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Differential responses of ectomycorrhizal fungi to pesticides in pure culture

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Abstract – The effects of several fungicides and herbicides on the growth of the ectomycorrhizal fungi *Lactarius deliciosus*, strain LDF5, and *Pisolithus tinctorius,* strains 30AM, 3SR and Mx, in pure culture were studied. The products tested were selected because they are commonly used in nurseries and afforestation practices. Five concentrations (0, 1, 10, 100 and 1000 µg/g) of each fungicide (benomyl, captan, hymexazol, iprodione, propamocarb hydrochloride and thiram) or herbicide (glyphosate, hexazinone and simazine) were prepared. Fungal growth was evaluated as colony diameter, mycelial biomass, and morphological features. Propamocarb hydrochloride was the most tolerated fungicide. Benomyl, captan and thiram reduced mycelial growth at concentrations above 100 µg/g. Hymexazol and iprodione had the highest inhibitory effect. In contrast, the herbicides produced little or no effect and they even stimulated the growth of *L. deliciosus*.

ectomycorrhizal fungi / *Lactarius deliciosus* / *Pisolithus tinctorius* / fungicide / herbicide / pesticide / mycelial growth

Resumen – Se estudian los efectos de varios fungicidas y herbicidas en el crecimiento en cultivo puro de los hongos ectomicorrícicos *Lactarius deliciosus* cepa LDF5 y *Pisolithus tinctorius* cepas 30AM, 3SR y Mx. Los productos se seleccionaron debido a su uso habitual en viveros y prácticas de aforestación. De cada fungicida (benomilo, captan, himexazol, iprodiona, propamocarb clorhidrato y tiram) y herbicida (glifosato, hexacinona y simazina) se prepararon cinco concentraciones (0, 1, 10, 100 y 1000 µg/g). Para evaluar el crecimiento del micelio se utilizaron los parámetros diámetro de la colonia, biomasa miceliar y características morfológicas. El fungicida más tolerado fue propamocarb clorhidrato. Benomilo, captan y tiram redujeron el crecimiento del micelio a concentraciones superiores a 100 µg/g. Himexazol e iprodiona tuvieron el mayor efecto inhibitorio. En cambio, los herbicidas utilizados apenas tuvieron efecto sobre el crecimiento e incluso produjeron un efecto estimulatorio sobre *L. deliciosus*.

hongos ectomicorrícicos / *Lactarius deliciosus* / *Pisolithus tinctorius* / fungicida / herbicida / pesticida / crecimiento miceliar

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INTRODUCTION

Forest seedlings in nurseries are highly susceptible to pathogen attack and in consequence, fungicide treatments are frequently and routinely applied in high quality forest plant production. Furthermore, herbicide application is also common after outplanting in order to improve plant survival in land afforestation.

However, the soil microbial activity, particularly the growth of mycorrhizal fungi, may be affected by these practices. Several studies have reported the effect of pesticides on mycorrhizae, but most are of agricultural rather than forestry interest. There are relatively few reports concerning the influence of fungicides or herbicides on the mycorrhizal forming Basidiomycetes in forestry and plant nursery (Colinas *et al*., 1994; Pawuk *et al*., 1980; Marx & Rowan, 1981; Pawuk & Barnett, 1981; Marx *et al*., 1982; Garbaye *et al*., 1992; Manninen *et al.,* 1998; Massicotte *et al*., 1998; O´Neill & Mitchell, 2000).

Prior to the use of fungicides in the nursery, an evaluation of their possible side effect on non-target organisms, particularly on mycorrhizal forming fungi, would seem advisable for the development of inoculation programs. The selection of the fungicides which minimize the negative side effect on the natural microbial population is important to preserve spontaneous mycorrhiza formation in environmentally friendly forest practices. In this sense, the different sensitivitiy of mycorrhizal fungi to pesticides in controlled *in vitro* conditions can be used as a preliminary approach to estimate their impact in natural conditions *in vivo*.

