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Evaluation of some fungicides on mycorrhizal symbiosis between two *Glomus* species from commercial inocula and *Allium porrum* L. seedlings

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Abstract

This paper reports the effect of twenty-five commonly used fungicides in agriculture on two arbuscular mycorrhizal fungi (AMF) present in commercial products of ATENS, S.L.: *Glomus intraradices* (Schenck & Smith) and *Glomus mosseae* [(Nicol. & Gerd.) Gerdemann & Trappe], forming the symbiosis with leek plants. Systemic fungicides (Aliette, Beltanol, Caddy 10, Forum, Moncut, Ortiva, Previcur, Ridomil Gold MZ, Ridomil Gold SL, Rubigan, Sinthane, Stroby, Swich, Tachigarem, Teldor, Topas 10 EC, Frupica) and non systemic fungicides (Daconil 75%, Ditiver, Euparem, INACOP, Octagón, Parmex, Terrazole and Metaram), started to be applied to soil and leaves at recommended concentrations and frequencies 4 weeks after transplant and AMF inoculation. The effect of the fungicides was assessed by comparing treated and untreated plants that were inoculated with the AMF through quantification of root mycorrhizal colonization. Among the fungicides applied to the soil, Octagon, Ditiver, Parmex and Metaram virtually eliminated the mycorrhizal symbiosis in treated plants, while the mycorrhizal colonization was not affected by the soil treatment with Beltanol, INACOP and Previcur. Three fungicides of foliar recommended application: Rubigan, Frupica, and Sinthane, strongly inhibited mycorrhizal colonization, but Aliette, Forum, Teldor, Swich and Ortiva, did not seem to reduce it substantially. In addition, the work describes the individual effect of each fungicide applied on both, foliage and soil.

Additional key words: chemical control, endomycorrhiza, *Glomus intraradices, Glomus mosseae*, leek seedling.

Resumen

Evaluación del efecto de varios fungicidas sobre la simbiosis micorrícica entre dos especies de *Glomus* presentes en inóculos comerciales y plántulas de *Allium porrum* L.

Se han evaluado 25 fungicidas comerciales usados comúnmente en agricultura, sobre dos especies de hongos formadores de micorriza arbuscular (HMA) presentes en productos comerciales de ATENS, S.L (*Glomus intraradices* (Schenck & Smith), y *Glomus mosseae* [(Nicol. & Gerd.) Gerdemann & Trappe], en simbiosis con las raíces de plántulas de puerro. Tanto los fungicidas sistémicos (Aliette, Beltanol, Caddy 10, Forum, Moncut, Ortiva, Previcur, Ridomil Gold MZ, Ridomil Gold SL, Rubigan, Sinthane, Stroby, Swich, Tachigarem, Teldor, Topas 10 EC y Frupica) como los no sistémicos (Daconil 75%, Ditiver, Euparem, INACOP, Octagón, Parmex, Terrazole y Metaram), comenzaron a aplicarse al suelo y a las hojas de las plantas, 4 semanas después del trasplante e inoculación con HMA, usando las dosis y frecuencias recomendadas por el fabricante. Se compararon los efectos de los compuestos fungicidas por medio del análisis de la colonización micorrícica de las raíces en plantas tratadas y no tratadas. Los fungicidas ensayados con aplicación recomendada al suelo: Octagón, Ditiver, Parmex y Metaram, eliminaron prácticamente la simbiosis micorriza en las plantas tratadas. Sin embargo, la micorriza no sufrió ningún tipo de afectación con los tratamientos dirigidos a suelo de Beltanol, INACOP y Previcur. Tres de los fungicidas de aplicación recomendadas por vía foliar: Rubigan, Frupica y Sinthane, inhibieron fuertemente la colonización micorrícica, a diferencia de Aliette, Forum, Teldor, Swich y Ortiva, que no parecen inhibirla de manera substancial. Se describe además en el trabajo el efecto individual de cada fungicida, aplicado tanto a nivel foliar como de suelo.

Palabras clave adicionales: control químico, endomicorriza, *Glomus intraradices, Glomus mosseae*, plántulas de puerro.

