective effect and almost equal to level obtained at *P. sylvestris* assay (2.06%) in which protection was also effective (Machon et al., 2006). This could indicate that the minimum mycorrhization level required for protection of some *Pinus* spp. should be around 2 percent or that the influence of mycorrhization became less important as plant grows and the mycorrhizal protection could be more effective at early stages of seedlings.

Morin et al. (1999) found a correlation between mycorrhizal colonization rate and infection rate of *Cylindrocladium floridanum*; and even at low percentage of mycorrhization, the plant protection was effective. Martin-Pinto et al.(2006b), pointed out that the preinoculation of substrate with *Laccaria laccata* provided statistically significative reduction of disease of *Pinus nigra* but only in the case of two months of preculture, and longer periods (four and six months) did not produce better results. Other studies on *Laccaria laccata* have also shown that a physical barrier around the roots was not involved in the protective effect (Stack and Sinclair, 1975; Chakravarty et al., 1991; Hwang et al., 1995).

On the other hand, it has been shown that conifer root exudates stimulate the production of antifungal compounds by *L. laccata* inhibiting *Fusarium* spp. (Chakravarty and Unestam, 1985). Several authors have reported that disease suppression by ectomycorrhizal fungi (including *L. laccata* against *Fusarium*) is associated with plant-produced antimicrobial substances (Chakravarty and Hwang, 1991; Chakravarty et al., 1991; Sylvia and Sinclair, 1983a and 1983b; Sampagne and Perrin, 1985) and in the rhizosphere of pine seedlings inoculated with *Paxillus involutus* the presence of fungitoxic substances has been reported (Duchesne et al., 1987).

The mycorrhization analysis showed that the percentage of mycorrhization was significantly lower when *Fusarium* was present. That could be explained by a direct

competition for nutrients and space, and the low level of mycorrhizae could reflect the fast colonization by *Fusarium* (even when the mycorrhizal fungus was ground earlier).

In nurseries, microbiological activity in the soil is the principal factor affecting mycorrhizae formation (Kropp and Langlois, 1990). For bare-root production, soil fumigation should be carried out to reduce pathogen populations before sowing and introducing mycorrhizal fungi (Marx and Cordell, 1987). To eliminate chemical products and inoculate seedlings with ectomycorrhizal fungi before exposing them to pathogens, containerized inoculated seedlings should be produced and transplanted in infested soil, since the inhibition of mycorrhizal formation by substrate microbial activity is rarely a problem in containers where excellent results have been obtained (Morin et al., 1999).

The present study suggests that inoculation of *P. pinea* seedlings by ectomycorrhizal fungi in nurseries reduces both damage intensity and seedlings mortality caused by *Fusarium* damping-off. However several points should be taken into account: (a) *Fusarium verticillioides* isolate Fm6 that has been reported as pathogen against *Pinus nigra* and *P. sylvestris* (Martin-Pinto et al., 2006a; Machon et al., 2006], did not produce severe damage in *Pinus pinea* seedlings; (b) statistically, the pre-emergence damping-off damage was no significant; (c) the percentage of mycorrhization was low in all cases and it did not reached significant level in *F. oxysporum* late damping-off assays.

It could be possible that *Pinus pinea* was more resistant to *Fusarium* damping-off than other *Pinus* species; despite the Fm6 isolate was recovered from *Pinus pinea* diseased plants [10]. Our results showed that percentage of mycorrhization as low as 2.6 percent or 2.06 percent in *P. sylvestris* (Machon et al., 2006) could be enough to provide effective protection, thus indicating that the principal method of protection could be not

the barrier effect, nor the metabolites produced by ectomycorrhizal fungi. According to our results, the hypotheses of plant-produced antifungal products could be the most suitable explanation. Nevertheless, more detailed biochemical examination should be carried out in order to confirm this hypothesis.

The protective effect of mycorrhizae in *F. oxysporum* post-emergence damping-off assays was not complete. Previous studies (Morin et al., 1999) suggested a correlation between mycorrhizal colonization rate and infection rate, therefore higher level of mycorrhization should be attempted in order to achieve mycorrhization percentage that should completely restore the seedlings health as found in *F. oxysporum* post emergence damping-off on *P. sylvestris* seedlings (Machon et al., 2006).

Our results suggest that mycorrhization can protect pine seedling and could be used as biological control of damping-off in forest nurseries, but further studies are required to establish the effectiveness of *L. laccata* against other pathogens, the level of mycorrhization required and the mechanisms involved in mycorrhizal protection in *Pinus* species

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Table 1. Pre-emergence damping-off of *Pinus pinea* (expressed as mean percentage of seed germinating 13 weeks after sowing in three inoculation experiments) in media with and without *Laccaria laccata* inoculum and with *Fusarium verticillioides*, *F. oxysporum* or no *Fusarium*. Means are followed by the standard deviation. No significant differences were found.