Information concerning the impact of fungicides on the mycelial growth of ectomycorrhizal fungi in pure culture is limited. Previous studies have demonstrated variable levels of inhibition, or sometime even stimulation, of ectomycorrhizal (ECM) fungi, depending on the fungal species, the fungicides, and the concentration studied (Trappe *et al*., 1984; Zambonelli & Iotti, 2001; Laatikainen & Heinonen-Tanksi, 2002). Several studies demonstrated that Benomyl, for instance, had a negative effect on the mycelial growth of *Pisolithus tinctorius* (Pers.) Coker & Couch, *Thelephora terrestris* Fr.:Fr., *Tricholoma matsutake* (S. Ito & Imai) Sing., *Suillus luteus* (L.: Fr.) S. F. Gray, and others species, at concentrations ranging from 10 µg/g (Marx & Rowan, 1981) to 200 µg/g (Edgington *et al*., 1971; Kawai & Ogawa, 1977). Laatikainen & Heinonen-Tanski (2002) also observed that the effect of benomyl was variable and depended on the species tested, *Cenococcum geophilum* Fr., *Cantharellus cibarius* Fr., *Amanita muscaria* (L.: Fr.) Hooker, and *Leccinum* sp. being inhibited, while *Paxillus involutus* (Batsch) Fr., *Suillus variegatus* (Swartz.: Fr.) O. Kuntze, and *S. bovinus* (L.: Fr.) O. Kuntze were stimulated. Captan had no effect at doses lower than 10 µg/g (Marx & Rowan, 1981) but tended to inhibit mycelial growth at very high concentrations (up to 15000 µg/g) (Trappe *et al*., 1984). Hymexazol and thiram have also been reported to inhibit mycelial growth of ECM fungi at doses of 200 and 100 µg/g respectively (Kawai & Ogawa, 1977). Laatikainen & Heinonen-Tanski (2002) tested the sensitivity of benomyl, chlorothalonil, copper oxychloride, maneb, and propiconazole on 64 strains of ectomycorrhizal fungi in pure culture. They concluded that chlorothalonil and propiconazole had the strongest inhibitory effect on the mycelial growth of all the species tested. Moreover, maneb also stimulated the growth of *P. involutus, S. variegatus*, and *S. bovinus*.

The impact of herbicides on mycelial growth is also variable. Some herbicides such as linuron may negatively affect mycelial growth (Laatikainen & Heinonen-Tanski, 2002) whereas others have either no effect or even a stimulatory effect, e.g. glyphosate, hexacinone, simazine and terbuthylazine (Kelley &

South, 1980; Trappe *et al*., 1984; Lake *et al*., 1981; Landis *et al*., 1990; Laatikainen & Heinonen-Tanski, 2002).

The experiment described below is a part of wider study of factors affecting the controlled mycorrhization of *Pinus halepensis* Miller in nursery conditions (Honrubia *et al*., 1998; Honrubia *et al*., 1999).

The objective of the present study was to evaluate the effect of several fungicides and herbicides at different doses on the *in vitro* growth of two ectomycorrhizal species (*Lactarius deliciosus* (L. ex Fr.) Gray and *Pisolithus tinctorius* (Pers.) Coker & Couch), with the aim of selecting the least damaging combination fungicide / dose for the development of these fungi and for their subsequent use in nursery inoculations.

MATERIALS AND METHODS

The fungal species used were *Lactarius deliciosus* (L. ex Fr.) Gray (strain LDF5) and *Pisolithus tinctorius* (Pers.) Cooker & Couch (strains 30AM, 3SR, and Mx) (Table 1). Pure cultures were obtained by isolation from basidiome on solid modified Melin-Norkrans medium (MMN) (Marx, 1969), then transferred on fresh medium every 3 months and maintained at the culture collection of the Mycology Laboratory, University of Murcia.