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Abbreviation used: AM (arbuscular mycorrhiza), AMF (arbuscular mycorrhizal fungi), Fosethyl-Al (Fosethyl aluminium).

Introduction

Growing social demands for a sustainable agriculture, high-quality food products, and more information concerning food industry protocols are causing a decrease in chemical inputs, *e.g.* chemical fertilizers and pesticides used (Boiffin *et al.*, 2001). Consequently, important positive changes have occured in agriculture and landscaping management, and many commercial products based on the use of beneficial soil microorganisms are now available.

Arbuscular mycorrhiza (AM) is the most frequent type of endomycorrhizal symbiosis, and the one formed by most vegetable crops. In horticultural systems, these crops are traditionally treated with large amounts of different fungicide agents in order to eliminate phytopathogenic fungi but these agents may have a detrimental effect on beneficial microorganisms associated to the plant (Carrenho *et al.*, 2000). Many fungicides exerting systemic or contact effects on pathogenic microorganisms are approved and registered in Europe for agricultural use but the damage that these products can cause on beneficial fungi such as AMF is not documented. Concerns over the non target effects of biocides employed in plant production has shifted the focus of pest and disease control towards integrated management techniques that employ combinations of cultural practices, biological control, and the use of chemicals against pests and pathogens (Schreiner and Bethlenfalvay, 1997).

Opposite observations concerning AMF and fungicides have been reported in the literature. Systemic fungicides are expected to have detrimental effects on endomycorrhizal fungi but some systemic fungicides actually stimulated root colonization by *Glomus* sp. (Jabaji-Hare and Kendrick, 1985).

Considering the role of AMF in plant growth stimulation and protection (Pinochet *et al.*, 1996; Calvet *et al.*, 2001; Hernández-Dorrego, 2002; Sorensen *et al.*, 2005; Jaizme-Vega *et al.*, 2006; Barea *et al.*, 2008), the objective of this work was to test the effect of twenty-five commercial fungicides which are among the most active chemicals currently applied in horticultural crops on the mycorrhizal symbiosis established by *Glomus intraradices* and *Glomus mosseae* isolates from commercial inocula, in leek (*Allium porrum* L.) seedlings under controlled conditions.

Material and methods

Plant material and growth conditions

The study was conducted all along a growing season in a greenhouse located in the Mediterranean area of Northeastern Spain (Lat 41°09'49.81" N and Long 1°22'26.91" E, in La Riera de Gaià, Tarragona). Plants were grown under natural light conditions. The greenhouse was maintained at daily temperatures between 20°C and 35°C, and day/night relative humidity of 75/85%, respectively. The experiment was conducted in plastic containers with leek seedlings (*Allium porrum* L. cv. Lancelot). One 45 days old rooted seedling was transplanted into a 400-mL container filled with a pasteurized substrate mixture 1:1 (v/v): Terragreen soil conditioner, calcined attapulgite clay (Oil Dri UK Ltd.) and peat TKS-1 (Floratorf® Floragard GmbH, Germany), with pH 7.35 and 10 mg kg^{-1} Phosphorus (P) content. After transplanting the leek plantlets, trays were watered daily by irrigation sprinkler systems with fertilizer (N: 0.90 mg L^{-1}; P_2O_5: 22.5 mg L^{-1}; K_2O: 90 mg L^{-1}; pH: 6.5).

Mycorrhizae and inoculation procedures

A mixed inoculum including two AM fungi present in commercial products from ATENS, S.L. was evaluated. The isolates were: *Glomus intraradices* (Schenck & Smith), and *Glomus mosseae* [(Nicol. & Gerd.) Gerdemann & Trape], originally provided by «Departament de Patologia Vegetal, IRTA» (Cabrils, Barcelona) and by «Departamento de Protección Vegetal, ICIA» (Tenerife, Canary Islands) respectively. The inoculum consisted in mixed rhizosphere samples from plant cultures containing 50 spores per gram of each fungus, hyphae and heavily infected root fragments with many internal spores. At transplant, leek plantlets were inoculated with 3 g of commercial inoculum, which was placed under the roots.

