In vitro antagonism of edible ectomycorrhizal fungi against *Fusarium oxysporum* and *Fusarium verticillioides*

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ABSTRACT

Twenty-one isolates of edible ectomycorrhizal fungi (ECM) of 15 different species were tested *in vitro* for mycelial growth and spore germination against two isolates each of *Fusarium oxysporum* and *F. verticillioides*. The growth of *Fusarium* isolates was significantly inhibited when co-cultured with most of the 21 ECM fungi tested. Two ECM fungi (Ba-2 and Xf-2) failed to reduce the growth of *Fusarium* isolates. In paired cultures, growth of all *Fusarium* isolates was significantly reduced by *Rhizopogon roseolus*, *Suillus luteus*, *Tricholoma portentosum*, *Amanita rubescens*, *Amanita ovoidea*, *Boletus fragrans* and *Laccaria laccata*. Spore germination of all *Fusarium* isolates was strongly inhibited by culture filtrates of *R. roseolus* and the two *S. luteus* isolates. Different behavior between ectomycorrhizal fungi and *Fusarium* species, as well as among isolates of the same species was observed in both assays. Inhibition of *Fusarium* species suggests that several isolates of edible ectomycorrhizal fungi have a high potential for biological control of damping off. The effect of ECM fungi was less evident in the conidial germination assay than in the growth assay, although inhibition was also observed. The methodology presented here can be used as an effective tool for *in vitro* selection of ectomycorrhizal fungi and in nursery assays.

Keywords: biological control, edible ectomycorrhizal fungi (ECM), seedling diseases, *in vitro* selection, culture filtrates

RÉSUMÉ

On a testé *in vitro* 21 isolats de champignons ectomycorhiziens comestibles (ECM) de 15 espèces différentes sur leur capacité à inhiber la croissance mycélienne et la germination des spores de deux isolats de *Fusarium oxysporum* et de deux isolats de F. *verticillioides*. La croissance des isolats de *Fusarium* a subi une forte diminution lorsqu'ils étaient cultivés en paires avec presque tous les 21 champignons ECM testés. Deux champignons ECM (Ba-2 and Xf-2) n'ont pas réduit la croissance des isolats de *Fusarium*. Dans les cultures en paires, tous les isolats de *Fusarium* ont vu leur croissance fortement diminuée par *Rhizopogon roseolus*, *Suillus luteus*, *Tricholoma portentosum*, *Amanita rubescens*, *Amanita ovoidea*, *Boletus fragrans* et *Laccaria laccata*. La germination des spores de tous les isolats de *Fusarium* a été considérablement réduite par *R. roseolus* et des filtrats de culture de deux isolats de *S. luteus*. Le comportement de chacun des champignons ectomycorhiziens a varié selon l'espèce de *Fusarium* et l'isolat de chacun. L'effet d'inhibition exercé sur les espèces de *Fusarium* laisse voir que certains isolats de champignons ectomycorhiziens comestibles pourraient être utilisés dans la lutte contrôle biologique contre la fonte des semis. Les effets des champignons ECM auront moins manifestes lors des essais de germination des conidies que lors des essais de croissance, même s'il y a eu une certaine réduction de la germination. La méthodologie que nous présentons ici pourrait aussi servir pour la sélection *in vitro* de champignons ectomycorhiziens et pour diverses expériences en pépinière.

Mors-clés : lutte biologique, champignons ectomycorhiziens comestibles (ECM), maladies des semis, sélection *in vitro*, filtrats de culture

Introduction

The fungal genus *Fusarium* includes species that cause serious plant diseases which cannot be easily controlled in forest nursery crops and plantations all over the world, being responsible for substantial damage (Axelrood *et al.* 1995; Neff and Perrin 1999). *Fusarium* inoculum can be carried on seeds, containers, peat moss or in irrigation water (Hwang *et al.* 1995). Some species of this genus, for instance susceptible pines, can be seriously impacted by *F. circinatum* Nirenberg & O'Donnell. threatening the sustainability of forests (Wikler *et al.* 2003; Landeras *et al.* 2005).

Several fungicides have been used to control damping off caused by *Fusarium*, but the suitability of chemical applications to manage this disease remains doubtful due to environmental consequences and low efficiency (Lamichhane *et al.* 2017), especially during the first stages of seedling growth in nurseries (Sinclair *et al.* 1975). Furthermore, several pathogenic fungi have developed resistance mechanisms against many fungicides (Lucas *et al.* 2015; Hu et al. 2016). Evidence suggests that some ectomycorrhizal (ECM) fungi behave as biological control agents and are able to reduce the damaging effects of plant pathogens. (Marx and Davey 1969; Duchesne

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1994; Annesi and Motta 1998; Pedersen *et al*. 1999; Chakravarty *et al*. 1999; Rudawska 2000; Martín-Pinto *et al*. 2006*a*; Zhang *et al*. 2011). The inhibitory effect caused by ECM fungi can be expressed through nutrient competition or the production of antifungal exudates, which may induce antibiosis and/or hyperparasitism when ECM fungi colonize the pathogen (Duchesne *et al*. 1987a; 1987b; 1988; 1989a; 1989b; Chakravarty *et al*. 1990; 1991). These mechanisms can be directly tested *in vitro* by ECM-pathogen co-cultures, or by ECM culture filtrates containing active substances involved in the process (Chakravarty *et al*. 1999).