The fungicides tested were benomyl (benzimidazole-Zetamilo®), captan (thiophtalimide-Captan®), hymexazol (oxazole-Tachigaren LS®), iprodione (dicarboximide-Rovral®), propamocarb hydrochloride (carbamate-Previcur®), and thiram (dithiocarbamate-Thiurox®), which are all commonly used in nurseries, particularly for damping-off and for controlling *Botrytis*. The herbicides tested were selected on the basis of their common use in the case of glyphosate (phosphonoglycine-Zeltrone®) and due to the good results obtained in afforestation experiments in the case of simazine (triazine-Gesatop®) and hexacinone (triazine-Velpar®) (Ortega *et al*., 1999).

The formulated pesticides were first dissolved in sterile water to a concentration of 100 mg/g (based on the active ingredient) and then diluted several times. Five concentrations (0, 1, 10, 100 and 1000 µg/g) of each fungicide or herbi-

Table 1. Ectomycorrhizal species used in the experiment, culture code, origin, associated tree and date of isolation.

Fungal specie	*Culture code*	*Origin*	*Associated tree*	*Date of isolation*
Lactarius deliciosus	LDF5	Valencia (Spain)	*Pinus halepensis*	11/1995
Pisolithus tinctorius	30AM	Moratalla, Murcia (Spain)	*Pinus halepensis*	10/1995
Pisolithus tinctorius	3SR	Uceda, Guadalajara (Spain)	*Quercus rotundifolia*	10/1995
Pisolithus tinctorius	Mx	Tlxacala (Mexico)	*Pinus* sp.	7/1995

cide were prepared in MMN agar medium (Marx, 1969). For this, after autoclaving, the medium was maintained in subfusion state (40 ºC) and the appropriate amounts of the aqueous suspensions of each pesticide were added and homogenized in a shaker to obtain the final concentrations. This was then dispensed on Petri dishes in sterile conditions.

Mycelial plugs (7 mm diameter) were taken from the margin of the colony of pure cultures, subcultured in the dishes, and incubated in the dark at 23 ºC for 6 weeks. Five replicates for each experimental treatment were prepared.

Colony diameter was measured every week. At the end of the experiment, mycelia were taken from the dishes, filtered through Whatman® glass microfiber filters (4 cm diameter) with hot water to facilitate agar dissolution, then dried at 160 ºC for 24 h and weighed to obtain the mycelial biomass values. Macroscopic and microscopic features of the cultures, such as colour, the presence of exudates and hyphae microfeatures, under the different treatments, were also noted.

RESULTS AND DISCUSSION

Effects of fungicides on mycelial growth

The ectomycorrhizal fungi tested reacted variably to the different fungicides assayed. However, some general trends were observed. In general, all the fungicides tested negatively affected the mycelial growth at the highest concentration used (100 and 1000 µg/g) (Table 2, Fig. 1).

Hymexazol and iprodione had the strongest inhibitory effect on the growth of ECM fungi. The mycelial growth of the four strains used was reduced by hymexazol at all the concentrations assayed and was completely inhibited at 1000 µg/g. The strains *P. tinctorius* 3SR and 30AM were the most sensitive, with significant reduction of colony diameters at a concentration as low as 1 µg/g. The colony diameter of *L. deliciosus* LDF5 was significantly reduced with respect to the control at a concentration as low as 10 µg/g, whereas mycelial biomass was significantly reduced only at 100 µg/g.

Kawai & Ogawa (1977) previously observed that hymexazol suppressed mycelial growth of *T. matsutake* and *S. luteus* at 200 µg/g, but our results demonstrated a toxic effect of this fungicide at lower concentrations, but on different species.

The sensitivity to iprodione was variable depending on the fungal species and strain. Significant decreases with respect to the control were observed in *L. deliciosus* at 100 µg/g, in *P. tinctorius* 3SR and Mx at 10 µg/g, and in 30AM at 1 µg/g.

The reactions of the four strains tested to captan, thiram, and benomyl were similar, with significant growth reductions observed at the concentration of 100 µg/g or higher. These results are in accordance with those of Kawai & Ogawa (1977), who reported a deleterious effect of thiram on ectomycorrhizal fungi at the same concentration. In relation to benomyl, similar findings were reported by Edgington *et al*. (1971) and Kawai & Ogawa (1977), although some authors (Marx & Rowan, 1981; Laatikainen & Heinonen-Tanski, 2002) have found a negative effect at lower dose (10 µg/g) but on other fungal species.