Application of fungicides

Plants were grown during 4 weeks prior to the application of fungicides to ensure a well established mycorrhizal colonization. Twenty-five fungicides commonly used in agriculture were tested at the highest concentration of active ingredient recommended and with the frequency suggested: every 15 days for all fungicides except Daconil and Ditiver that were applied every 7 days (Table 1). As many field applications against soil pathogens are made through the irrigation system, where the foliage is in contact with active ingredient and inversely, some fungicides are prepared for foliar

Table 1. Fungicides, recommended application, and concentrations of active ingredients used

Commercial name/ S or F[1]	Active ingredient and concentration (%)	Chemical name	Concentration tested
Systemic			
Aliette/F	Fosethyl-aluminium 80	Aluminium tris(ethyl phosphonate)	2.5 g L^{-1}
Beltanol/F&S	Chinosol 50	Quinolin-1-ium-8-ol sulfate	2.0 mL L^{-1}
Caddy/F	Ciproconazole 10	(2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol	0.2 g L^{-1}
Moncut/F&S	Flutholanil 50	α,α,α-trifluoro-3'-isopropoxy-o-toluanilide	1.2 g L^{-1}
Ortiva/F	Azoxystrobin 25	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	1.5 mL L^{-1}
Previcur/S	Propamocarb 60.5	Propyl 3-(dimethylamino)propylcarbamate	5.0 g L^{-1}
Ridomil Gold MZ/F	Methalaxyl 64 + Mancozeb 3.9	Methyl N-(methoxyacetyl)-N-(2,6-xylyl)-D-alaninate (64%) + Manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt (3,9%)	2.5 g L^{-1}
Ridomil Gold SL/S	Methalaxyl = Mephenoxam 46.5	Methyl N-(methoxyacetyl)-N-(2,6-xylyl)-D-alaninate (46,5%)	1.2 ml L^{-1}
Rubigan/F	Fenarimole 12	a-(2-chlorophenyl)-a-(4-chlorophenyl)-5-pyrimidine methanol	0.5 g L^{-1}
Sinthane/F	Miclobutanil 24	(RS)-2-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl) hexanenitrile	0.4 mL L^{-1}
Stroby/F	Kresoxim-methyl 50	Methyl (E)-methoxyimino[_-(o-tolyloxy)-o-tolyl]acetate	0.5 g L^{-1}
Tachigarem/S	Hymexazole 36	5-methylisoxazol-3-ol	2.0 mL L^{-1}
Teldor/F	Fenhexamide 50	2',3'-dichloro-4'-hydroxy-1-methylcyclohexanecarboxanilide	1.5 g L^{-1}
Topas/F	Penconazole 10	(RS)-1-[2-(2,4-dichlorophenyl)pentyl]-1H-1,2,4-triazole	0.4 mL L^{-1}
Non-systemic			
Daconil/S	Chlorothalonil 75	Tetrachloroisophthalonitrile	2.0 g L^{-1}
Ditiver/S	Mancozeb 80	Manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt	4.0 g L^{-1}
Euparem/S	Dichlofluanide 50	N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulfamide	2.0 g L^{-1}
INACOP/S	Copper oxychloride 50	Dicopper chloride trioxide	4.0 g L^{-1}
Octagón/S	Prochloraz 45	N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide	2.0 mL L^{-1}
Parmex/F&S	Iprodione 50	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	1.5 g L^{-1}
Terrazole/S	Etridiazole 48	Ethyl 3-trichloromethyl-1,2,4-thiadiazol-5-yl ether	2.0 mL L^{-1}
Metaram/F&S	Tetramethylthiuram-disulfide 80	Tetramethylthiuram disulfide	3.0 g L^{-1}
Systemic and non-systemic			
Swich/F	Ciprodinyl 37.5 + Fludioxonyl 25	4-cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine (37,5%) + 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile (25%)	1.0 g L^{-1}
Forum/F	Dimetomorph 11.3 + Folpet 60	(EZ)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl] morpholine (11,3%) + N-(trichloromethylthio)phthalimide (60%)	1.6 g L^{-1}
Unknown			
Frupica/F	Mepanipyrim 50	N-(4-methyl-6-prop-1-ynylpyrimidin-2-yl)aniline	0.8 g L^{-1}

[1] S: soil recommended application. F: foliar recommended application.

treatments but excessive inputs can cause a delivery of active ingredient to the soil; that is why the majority of the fungicides were applied by foliar pulverization and by irrigation despite its mode of action. Moncut was applied only by immersion of the plant's root system in the fungicide's solution prior to transplant, and this mode of application was considered as well for Ortiva and Metaram.