	No *Fusarium*	*F. verticillioides*	*F. oxysporum*
No Myc	40.28±7.3	32.64±6.7	40.97±2.4
Myc	45.14±2.4	37.50±5.5	47.22±3.2

Table 2. Post-emergence damping-off of *Pinus pinea*. Measures were taken 18 weeks after sowing. Diameter in mm was measured at the shoot base and dry weight was used. Within columns values sharing the same letter did not show significant differences (LSD test $p<0.05$)

Treatment	Shoot height (cm)	Shoot weight (g)	Diameter (mm)	Root length (cm)	Root weight (g)
Control	11.14 a	0.300 a	1.593 b	13.31 b	0.107 b
Fm6	10.95 a	0.348 ab	1.553 ab	12.93 ab	0.110 b
Fo4	10.89 a	0.328 ab	1.463 ab	12.30 a	0.098 ab
Myc	10.94 a	0.347 ab	1.573 ab	12.95 ab	0.105 b
Fm6 + Myc	11.33 a	0.372 b	1.597 b	12.25 a	0.101 ab
Fo4 + Myc	10.57 a	0.296 a	1.410 a	12.80 ab	0.078 a

Table 3. Late damping-off of *Pinus pinea*. Measures were taken 18 weeks after planting. Diameter in mm was measured at the shoot base and dry weight was used. Within columns values sharing the same letter did not show significant differences (LSD test $p<0.05$)

Treatment	Shoot height (cm)	Shoot weight (g)	Diameter (mm)	Root length (cm)	Root weight (g)
Control	15.52 c	0.448 b	1.543 b	13.19 ab	0.145 b
Fm6	12.43 a	0.387 ab	1.503 b	13.20 ab	0.141 b
Fo4	11.84 a	0.332 a	1.427 ab	14.65 b	0.119 ab
Myc	13.61 b	0.343 a	1.450 ab	12.25 a	0.103 a
Fm6 + Myc	11.69 a	0.325 a	1.440 ab	12.98 a	0.101 a
Fo4 + Myc	11.55 a	0.318 a	1.333 a	13.18 ab	0.095 a

Table 4. Mycorrhization percentage of *Pinus pinea* 18 weeks after sowing (Post emergence Damping-off) and 18 weeks after planting (Late Damping-off). The intensity of root colonization was expressed as percentage of mycorrhized apexes within 250 observed apexed plants. Means followed by the same lower case letter (a-b) within each file (*Fusarium* treatments) or by the same capital case letter (A-B) within each column (*L. laccata* treatment) are not significantly different at p=0.05 (LSD test).

Post-emergence Damping-off	**No *Fusarium***	***F. moniliforme***	***F. oxysporum***
No *L. laccata*	0.0 aA	0.0aA	0.0 aA
L. laccata	6.81aB	4.52bB	2.58 bB
Late damping-off	**No *Fusarium***	***F. moniliforme***	***F. oxysporum***
No *L. laccata*	0.0 aA	0.0aA	0.0 aA
L. laccata	9.28aB	3.39 bB	2.03 bA

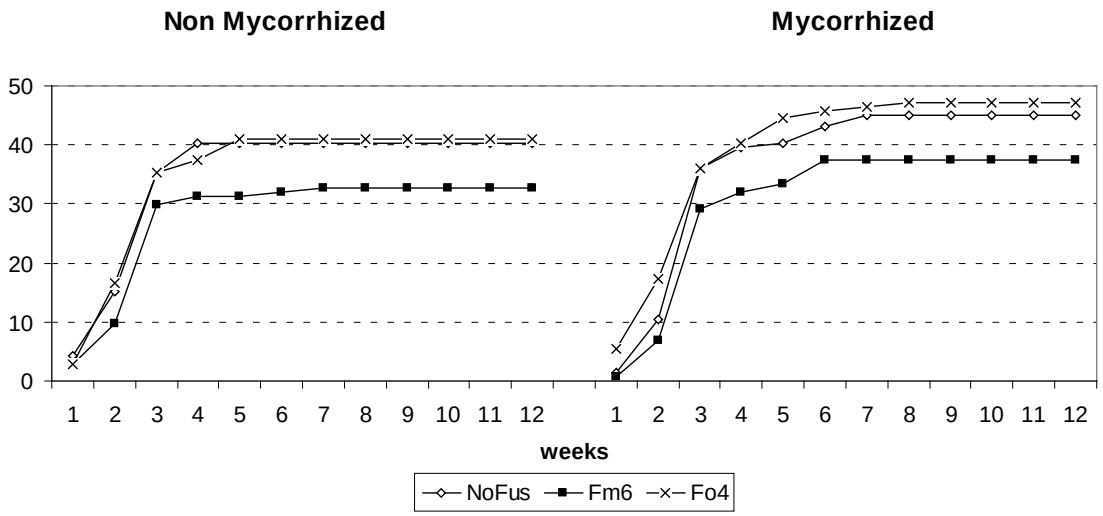

Figure 1. Pre-emergence damping-off every week after *Fusarium* inoculation measured as percentage of germinated plants. *Fusarium* treatments are represented as NoFus (no *Fusarium*); FM6 (*F. verticillioides*) and FO4 (*F. oxysporum*).