Table 2. Mycelial biomass (μg) of ectomycorrhizal fungi grown in pure culture under different fungicide treatments. Data are mean of five replicates. Data in a column followed by the same letter are not significantly different (Duncan´s test $p < 0.05$).

Fungicide	*Concentration*	*Fungal strain*			
		L. deliciosus LDF5	*P. tinctorius 30AM*	*P. tinctorius 3SR*	*P. tinctorius Mx*
Benomyl	0 μg/g	202.9 ab	91.8 a	76.0 a	113.6 a
	1 μg/g	285.0 a	77.4 a	76.1 a	102.7 a
	10 μg/g	226.0 ab	75.7 a	72.9 a	116.9 a
	100 μg/g	25.7 b	71.7 a	0 b	99.3 a
	1000 μg/g	19.0 b	0 b	0 b	57.2 b
Captan	0 μg/g	202.9 a	91.8 a	76.0 a	113.6 a
	1 μg/g	178.0 a	68.4 ab	81.6 a	118.8 a
	10 μg/g	172.2 a	75.3 a	66.3 a	100.2 a
	100 μg/g	34.0 b	63.8 a	45.8 a	87.0 a
	1000 μg/g	9.4 c	53.0 b	45.2 a	28.9 b
Hymexazol	0 μg/g	202.9 a	108.7 a	62.4 a	115.3 a
	1 μg/g	105.8 ab	65.3 b	60.0 a	106.1 a
	10 μg/g	39.8 ab	44.7 b	73.2 a	97.1 a
	100 μg/g	15.4 b	39.6 b	83.7 a	80.2 a
	1000 μg/g	0 b	6.2 c	6.2 b	0 b
Iprodione	0 μg/g	202.9 a	91.8 a	76.0 a	113.6 a
	1 μg/g	257.0 a	77.8 ab	72.0 a	101.4 ab
	10 μg/g	198.9 a	59.6 ab	40.3 a	63.5 b
	100 μg/g	147.9 a	51.8 ab	0 b	16.6 c
	1000 μg/g	99.1 a	42.5 b	0 b	4.5 c
Propamocarb hydrochloride	0 μg/g	202.9 a	108.7 a	62.4 a	115.3 a
	1 μg/g	127.4 a	76.6 b	53.7 a	125.6 a
	10 μg/g	219.3 a	68.7 b	61.3 a	110.3 a
	100 μg/g	161.2 a	52.7 b	67.9 a	131.2 a
	1000 μg/g	174.8 a	68.7 b	73.3 a	125.7 a
Thiram	0 μg/g	202.9 ab	91.8 a	76.0 a	113.6 a
	1 μg/g	247.3 a	68.2 a	84.1 a	87.1 a
	10 μg/g	191.2 ab	66.9 a	90.6 a	103.2 a
	100 μg/g	33.0 ab	18.2 b	0 b	23.2 b
	1000 μg/g	21 b	0 b	0 b	18.0 b

In contrast, the fungicide propamocarb hydrochloride did not affect the mycelial growth (both, colony diameter and biomass) of the fungi tested at any of the assayed concentrations, making it the most compatible fungicide with our ectomycorrhizal fungi under the particular experimental conditions assayed.

Pisolithus tinctorius 3SR seemed to be the most sensitive ectomycorrhizal fungi to the different fungicides used. Its growth, either in term of biomass or radial growth, was completely suppressed by benomyl, iprodione, and thiram at concentrations of 100 μg/g or higher, and by hymexazol at 1000 μg/g (only radial growth).