The fungicides were mixed with water and applied to the foliage with a hand sprayer without covering the potting media, applied directly to soil, or by root immersion, according to the producer's recommendation. Mycorrhizal plants with no fungicide application were used as controls, and an additional treatment including nonmycorrhizal plants was also considered.

Each treatment was replicated five times in a completely randomized design. At harvest, 3 months after starting the fungicide's application, shoot dry weight (after drying at 60°C during 72 h) and arbuscular mycorrhizal (AM) colonization for each plant were assessed. Root samples were clarified and stained (Koske and Gemma, 1989) to estimate internal mycorrhizal colonization under the dissecting microscope. The extent of infection in leek roots was quantified with the gridline intersect method (Giovannetti and Mosse, 1980).

Results were examined using analysis of variance (ANOVA) and treatment means were compared according to Tukey's multiple range test ($P < 0.05$). Data on percentage of AM colonization were transformed to arcsin for analysis.

Results

The nomenclature of the active ingredients and their concentration will be used in the text from now onwards for a better understanding of their performances as well as a clear discussion of the results obtained (Table 1 and Table 2).

The fungicides had no significant effects on shoot growth of mycorrhizal leek plants. However, the mycorrhizal inoculation caused growth stimulation in leeks. Mean dry matter contents of 2.31 g and 0.50 g per plant for mycorrhizal and non mycorrhizal plants were respectively quantified.

There is no direct relationship between the application of systemic fungicides and a detrimental effect on mycorrhizal symbiosis, and there is no evidence either that the foliar application of fungicides are inoquous for the mycorrhizal fungi (Table 2).

Fungicides with soil recommended application (Table 2)

The application of 50% Chinosol, 60.5% Propamocarb, and 50% Cooper oxychloride had no influence on the percentage of root length colonized when the plants were treated by fungicide soil application. All mycorrhizal plants treated with these fungicides and the mycorrhizal control plants showed a high level of root colonization without significant variations among mean values (above 80%). However, the application of 45% Prochloraz, 80% Mancozeb, 50% Iprodione and 80% Thiram virtually eliminated the mycorrhizal symbiosis in treated plants (values below 10%). 46.5% Methalaxyl, 36% Hymexazole, 75% Chlorothalonil, 50% Dichlofluanide and 48% Etridiazole applications on mycorrhizal plants produced a significant reduction of the percentage of AM root colonization, but the fungus was still present in the root cortex (from 30.9 to 50.2%).

Fungicides with foliar recommended application (Table 2)

The application of 80% Fosethyl-Al, 11.3% Dimetomorph + 60% Folpet, 37.5% Ciprodinyl + 25% Fludioxonyl, 50% Fenhexamide and 50% Cooper oxychloride, did not show a deleterious effect on AMF when the plants were treated by foliar spraying. All mycorrhizal plants treated with these fungicides and mycorrhizal control plants achieved a high level of root colonization without significant variations between mean values (above 70.2%). Although there is not a clear inhibition of established AM in 25% Azoxystrobin, 64% Methalaxyl + 3.9% Mancozeb, 50% Iprodione and 50% Kresoxym-methyl, the AMF colonization (from 52.7 to 65.3%) is reduced in front of M+Control plants. Nevertheless, the application of 12% Fenarimole and 24% Miclobutanil strongly inhibits the mycorrhizal symbiosis in treated plants (below 8.1%). The application of 10% Ciproconazole, 10% Penconazole, 50% Mepanipirim and 80% Thiram on mycorrhizal plants produced significant reductions of the percentage of AM root colonization (from 22.3 to 40.4% for 50% Mepanipirim, and 10% Penconazole, respectively; and from 49.8 to 50.7% for 10% Ciproconazole and 80% Thiram, respectively); but again, the fungus was still present in the root cortex.