Previous studies reported that the efficacy of these symbiotic fungi for plant protection against root pathogens varies among mycorrhizal species and among different isolates within the same species (Sampagni and Perrin 1985; Perrin and Soulas 1996; Zhang et al. 2017). Thus, considering such inter and intraspecific variation, it is advisable to test the highest number of ECM species and isolates for selecting candidates with effectivity against *Fusarium* spp. (Trappe 1977; Zhao *et al*. 1988).

Many studies on ECM fungi as biological control agents have tested poisonous species such as *Paxillus involutus* (Batsch ex Fries) Fries (Duchesne *et al*. 1987a; 1987b; 1988; 1989a; 1989b; Chakravarty *et al*. 1990; 1991) and only few studies have used edible species as *Laccaria laccata* (Scop. Ex Fr.) Berk. & Br. (Chakravarty and Hwang 1991) or *Suillus tomentosus* (Kauff.) Singer, Snell and Dick (Hwang *et al*. 1995), therefore, the efficacy of edible ECM for control of damping off caused by *Fusarium* is largely unknown.

However, some edible ECM fungi with recognised economic value may be promising for protection of damping off in nurseries (Martín-Pinto *et al*. 2006a). These species may provide protection against pathogens and add economic value through the production of edible fruiting bodies following after planting.

Several species of the genus *Lactarius* (*L. deliciosus* L. Gray, *L. semisanguifluus* Heim & Leclair. and *L. sanguifluus* (Paulet) Fr.) and *Boletus* (*B. pinophilus* Pilat & Derm. *B. edulis* Bull.:Fr., *B. aestivalis* (Paulet) Fr. and *B. aereus* Bull.) are valued and consumed all over the world (Boa 2004). Moreover, other species such as *Suillus luteus* (L.) Roussel are commonly used in the food industry, as well as other less known species, for instance *Amanita rubescens* (Pers.: Fr.) S. F. Gray, *Amanita ovoidea* (Bull.: Fr.) Quèl., *Boletus fragans* Vittad., *Laccaria laccata*, *Leccinum lepidum* (Bouchet ex Ess.) Quadr., *Rhizopogon roseolus* (Corda) Th. M. Fr., *Xerocomus ferrugineus* (Schaeffer) Bon and *Tricholoma portentosum* (Fries) Quélet., which are consumed in some regions of Spain and in other countries (Martínez de Azagra *et al*. 1997).

The main goal of our work was to investigate the *in vitro* interactions between isolates of various edible ECM fungi against pathogenic strains of *F. oxysporum* Schlecht. Emend. Snyd. & Hans. and *F. verticillioides* (Sacc) Niremberg (syn. *F. moniliforme* Sheldon), the main causal agents of damping off in Spanish forest nurseries (Martín-Pinto *et al*. 2006b). Specifically, the study aimed (1) to evaluate the effect of edible ECM fungi on growth of *Fusarium* spp. colonies, and (2) to assess the effect of edible ECM fungi culture filtrates on *Fusarium* spp. conidial germination.

Materials and Methods

Fungal organisms

Fifteen species of edible ECM fungi were selected by their ability to form ectomycorrhizal short roots (Trappe 1962) and their edible value (Sanchez and García 2006). ECM fungi were isolated using the Molina and Palmer (1982) methodology (Table 1), except Rr-1 and Ll-1 isolates that were provided by the Valonsadero Forest Research Center in Soria, Spain. A total of four virulent isolates of *Fusarium* were used, two *F. oxysporum* (Fo-4P and Fo-5P) and two *F. verticillioides* (Fm-5P and Fm-6P) (Martín-Pinto *et al*. 2006a; 2008; Olaizola 2007). Single-spore cultures were obtained and subcultured every seven days on Difco PDA medium.

Effect of ECM fungi on *Fusarium* growth

The antagonistic effect of the 21 ECM isolates against *F. verticillioides* and *F. oxysporum* was studied on Modified Melin Norkrans (MMN) medium (Marx 1969) in 90-mm Petri dishes. The media was inoculated on the edge of the plates with ECM fungi by placing 5-mm agar plugs of each isolate taken from the periphery of 30-day-old mycelial mats. Cultures were allowed to grow at 22 ºC in the dark, and after fifteen days 5-mm mycelial plugs from each *Fusarium* isolate were placed on the opposite side of the plate at a distance of 4 cm. Antagonism was recorded daily for 10 days by measuring the radial growth of the *Fusarium* colonies. Each combination of *Fusarium* and ECM fungi was replicated five times.

Effect of ECM fungi on *Fusarium* conidial germination

The assay was carried out using Chakravarty and Hwang (1991) methodology with some variations as described next.