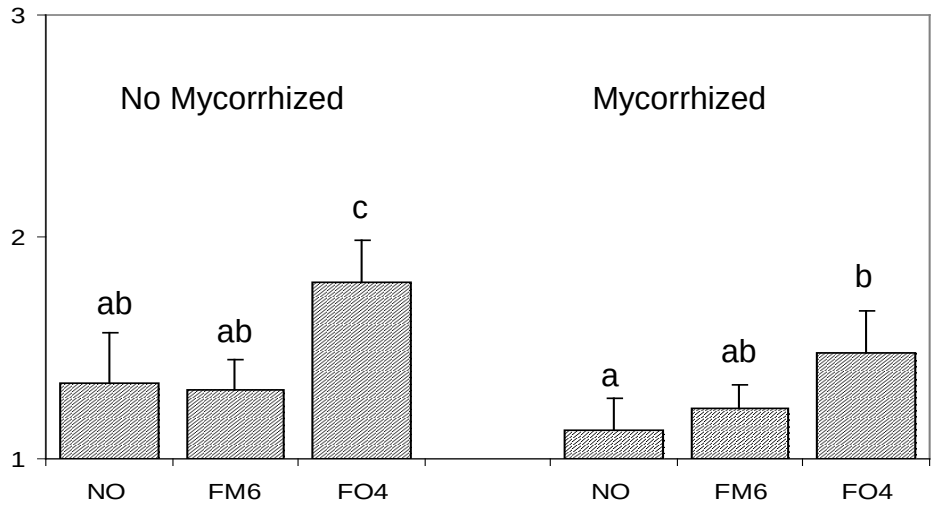

Figure 2. Post-emergence damping-off of *Pinus pinea* seedlings 18 weeks after sowing. Severity was expressed as mean damage of seedlings in three inoculation experiments by using the scale: 1 (no symptoms), 2 (moderate damage) and 3 (dead plant). *Fusarium* treatments are represented as NO (no *Fusarium*); FM6 (*F. moniliforme*) and FO4 (*F. oxysporum*). Error bars are standard deviation. Columns sharing the same lower case letter (a-c) are not significantly different at $p = 0.05$ (LSD multiple range test).
* Damage in mycorrhized *F. oxysporum* treatment was significatively lower than no mycorrhized FO treatment.

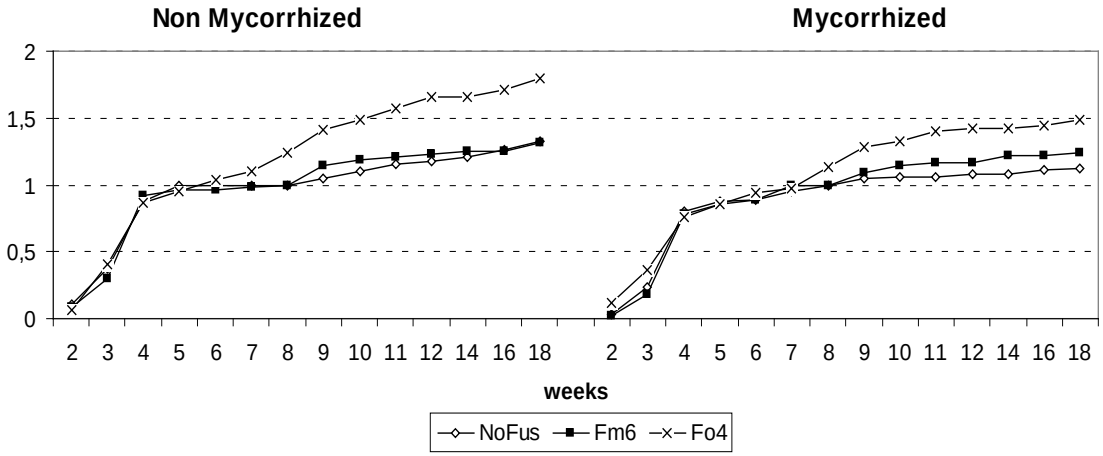

Figure 3. Post-emergence damping-off damage after *Fusarium* inoculation. Severity was expressed as mean damage of seedlings in three inoculation experiments on the following scale: 0 (no symptoms), 1 (slight damage), 2 (moderate damage) and 3 (dead plant). *Fusarium* treatments are represented as NoFus (no *Fusarium*); FM6 (*F. verticillioides*) and FO4 (*F. oxysporum*).