Table 2. Effect of fungicides on mycorrhizal colonization of leek plants. Within the same column, values followed by the same letter do not differ statistically according to Tukey's multiple range test ($P \leq 0.05$)

Commercial name/ fungicides S or F[1]	Active ingredient and concentration (%)	Root infected length (%)		
		Foliar application	Soil application	Root inmersion
M+Control (without fungicide)		85.0^{g}	85.0^{gh}	85.0^{b}
Systemic				
Aliette/F	Fosethyl-aluminium 80	70.2^{fg}	50.9^{f}	
Beltanol/S	Chinosol 50	88.3^{g}	90.4^{h}	
Caddy/F	Ciproconazole 10	49.8^{d}	38.1^{e}	
Moncut/F&S	Flutholanil 50			80.7^{b}
Ortiva/F	Azoxystrobin 25	65.3^{ef}	15.8^{c}	30.2^{a}
Previcur/S	Propamocarb 60,5	87.5^{g}	90.4^{h}	
Ridomil Gold MZ/F	Methalaxyl 64 + Mancozeb 3,9	55.3^{d}	50.7^{f}	
Ridomil Gold SL/S	Methalaxyl = Mephenoxam 46,5	56.4^{d}	38.3^{e}	
Rubigan/F	Fenarimole 12	8.1^{a}	0.0^{a}	
Sinthane/F	Miclobutanil 24	8.4^{a}	4.3^{ab}	
Stroby/F	Kresoxim-methyl 50	52.7^{d}	9.3^{b}	
Tachigarem/S	Hymexazole 36	55.2^{d}	50.5^{f}	
Teldor/F	Fenhexamide 50	73.0^{fg}	30.4^{de}	
Topas/F	Penconazole 10	40.4^{c}	14.0^{c}	
Non-systemic				
Daconil/S	Chlorothalonil 75	85.5^{g}	50.2^{f}	
Ditiver/S	Mancozeb 80	65.9^{e}	0.0^{a}	
Euparem/S	Dichlofluanide 50	60.5^{de}	38.4^{e}	
INACOP/F&S	Copper oxychloride 50	87.0^{g}	81.4^{gh}	
Octagón/S	Prochloraz 45	30.2^{b}	10.4^{bc}	
Parmex/F&S	Iprodione 50	58.7^{d}	0.0^{a}	
Terrazole/S	Etridiazole 48	60.4^{de}	30.9^{e}	
Metaram/F&S	Tetramethylthiuram disulfide 80	50.7^{d}	2.7^{ab}	83.2^{b}
Systemic and non-systemic				
Forum/F	Ciprodinyl 37,5 + Fludioxonyl 25	88.2^{g}	26.4^{d}	
Swich/F	Dimetomorph 11.3 + Folpet 60	73.0^{fg}	1.1^{a}	
Unknown				
Frupica/F	Mepanipyrim 50	22.3^{b}	12.4^{bc}	

The data (showed untransformed) are means of 5 replications. Values have been transformed to arcsin for analysis. [1] S: soil recommended application. F: foliar recommended application.

Fungicides with root immersion recommended application (Table 2)

The application of 50% Flutholanil and 80% Thiram, prior to transplant did not produce any change in the rate of colonization observed at harvest. The percentage of root colonization after 25% Azoxystrobin root immersion treatment was significantly reduced to 30.2%, and thus, that fungicide treatment significantly inhibited colonization when compared with the latter.

Fungicides with non recommended application treatment (foliar or soil)

The experiment was carried out by testing the soil fungicides in foliage and the foliar fungicides to soil. In the first case, soil fungicides 60.5% Propamocarb, 75% Chlorothalonil and 50% Cooper oxychloride did not influence the mycorrhizal symbiosis when they were applied by foliar spraying; 80% Mancozeb, 50% Dichlofluanide and 48% Etridiazole caused some inhibitory effect on AMF; and 36% Hymexazole, 46.5%

Methalaxyl and 45% Prochloraz showed an AMF restricted development (Table 2).