The effect of the fungicides on mycelial growth differed slightly according to the measured parameter considered. Results concerning colony diameter

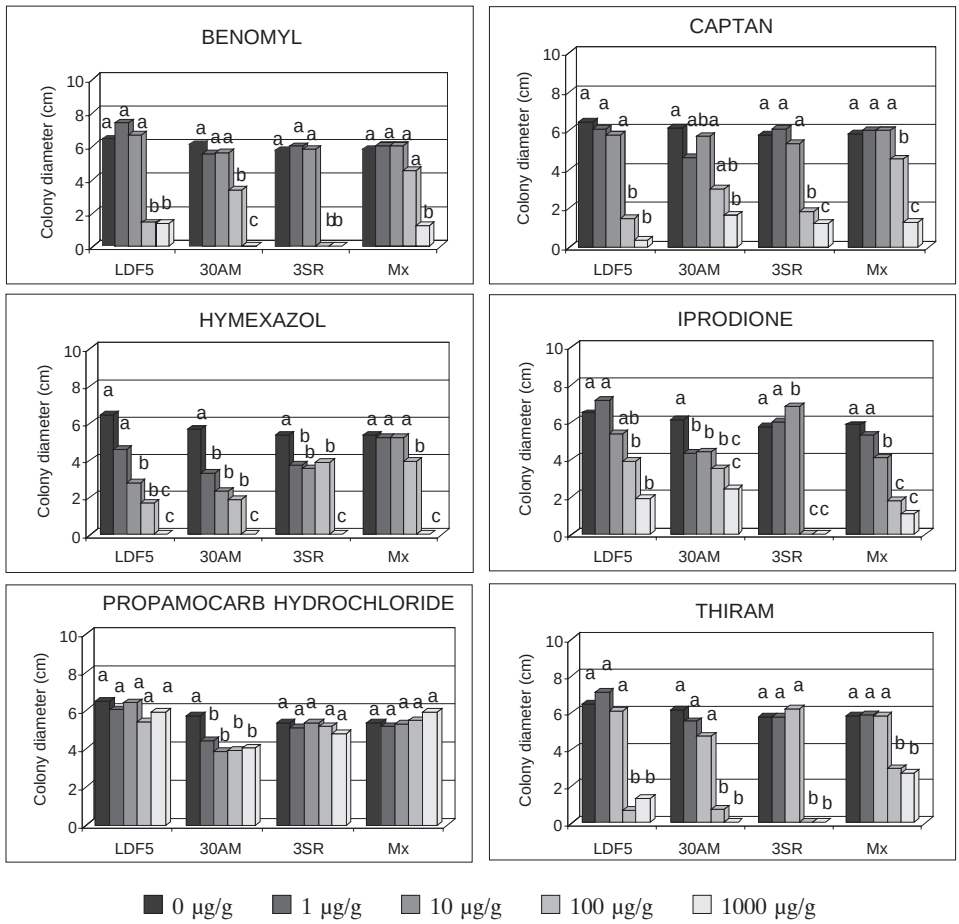

Fig. 1. Colony diameter (cm) of ectomycorrhizal fungi (*Lactarius deliciosus* strain LDF5, *Pisolithus tinctorius* strains 30AM, 3SR, Mx) grown in pure culture under different fungicide treatments. Data are mean of five replicates. Values in a group sharing the same letter are not significantly different (Duncan´s test $p < 0.05$).

were, in general, more homogeneous than those for mycelial biomass. In many treatments, the negative effect on mycelial growth was observed at lower concentrations when colony diameter was measured than when mycelial biomass was considered. The explanation for this could be that fungi which show high growth rates *in vitro* usually form colonies with an aerial mycelia made up of loose, scarce hyphae and with correspondingly low biomass values. However, under stress conditions, the reduction in radial growth in these fungi may be accompanied by formation of very dense, felty or fibrous hyphae and consequently a relative increase in biomass production. Furthermore, mycelium is often submerged in the medium as a protection mechanism against unfavourable conditions (Sánchez *et al*., 2001).