Table 1. Isolate code, species and main tree species of edible ectomycorrhizal fungi tested against pathogenic isolates of *Fusarium*

Isolate code	Species	Dominant tree species
Ld-0	*Lactarius deliciosus*	*Pinus sylvestris*
Ld-1	*Lactarius deliciosus*	*Pinus pinaster*
Ld-2	*Lactarius deliciosus*	*Pinus sylvestris*
Ls-3	*Lactarius sanguifluus*	*Pinus pinaster*
Lm-1	*Lactarius semisanguifluus*	*Pinus pinaster*
Ao-1	*Amanita ovoidea*	*Quercus ilex*
Ar-1	*Amanita rubescens*	*Quercus ilex*
Tp-1	*Tricholoma portentosum*	*Pinus sylvestris*
Tp-2	*Tricholoma portentosum*	*Pinus sylvestris*
Ll-1	*Laccaria laccata*	*Pinus sylvestris*
Rr-1	*Rhizopogon roseolus*	*Pinus sylvestris*
Bf-1	*Boletus fragrans*	*Quercus ilex*
Ba-2	*Boletus aereus*	*Quercus ilex*
Bv-1	*Boletus aestivalis*	*Quercus pyrenaica*
Bv-2	*Boletus aestivalis*	*Quercus pyrenaica*
Bl-1	*Leccinum lepidum*	*Quercus ilex/Q. faginea*
Bl-4	*Leccinum lepidus*	*Quercus ilex/Q. faginea*
Bp-1	*Boletus pinophylus*	*Castanea sativa*
Sl-1	*Suillus luteus*	*Pinus sylvestris*
Sl-2	*Suillus luteus*	*Pinus sylvestris*
Xf-2	*Xerocomus ferrugineus*	*Quercus ilex*

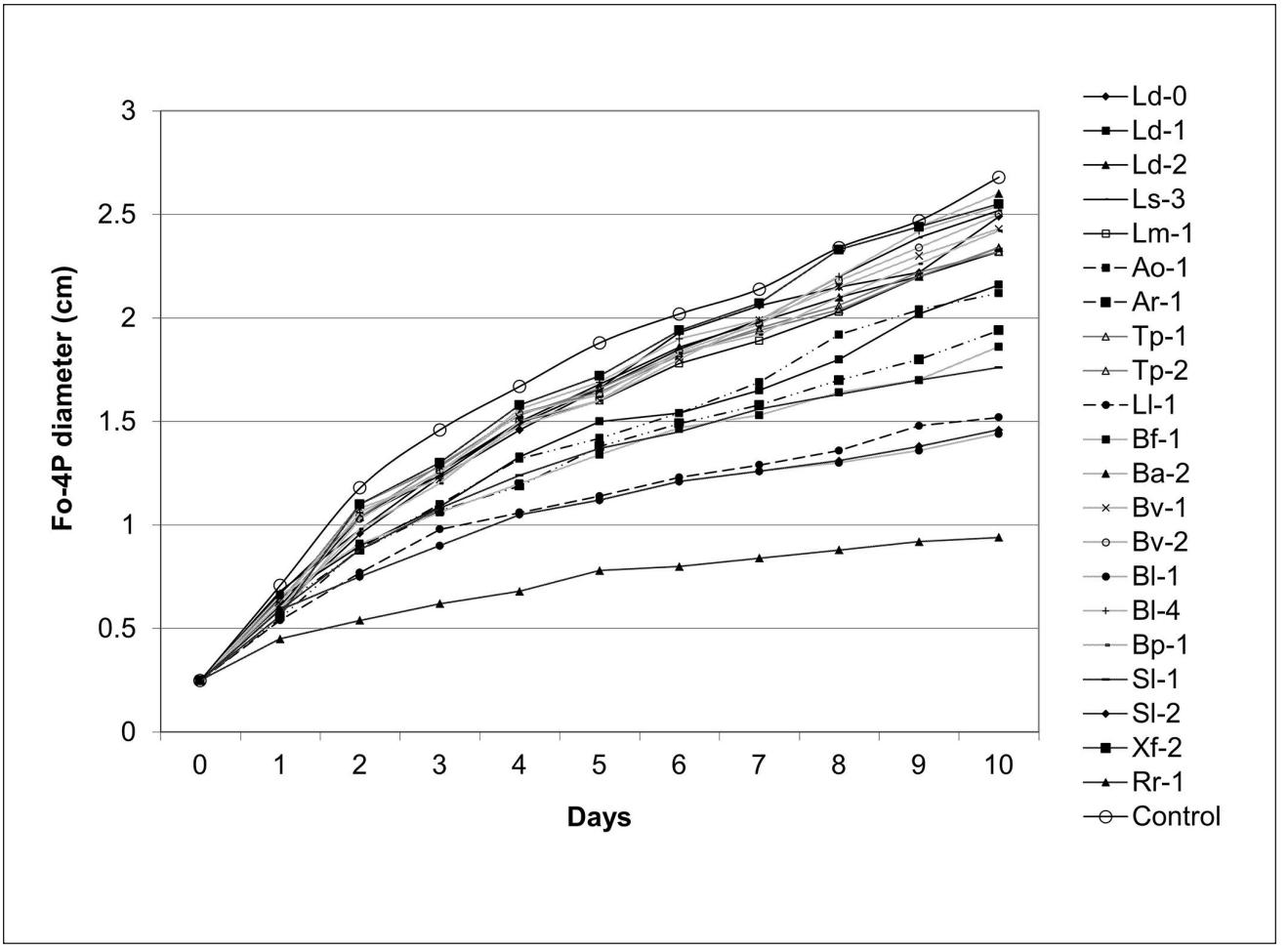

Fig. 1. Radial growth of *Fusarium oxysporum* (Fo-4P) in co-culture with 21 ectomycorrhizal fungal isolates (see Table 1 for the list of names)