In the second case, as expected, the effect of foliar fungicides in soil applications caused in general a harmful effect on mycorrhizal fungi: a slight inhibition (about 40% inhibition) was produced by 80% Fosethyl-Al and 64% Methalaxyl + 3.9% Mancozeb. A strong reduction of root colonization by AMF was observed for 10% Ciproconazole, 50% Fenhexamide, 11.3% Dimetomorph + 60% Folpet, 25% Azoxystrobin, 10% Penconazole and 50% Mepanipirim applications. Finally, the presence on soil of 37.5% Ciprodinyl + 25% Fludioxonyl, 50% Kresoxym-methyl, 24% Miclobutanil and 12% Fenarimole, produced an almost complete elimination of mycorrhizal symbiosis in treated plants (values below 7.5%).

Discussion

Scientists have stated that fungicides affect the AM symbiosis with the host plant in different manners: negatively, neutrally and positively (Samarbakhsh *et al.*, 2009). To start with non detrimental fungicides, in these experimental conditions there are three active ingredients that do not affect mycorrhizal symbiosis regardless their application method (soil or foliar): two systemic fungicides, 50% Chinosol and 60.5% Propamocarb, and one non systemic fungicide 50% Copper oxychloride. Marin *et al.* (2002) and Fontanet *et al.* (1998) observed the same result with Propamocarb application in cardoon seedlings and nursery peach-almond rootstocks, respectively. 50% Chinosol is known for its brief action period on soil or plant, and that may be a cause of its inoquity on the mycorrhizal fungus. Experiments with Copper oxychloride reported negative effects for *Glomus* sp. and *Arachis hypogea* L. (Sreenivasa and Bagyaraj, 1989) and neutral effects for *Glomus fasciculatum* and *Agrostis palustris* L. (Rhodes and Larser, 1981), thus results were dependent on the crop and the AMF involved.

Fungicides like Fosethyl-Al did not affect the AMF symbiosis when applied to plant leaves. Similar observations were made by Cardoso and Lambais (1993) and Carrenho *et al.* (2000). Jabaji-Hare and Kendrick (1985) related this lack of effect to the fact that Fosethyl-Al increases root exudation, which seems to facilitate the formation and penetration of the spore germinative tube into the root, helping the fungal establishment inside the root cortex. However, the extent of AM colonization detected in Fosetyl-Al foliar sprayed plants were not higher or significantly different from those observed in untreated plants. It is important to know that if Fosethyl-Al drips to soil a little inhibition of the mycorrhizal fungi can be expected.

In foliar application, Chlorothalonil has been used in a number of experiments, where its effectiveness in reducing the abundance of AM fungi was demonstrated (Aziz *et al.,* 1991; Wan *et al.,* 1998; Laatikainen and Heinonen-Tanski, 2002). These reports are in contrast with the observations made in this study because this active ingredient did not affect the AM fungi. Chlorothalonil, like Forum (11.3% Dimetomorph + 60% Folpet), contains more than 50% of active ingredients that belong to the group of phthalimide, and act similarly on mycorrhizal fungi involved in the experiment (percentage of root infected length, between 73 and 85%).

The foliar application of active ingredients belonging to other chemical groups (pyrimidine + phenilpyrrole and anilide), does not affect the mycorrhizal fungus. Swich (37.5% Ciprodinyl + 25% Fludioxonyl) and Teldor (50% Fenhexamide) exert an inhibitory effect on some of the processes occurring during the biological synthesis of ergosterol, a basic component of the cellular membrane in fungi. Both fungicides are normally used for *Botrytis* control and are documented as environmentally friendly because they are not toxic and do not produce the emergence of cross resistances.