In addition to its effect on mycelial growth, the presence of fungicides also affect the macro-morphological aspect of the colony and the micromorphology of the mycelium. In this sense, some important parameters that should be taken into account are the colour and aspect of the aerial mycelia or exudates (Table 3). The strain of *L. deliciosus* showed an orange to brown mycelium when treated with captan and thiram, instead of the white to cream-orange of the controls. Variations in colour were also observed in the aerial mycelia and the colony reverse of *P. tinctorius* for some of the treatments. It is worth mentioning the general change in the aspect of aerial mycelia from cottony (*P. tinctorius*) or fibrous (*L. deliciosus*) in the controls to fibrous, felty or even spiny in the presence of captan, thiram or iprodione. The presence and colour of exudates was also affected by pesticides, their production being inhibited in the strains of *P. tinctorius* in presence of iprodione. In addition, microscopic hyphal changes (reduction of hyphal diameter and increase of wall thickness) were also noted, in particular in *L. deliciosus*. These morphological changes in presence of fungicides may well be attributed to the same defence response.

Effects of herbicides on mycelial growth

In general, the herbicides tested had little or no effect on fungal growth. However, in some cases, and depending on the fungal strain, growth was stimulated or inhibited (Table 4, Fig. 2).

Glyphosate, simazine, and hexacinone stimulated the mycelial growth of the strain of *L. deliciosus* in terms of biomass production and colony diameter at doses inferior to 1000 µg/g. At the highest dose applied (1000 µg/g), this effect disappeared and the growth measured as colony diameter was significantly inhibited. This stimulatory effect of glyphosate, a fact also observed for other herbicides such as simazine, terbuthylazine, atrazine, bifenox, nitrofen and triclopyr, has been reported previously (Trappe *et al*., 1984; Laatikainen & Heinonen-Tanski, 2002). However, the explanation of this effect remain uncertain. Laatikainen & Heinonen-Tanski (2002) suggested that the ectomycorrhizal fungi might be able to degrade the added herbicide to a compound that may act as a nutrient. Another possibility is that the fungal tissue may incorporate the pesticide molecule with no mineralization, as it has been reported by Donelly *et al*. (1993) for atrazine.

In any case, the concentration threshold seems to be decisive for producing a stimulatory or inhibitory effect and, in general, the herbicide concentration necessary to produce a depressive effect on the growth of ectomycorrhizal fungi would be higher than the application rate recommended for herbicide soil treatment, as Kelley & South (1980) have pointed out. These results contrast with the findings of Chakvravarty & Sidhu (1987), who reported inhibitory effects of glyphosate and hexazinone on *Hebeloma crustuliniforme* (Bull.: St. Amans) Quélet, *Laccaria laccata* (Scop.: Fr.) Berk. & Broome and *Suillus tomentosus* (Kauff.) Sing., Snell & Dick at concentrations higher than 10 µg/g. It is probable that the effect of a particular herbicide is depending on its concentration and the fungal species tested.

Simazine and hexazinone had no or little effect on *P. tinctorius* growth while glyphosate produced a depressive effect at low doses on this fungus.

Studies carried out in abandoned agricultural lands showed simazine and hexacinone to be effective herbicides for removing weeds without affecting *Pinus* species (Ortega *et al*., 1999). In a preliminary study on mycorrhizae carried out in Uceda (Guadalajara, Spain), plants treated with these herbicides showed a more

Table 3. Distinguishing morphological features of ectomycorrhizal fungi grown in pure culture under different fungicide treatments, in relation to controls.

L. deliciosus LDF5						
Fungicide	*Basal mycelium*	*Aerial mycelium*	*Margin*	*Reverse*	*Exudates*	*Hyphae-microfeatures*
Control	Cream-orange with concentric rings	White Fibrous Scarce	Regular Pale cream-orange	Cream	No	Colourless Thin-walled 4.8 µm wide
Captan	Dark orange-brown	Orange Felty	Irregular	Cream	No	Colourless Thin-walled 4.8 µm wide
Thiram	Brown Scarce	White-brown Fibrous to spiny Abundant	Irregular	Brown	Orange	Orange to green Thick-walled 4.0 µm wide with anastomoses
Iprodione	Cream Very scarce	Fibrous Abundant	Lobed	Pale cream-brown	No	Thick-walled 3.2 µm wide
P. tinctorius 30 AM						
Fungicide	*Basal mycelium*	*Aerial mycelium*	*Margin*	*Reverse*	*Exudates*	*Hyphae-microfeatures*
Control	Brown-cream	Pale brown Cottony Scarce	Regular Brown-yellowish	Dark brown in the center, cream in the margin	Yellowish-orange	Pale-brown 3.2 µm wide Scarce clamps
Captan	Brown Striated	Felty Abundant	Irregular	Brown-blackish	Orange-brown	Pale-brown 3.2 µm wide Scarce clamps
Iprodione	Pinkish-brown	Grey Scarce, loose	Lobed	Cream-brown	Little	Pale-brown 3.2 µm wide Scarce clamps