To produce fungal culture filtrates, three agar plugs (5-mm diameter), removed from the periphery of 30-day-old mycelial mats, were placed into 50 ml Erlenmeyer flasks and maintained in 25 ml liquid MMN medium at 22 °C in the dark on an orbital shaker (100 rpm). After 20 days, culture filtrates were collected by filtering through 0.20 µm cellulose acetate syringe filters (Albet®). The filtrates were then stored in sterile Eppendorf tubes at 4 °C in the dark. A conidial suspension was prepared with each *Fusarium* isolate by adding 3 ml of sterile distilled water into a six-day-old PDA culture, and the final conidium concentration was adjusted to $3{\cdot}10^6$ microconidia·ml^{-1} by using a haemocytometer.

One hundred µl of the ECM culture filtrates per isolate and 100 µl of the adjusted spore suspension of *Fusarium* were mixed in Eppendorf tubes and maintained in the dark at 22 °C. We also set up a control treatment that contained 100 µl of sterile liquid MMN and 100 µl of the *Fusarium* conidia suspension. Spore germination of *Fusarium* was observed under the microscope at 0, 6, 12 and 24 hours after confrontation. A total of 1200 spores were counted for each treatment (6 replicates of 200 spores each).

Statistical analysis

Data from both experiments were submitted to analysis of variance (ANOVA) to examine differences among *Fusarium* species and isolates, and to evaluate the effect of each ECM fungi against them using Statistica 5.5 software. The individual means were compared using the Least Significant Difference (LSD) test at $p<0.05$.

Results

Effect of ECM fungi on *Fusarium* growth

Growth of the *Fusarium* isolates was significantly inhibited when co-cultured with most of the 21 ECM fungi tested (Figs. 1, 2). Only two of the ECM fungi assayed (Ba-2 and Xf-2) failed to decrease the growth of any *Fusarium* isolate. ECM isolates Ao-1, Ar-1, Bf-1, Bl-1, Ld-2, Ll-1, Rr-1, Sl-2, Sl-1 and Tp-1 inhibited all *Fusarium* isolates. Of those, Bl-1, Rr-1 and Sl-2 had the highest inhibitory effect (LSD test: $p < 0.05$). In other cases, ECM fungi caused inhibition of three or two of the *Fusarium* isolates (Ld-1 or Lm-1 and Tp-2 isolates respectively) (Fig. 2).