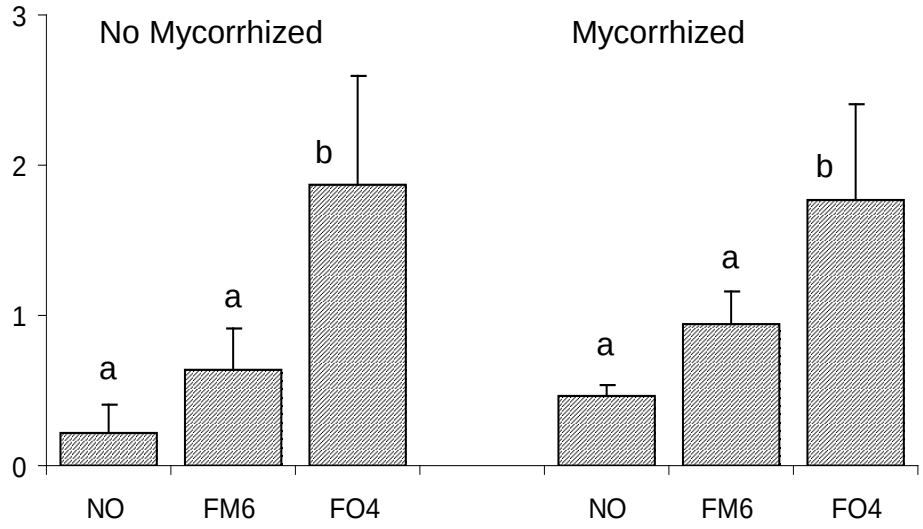

Figure 4. Late damping-off *Pinus pinea* seedlings 18 weeks after planting. Severity was expressed as mean damage of seedlings in three inoculation experiments by using the scale: 0 (no symptoms), 1 (slight damage), 2 (moderate damage) and 3 (dead plant). *Fusarium* treatments are represented as NO (no *Fusarium*); FM6 (*F. moniliforme*) and FO4 (*F. oxysporum*). Error bars represent standard deviation Columns sharing the same lower case letter (a-b) are not significantly different at p= 0.05 (LSD multiple range test).

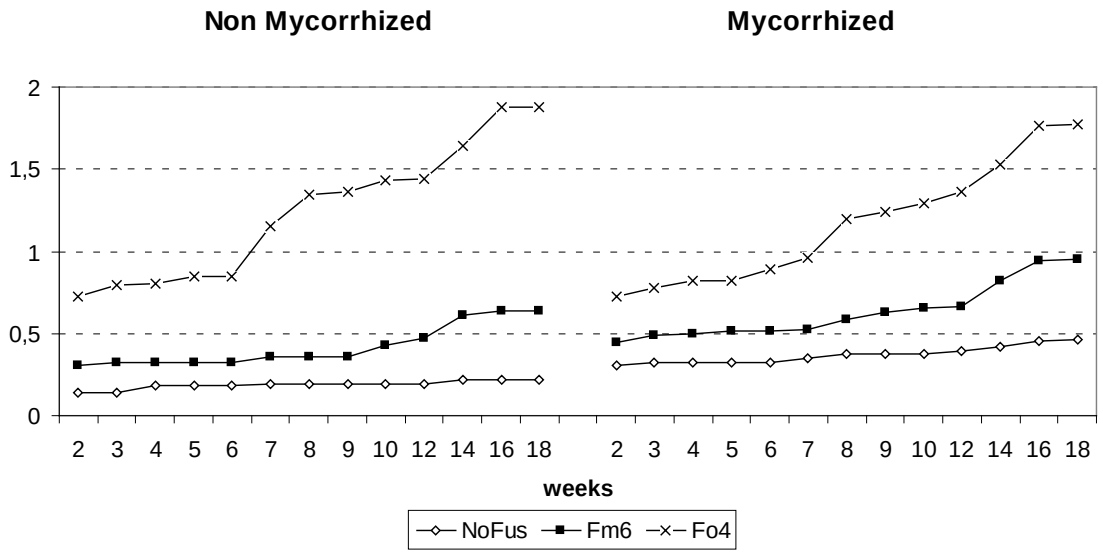

Figure 5. Late damping-off damage after *Fusarium* inoculation. Severity was expressed as mean damage of seedlings in three inoculation experiments on the following scale: 0 (no symptoms), 1 (slight damage), 2 (moderate damage) and 3 (dead plant). *Fusarium* treatments are represented as NoFus (no *Fusarium*); FM6 (*F. verticillioides*) and FO4 (*F. oxysporum*).