P. tinctorius 3 SR

Fungicide	*Basal mycelium*	*Aerial mycelium*	*Margin*	*Reverse*	*Exudates*	*Hyphae-microfeatures*
Control	Dark brown	Yellow-ochre Cottony, with concentric rings Abundant	Regular	Purple brown in the center, yellowish in the margin	Yellow-orange	Brown-yellowish 4.0 µm wide Abundant clamps Ornamented
Captan	Dark brown	Yellow Felty	Irregular	Dark brown	Brown	Yellowish 3.2 µm wide Scarce clamps
Iprodione	Brown	Dark yellow Loosly cottony withouth rings	Irregular	Brown	No	Brown yellowish

P. tinctorius Mx

Fungicide	*Basal mycelium*	*Aerial mycelium*	*Margin*	*Reverse*	*Exudates*	*Hyphae-microfeatures*
Control	Brown-orange	Cream-grey in the center, yellowish in the margin Cottony Scarce	Regular	Brown in the center, orange in the margin	Yellowish	Pale brown 5.6 µm wide Abundant clamps
Captan	Brown-orange	Yellow-green Velvety Abundant	Irregular	Brownish-green	Orange	Pale brown 5.6 µm wide Abundant clamps
Thiram	Brown-orange	Pale brown Abundant	Regular	Cream-green	Yellowish green	Colourless
Iprodione	Cream Very scarce	Brownish-green	Regular	Brown	No	Pale brown

Table 4. Mycelial biomass (µg) of ectomycorrhizal fungi grown in pure culture under different herbicide treatments. Data are mean of five replicates. Data in a column followed by the same letter are not significantly different (Duncan´s test $p < 0.05$).

Herbicide	*Concentration*	*Fungal strain*			
		L. deliciosus LDF5	*P. tinctorius* 30AM	*P. tinctorius* 3SR	*P. tinctorius* Mx
Glyphosate	0 µg/g	120.1 b	127.9 a	95.9 a	131.6 a
	1 µg/g	208.9 a	80.5 b	58.4 b	101.5 b
	10 µg/g	189.4 a	92.6 b	71.2 b	106.6 b
	100 µg/g	161.8 a	75.6 b	53.8 b	96.5 b
	1000 µg/g	69.8 b	68.4 b	0 c	33.3 c
Hexacinone	0 µg/g	120.1 b	75.6 a	57.7 ab	80.1 a
	1 µg/g	173.7 a	84.4 a	56.5 ab	80.5 a
	10 µg/g	224.4 a	87.1 a	68.1 a	95.0 a
	100 µg/g	150.2 a	71.8 ab	67.8 a	94.7 a
	1000 µg/g	65.8 b	45.9 b	34.6 b	57.9 b
Simazine	0 µg/g	120.1 b	75.6 ab	57.7 ab	80.1 a
	1 µg/g	242.9 a	88.6 ab	57.6 ab	88.2 a
	10 µg/g	230.7 a	89.0 ab	72.9 a	90.7 a
	100 µg/g	235.7 a	101.9 a	66.7 ab	89.0 a
	1000 µg/g	84.3 b	66.5 b	46.4 b	74.9 a

colonized root system than those treated with other herbicides (data not shown), which strongly suggests that these herbicides can be used in conjunction with mycorrhized seedlings in plantations with the aim of removing undesired seeds and stimulating the growth of natural or introduced mycorrhizae.