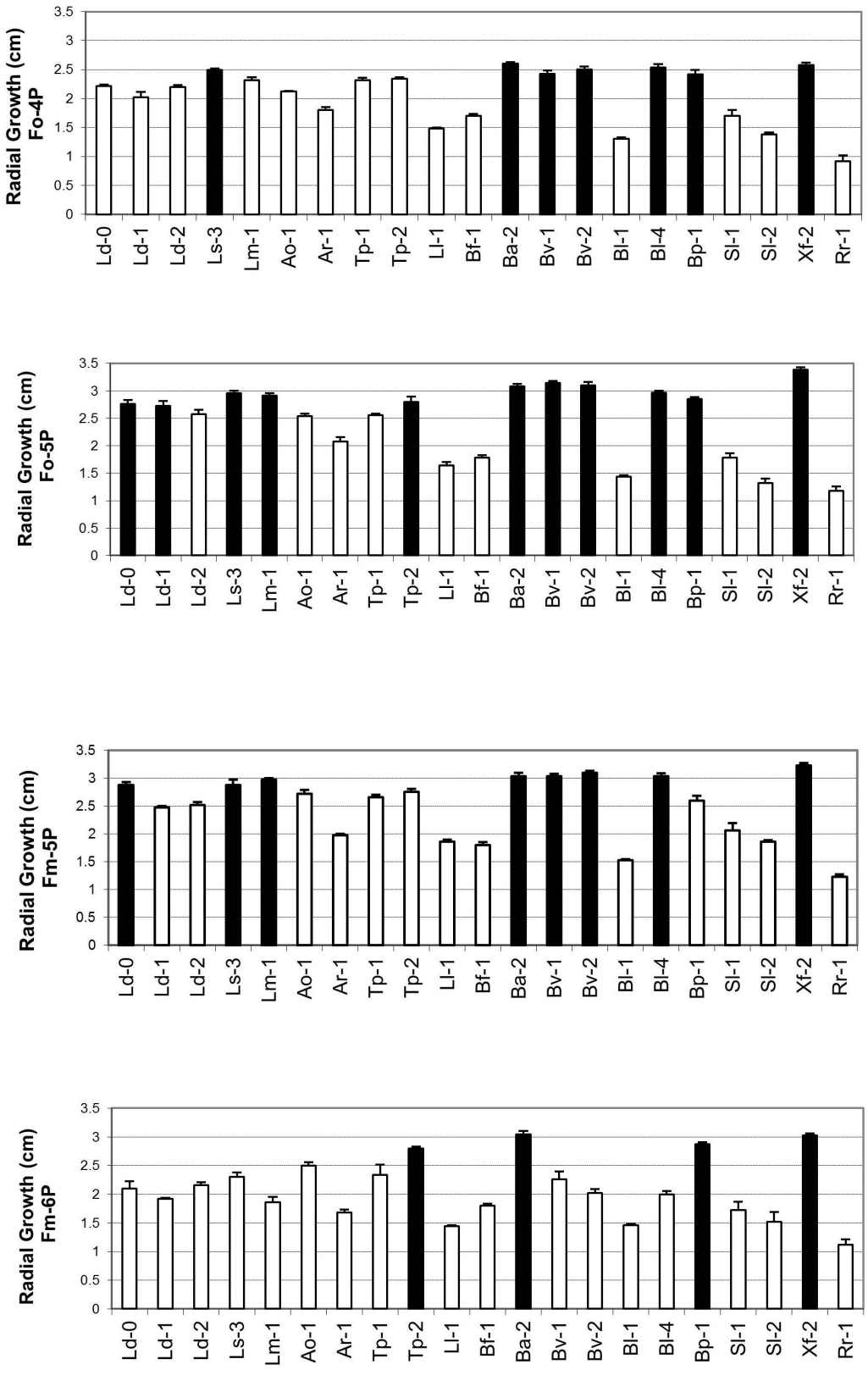

Fig. 2. Radial growth (cm) of two *F. oxysporum* isolates (Fo-4P and Fo-5P) and *F. verticillioides* isolates (Fm-5P and Fm-6P) in co-culture with 21 isolates of edible ectomycorrhizal fungi on agar plates after 10 days incubation. Black values are not significantly different compared to the control ($p < 0.05$)

Intraspecific variation was observed among ECM isolates. Three *Lactarius* isolates caused inhibition of Fo-4P, however, only one isolate repressed growth of Fo-5P. Similarly, Tp-1 inhibited growth of the four *Fusarium* isolates, compared to Tp-2 that affected only Fo-4P and Fm-5P isolates. Regarding the *Fusarium* isolates, significant differences in growth were found when the four strains were compared independently (ANOVA, $p<0.001$). However, we did not find significant differences in growth between the two *Fusarium* species when co-cultured with ECM fungi (ANOVA, $p=0.639$), despite the observation that ECM fungi caused growth inhibition on both *F. oxysporum* isolates in 24 treatments compared to 30 in *F. verticillioides* (Fig. 2).

Effect of ECM fungi on *Fusarium* conidial germination

The effect of ECM fungi on the *Fusarium* conidial germination assay was less evident than in the growth assay although some degree of inhibition was indeed observed. The effect of ECM filtrates on *Fusarium* after 6, 12 and 24 hours strongly differed among mycorrhizal isolates (Fig. 3). No spores of *Fusarium* had germinated at the beginning of the experiment (0 hours), while at 6 and 12 hours germination was variable amongst isolates. After 24 h, almost all spores in the control treatments germinated, therefore validating this time interval as optimal for comparison of *Fusarium* and ECM isolates (Fig. 4).

Many ECM fungal filtrates significantly inhibited spore germination of the four *Fusarium* isolates. There were significant differences in spore germination between both *Fusarium* species (ANOVA, $p = 0.004$) when treated with ECM culture filtrates. Twenty-five ECM isolates inhibited both isolates of *F. verticillioides* compared to 13 ECM isolates which inhibited germination of both *F. oxysporum* isolates (Fig. 4). Differences were observed among the four *Fusarium* isolates (ANOVA, $p < 0.0001$). Fm-6P was the most susceptible isolate with reduced germination when placed together with 16 ECM isolates, whereas Fo-4P was the most resistant with decreased germination when exposed to 6 ECM isolates. Fm-5P and Fo-5P responded similarly to ECM fungal filtrates with reduced germination when combined with 9 and 7 ECM isolates, respectively.

Quantitatively, the culture filtrates from *S. luteus* (Sl-1, Sl-2) and *R. roseolus* (Rr-1) were the most inhibitory, significantly reducing spore germination of all four *Fusarium* isolates (Fig. 4). ECM isolates greatly decreased spore germination of Fo-5P. Other ECM fungi appeared to cause a specific inhibition of particular *Fusarium* isolates. For instance, *Tricholoma portentosum* isolates (Tp-1 and Tp-2) produced the highest inhibition of Fm-5P, and *Leccinum lepidum* (Bl-1) was the most effective against Fm-6P. Four ECM culture filtrates (Bl-4, Ba-2, Ld-0, Lm-1) failed to affect either *Fusarium* isolate at the end of 24 hour period.

Similarly, as in the co-culture assay, intraspecific variation was observed between both *Fusarium* species and among ECM isolates (i.e., Ll-1 inhibited spore germination in Fo-5P, but not in Fo-4P, whereas Ld-2 inhibited spore germination in Fm-6P but not in Fm-5P).