Eventually, all foliar fungicides produced a strong inhibition on the development of mycorrhizal fungi when they were applied to the soil, despite their chemical group and the expected modes of action on the fungus. The application of azole fungicides (triazole or imidazole) and pirimidine caused a clear damage on mycorrhizal symbiosis, higher when the fungicides were in contact with the soil. As previously stated, the primary mode of action of derivatives of imidazole, pyrimidine and triazole fungicides is the inhibition of the biosynthesis of ergosterol in pathogenic fungi. This evidence is in agreement with the observations made in the present study for mycorrhizal colonization depressed in plants treated by soil application with 10% Ciproconazole, 37.5% Ciprodinyl + 25% Fludioxonyl, 12% Fenarimole, 50% Mepanipirim, 24% Miclobutanil, 10% Penconazole and 45% Prochloraz. Although azole fungicides have been reported to affect *in vitro* growth of *Rizopus* spp., zygomycota like *Mucor* spp. can be regarded as unsensitive to this group (Diedhiou *et al.*, 2004). Nevertheless, concerning symbionts such as mycorrhizal fungi, this effect does not show.

The detrimental effect of Iprodione on mycorrhizal development has been reported too by Gange *et al.* (1990). This fungicide interferes in nucleic acid metabolism, protein synthesis and cell division. Similarly, Mancozeb had a negative influence on the roots AM colonization when applied to soil. This effect could be attributed to its non-specific reaction with fungal cell components, particularly thiol groups, by inhibiting respiration. Plenchette and Perrin (1992) reported the same results on wheat roots.

The Methalaxyl application to soil or to the leaves produces a moderate inhibition on mycorrhizal symbiosis in these experimental conditions. Data reported by Musumeci *et al*. (1982) showed that the absorption and translocation occurred over approximately 60 days, and that after this period, it decreased due to its degradation in the soil and in the tissues of the plant. Previous studies have documented that Methalaxyl and its metabolites did not have negative effects on the development of AMF, but generally increased colonization (Afek *et al.*, 1990; Hetrick *et al.*, 1992). In contrast, Carrenho *et al.* (2000) documented a considerable reduction in percentage of colonization of citrus seedling roots with this active ingredient.

With reference to Azoxystrobin and Krexoxym methyl, both members of a class of fungicides derived from fungal secondary metabolite strobilurin A, did not excessively affect the AM colonization in foliar spraying. Strobilurins basically inhibit mitochondrial respiration. Diedhiou *et al.* (2004) proved that foliar applications of Azoxystrobin and Krexoxym methyl did not have negative effects on established mycorrhizal colonization of maize plants, but the application of these fungicides onto soil was harmful.

25% Azoxystrobin in the root immersion treatment is the only active ingredient tested that decreased the percentage of root colonization by AM fungi. This effect was initially expected as the substance acts on the spore germination and mycelium production. Thus, if the roots are submerged in fungicide solution and then plants are inoculated, the probability of exerting negative effects on spore germination of the mycorrhizal fungus is high. Von Alten *et al*. (1993) observed that if the foliar application of strobilurins causes an accumulation in the sandy substrate where the root tissue colonization occurs, the germination of AM spores would be inhibited.

On the other hand, 50% Mepanipyrim has an inexactly known mode of action on pathogenic fungi, but this active ingredient acts by contact and by translaminar movement. Mepanipyrim prevents the penetration of the fungus in the plant, the elongation of the germinative tube and the appressoria formation. It can thus be expected that its application affects the mycorrhizal symbiosis.

The results indicate that a group of fungicides may safely be applied with little effect on mycorrhizae; they do not eliminate the beneficial fungus and the symbiosis within the host roots. It has been found that many fungicides of foliar recommended application drenched to the soil have a pronounced inhibitory effect on the development of AM fungi (Plenchette and Perrin, 1992) and may act more drastically on soil infectivity that fungicide spraying.

Differences in the results of fungicidal effects on AM fungi may also be due to differences in the sensitivity of fungi or isolates to fungicides as reported for *Glomus* species (Fontanet *et al*., 1998; Kjoller and Rosendahl, 2000). Most probably, two AMF species present in commercial products will differ when submitted to different fungicides, but a combination of these fungi may be more efficient under certain circumstances, especially to maintain the beneficial endomycorrhizal activity for the plant even when deleterious substances are applied.

The observations of this study suggest that horticultural crops can be inoculated with AM fungi in the nursery (early infection) to take advantage of the symbiosis and then be treated with compatible fungicides, provided that the applications are conducted with great care, especially when foliar fungicides are spayed, because active ingredients accidentally delivered to the soil may detrimentally affect the development of the AM fungus.

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