CONCLUSIONS

The knowledge of the ECM fungi tolerance to fungicides or herbicides *in vitro* is a useful preliminary approach to decide which one can be subsequently used *in vivo* conditions.

As expected, fungicides were in general more toxic to ectomycorrhizal fungi in pure culture than herbicides. The fungicide propamocarb hydrochloride was the best tolerated by *L. deliciosus* and the three *P. tinctorius* strains, and its use could be therefore recommended for controlling diseases in nurseries. The commercially recommended rates of captan, thiram, and benomyl commonly used by foresters in nurseries could be compatible with the mycelial development of the ectomycorrhizal fungi tested. However, hymexazol and iprodione had a strong inhibitory effect and consequently, their use should not be recommended, or limited to a minimun.

In general, the use of the herbicides glyphosate, simazine, and hexacinone may be compatible with the use of mycorrhized plants both in nurseries and plantations.

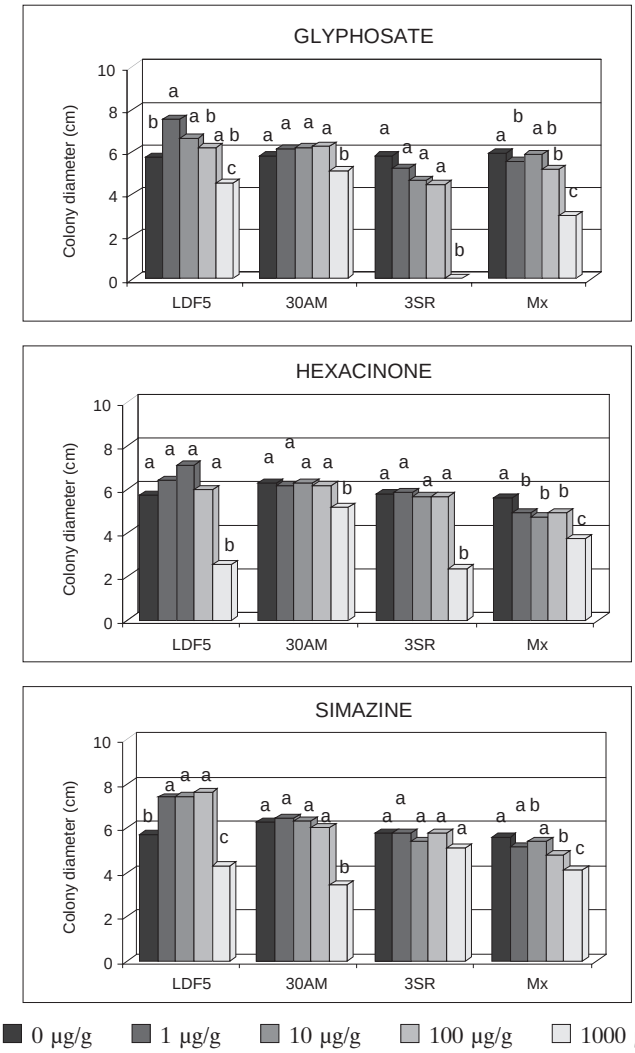

Fig. 2. Colony diameter (cm) of ectomycorrhizal fungi (*Lactarius deliciosus* strain LDF5, *Pisolithus tinctorius* strains 30AM, 3SR, Mx) grown in pure culture under different herbicide treatments. Data are mean of five replicates. Values in a group sharing the same letter are not significantly different (Duncan´s test $p < 0.05$).

Due to the differential responses of ECM fungi to pesticides, the particular combinations fungal species or strain / chemical compound / application dose should be tested in *in vitro* conditions before pesticide application in nurseries. However, results obtained from *in vitro* experiments with pure cultures do not necessary correspond entirely with the *in situ* performance. In natural conditions, pesticides may directly affect seedling root development, so that other effects such as mycorrhiza formation level or plant nutritional status should also be taken into

account when considering the usefulness of different pesticides in pest management. Further studies with field experiments should be conducted to evaluate and confirm the pesticide toxicity observed in our *in vitro* results.

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