Discussion

Our study showed that several genera of ECM fungi have the potential for biological control of plant pathogens. The growth of all isolates of *F. oxysporum* and *F. verticillioides* was inhibited in dual cultures with *L. deliciosus*, *S. luteus, A. rubescens, A. ovoidea, B. fragans, L. lepidum, L. laccata, T. portentosum* and *R. roseolus*. Similar results were observed by Kope and Fortin (1989) and Morin et al. (1999) when assaying the effect of antimicrobial substances produced by diverse ectomycorrhizal fungi against different pathogenic agents. *Rhizopogon roseolus* and both isolates of *S. luteus* were the most efficient ectomycorrhizal fungi of the 21 ECM fungal isolates tested, causing inhibition of growth and spore germination in all four strains of *Fusarium* spp. Additionally, some other ECM fungi significantly reduced growth and spore germination in three, two or one of the *Fusarium* spp. isolates.

ECM isolates from the same species behaved similarly, though some clear intraspecific variations on *Fusarium* inhibition was observed. This was the case for *Lactarius deliciosus* and *Tricholoma portentosum* where we found differences in the behaviour of isolates. Isolates within the same species with differential efficiency against *Fusarium* were also found for *Laccaria* spp. (Perrin and Soulas 1996). The interaction of ECM fungi with root pathogens of pines is not well understood. Several authors have reported disease suppression by ECM fungi associated with fungal production of antimicrobial substances (Duchesne *et al.* 1987a; Chakravarty and Hwang 1991). Toxic effects of mycorrhizal fungi have been linked not only to plant pathogens, but also to insects (Halldorsson *et al.* 2000) and nematodes (Diedhiou *et al.* 2003). The antagonism of some ECM, such as *L. laccata*, on growth and spore germination of *F. oxysporum* and of *F. verticillioides* by toxic-like compounds was reported in previous studies conducted in our laboratory (Martín-Pinto *et al.* 2006*a*).

Suppression of *Fusarium* growth by ECM is likely caused in part by the production of antifungal compounds. Our results suggest that some ECM species release inhibitory substances into the medium, which are toxic to *F. oxysporum* and *F. verticillioides*. However, *Boletus* species had a reduced inhibitory effect and *X. ferrugineus* had no effect on *Fusarium* isolates. This different behavior indicates that under the assayed conditions, these isolates did not produce substances that were detrimental to *Fusarium* growth. However, plants use various defense mechanisms, including the production of antimicrobial compounds in the presence of ECM and the protective barrier effect provided by the presence of fungal mantle in the rhizosphere (Machón *et al.* 2006). Therefore, it is essential to also develop *in vivo* assays to test the inhibitory effect of ECM species on damping off in nurseries. This approach has been successfully tested for *Laccaria laccata* (Machón *et al.* 2006; 2009), *Lactarius sanglifluus* and *Rhizopogon roseolus* (Olaizola 2007), *Suillus luteus* (Mateos *et al.* 2017) and *Amanita rubescens* (Martinez *et al.* 2006) isolates.

Spore germination of *F. oxysporum* and *F. verticillioides* isolates was inhibited by the culture filtrates of some of the ECM fungi we assayed, but the effect was in general less marked than in the co-culture test. Our conidial germination experiment may demonstrate that inhibition of spore germination in *F. oxysporum* and in *F. verticillioides* was related to the production of antifungal compounds by some ECM isolates. While *S. luteus* (Sl-1, Sl-2), *L. deliciosus* (Ld-1, Ld-2), *R. roseolus* (Rr-1) and *T. portentosum* (Tp-1) were the most active strains, other ECM fungi, such as *Boletus aereus* (Ba-2), *Leccinum lepidum* (Bl-4) or *Lactarius semi-*

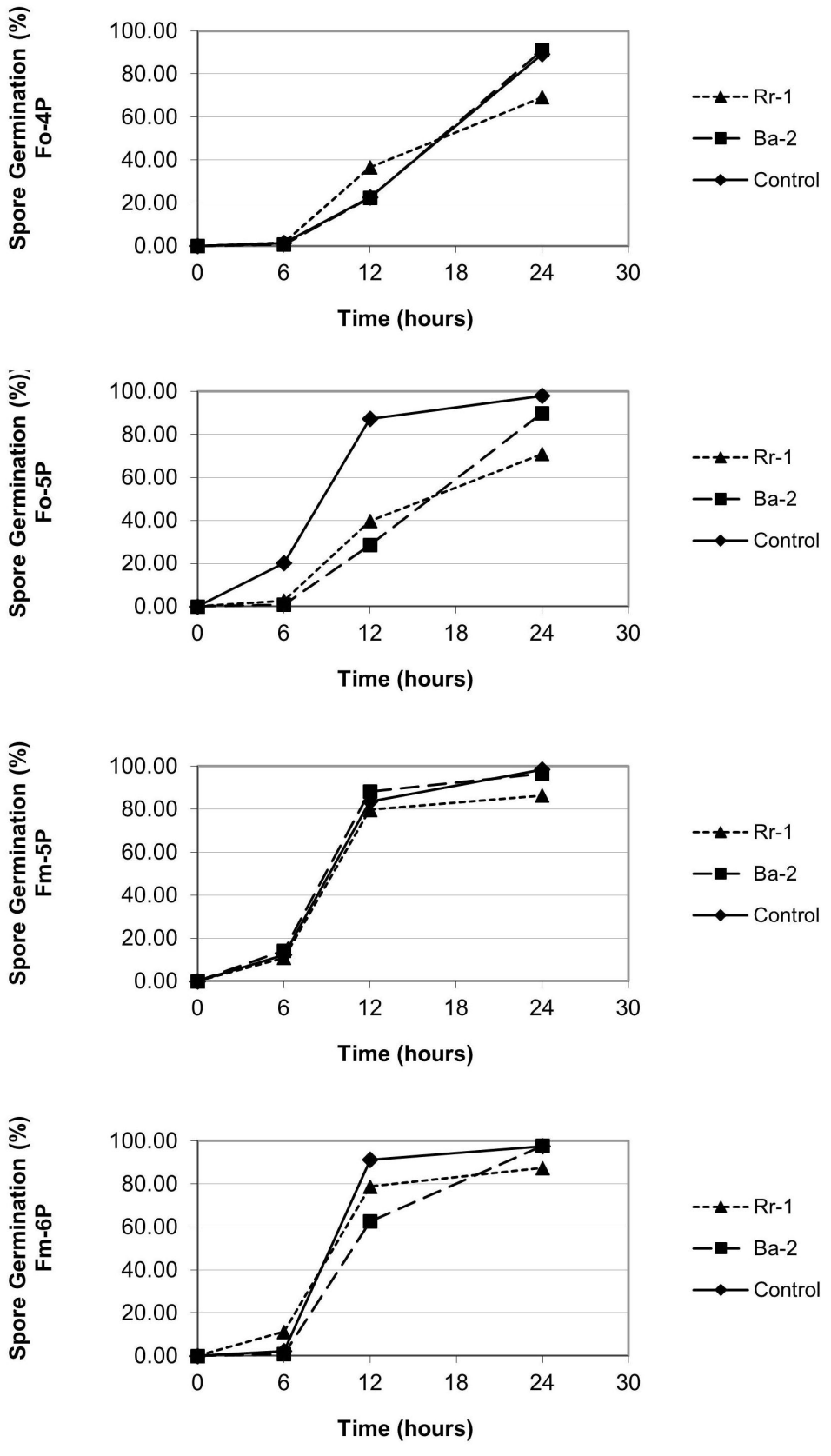

Fig. 3. Effect of culture filtrates from *R. roseolus* (Rr-1) and *B. aereus* (Ba-2) on *F. oxysporum* (Fo-4P and Fo-5P) and *F. verticillioides* (Fm-5P and Fm-6) on microconidial germination at 0, 6, 12 and 24 hours after confrontation

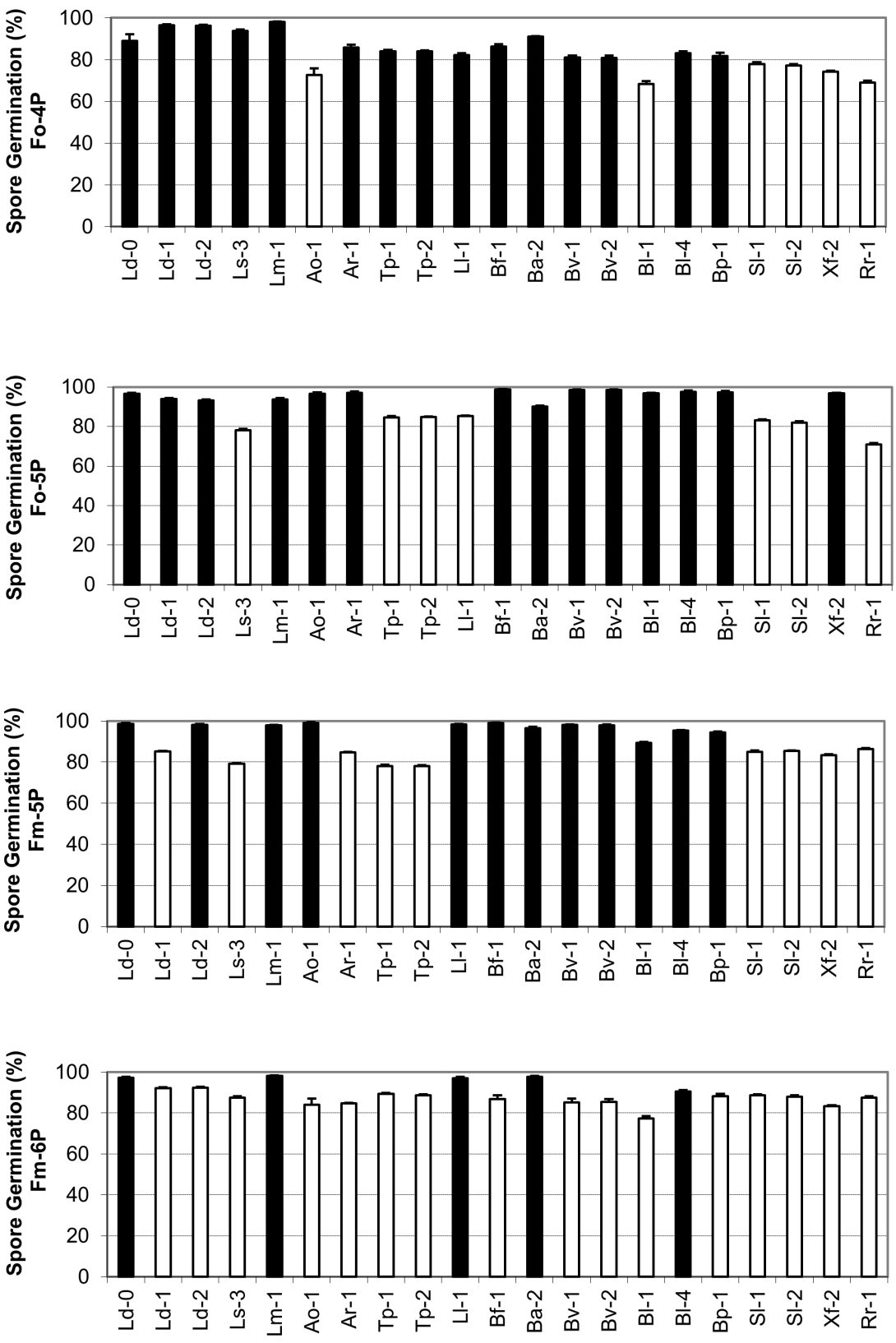

Fig. 4. Effect of culture filtrates from 21 isolates of edible ectomycorrhizal fungi on spore germination of two *F. oxysporum* isolates (Fo-4P and Fo-5P) and *F. verticillioides* isolates (Fm-5P and Fm-6P) 24 hours after addition of culture filtrate. Values are means of 1200 microconidia. Black bars represent values that are not significantly different from controls ($p < 0.05$)

sanguifluus (Lm-1), appeared to lack this ability, as their culture filtrates had no significant effects on *Fusarium* spp. spore germination.

Similar results were observed by Hwang et al. (1995) in *in vitro* assays, recording a reduction in *F. verticillioides* spore germination when treated with culture filtrates from *Paxillus involutus* and *Bacillus subtilis* (40.0 % and 55.2 %, respectively) and no effect on spore germination by other ECM fungi like *S. tomentosus*. Chakravarty and Hwang (1991) recorded that culture filtrates from *Laccaria laccata* reduced spore germination in *F. oxysporum* and postulated that this effect was probably related to the production of soluble phenols by ECM fungi. A similar *in vitro* mechanism of inhibition, involving the production of antimicrobial compounds, may be associated with our culture filtrates assay. Further studies should be performed to confirm the influence of the ECM phenols on the germination of *Fusarium* isolates. More ECM isolates showed effects against *Fusarium* isolates in the co-culture assay than in the conidial germination assay. It could be that the inhibitory substances produced in the agar medium, or how they are diffused, are qualitatively or quantitatively different than those produced on the culture filtrates or they affect *Fusarium* growth and spore germination differently. In the co-culture assay on solid media, not only antibiosis but also the effect of competition for nutrients might be involved. However, it is unlikely that the observed reduction of growth in the *Fusarium* isolates could be only due to a competition effect with the ECM fungi, as some isolates with vigorous growth (i.e., Xf-2) failed to cause any effect on the *Fusarium* colonies.

Acidification of the medium as the result of the ECM *in vitro* growth might be another reason for the decrease in *Fusarium* growth, so inhibitory effects observed using unbuffered media can be misinterpreted as antibiosis (Rasanayagam and Jeffries 1992; Schelkle and Peterson 1996). Unbuffered medium was used in this study and therefore the possibility of a pH decrease must be considered. Olaizola (2007) analyzed the acidification of BA medium by 12 different ECM fungi and found no relationship between pH fluctuation by ECM and *Fusarium* inhibition.

ECM species such as *Rhizopogon*, *Suillus*, *Laccaria* or *Lactarius* form ectomycorrhizal roots during the first stages of seedling growth (Sanchez and García 2006) and were more effective against *Fusarium* than *Boletus*, *Amanita* or *Xerocomus*. These fungi are commonly associated with mature forests and are difficult to manage for mycorrhizal root formation in nursery seedlings. The results are confirmed *in vivo*, and have practical implications for the production of healthy mycorrhizal plants in nurseries, where ECM species form ectomycorrhizal roots during the first stages of seedling growth.

In vitro antagonism assays are useful for studying the production of antifungal substances by ECM fungi, as interactions with other organisms are avoided and a high number of organisms can be tested for further *in vivo* assays. After screening 21 ECM fungi we concluded that isolates of *Suillus luteus* (Sl-1), *Lactarius deliciosus* (Ld-1 and Ld-2), *Rhizopogon roseolus* (Rr-1) and *Tricholoma portentosum* (Tp-1) reduced *Fusarium* growth and spore germination *in vitro*. Therefore, these ECM fungi are considered potential candidates for *in vivo* biological control of *F. oxysporum* and *F. verticillioides*. Four of these fungi have already been tested *in vivo* (Machón *et al*. 2006; 2009; Olaizola 2007; Mateos *et al*. 20017) and we are investigating other species to determine their efficiency against *Fusarium* species, and their interaction with soil microorganisms, pathogens, and naturally occurring mycorrhizal fungi.

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