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Mycorrhiza

Evaluation in the greenhouse of the effects of fungicides on the development of mycorrhiza on leek and wheat

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Summary. The effect of 11 fungicides, viz Aliette, Bayleton 5, Benlate, Dithane M45, Euparene, Karathane, Orthocide 83RP, Pelt 44, Tilt C, Tilt 125 and Tripomol 80, on vesicular-arbuscular mycorrhizal symbiosis in leek and wheat growing in a calcined clay was studied in the greenhouse at four concentrations applied to the foliage. Fungicides had minimal effects when applied to leek or wheat plants with well-established mycorrhiza. Mycorrhizal infection was not drastically affected by any fungicide at any application level. However, mycorrhizal infection of leek plants was reduced by systemic fungicides, and that of wheat by non-systemic fungicides. Aliette was the only fungicide that stimulated mycorrhizal development.

Key words: Fungicide - Leek - Wheat - Vesicular-arbuscular mycorrhiza

Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi have been found associated with the roots of most crops and their beneficial effects have been well documented. The lack of host specificity of VAM fungi (Smith 1980) is mainly responsible for their ubiquity in natural and in agro-ecosystems. However, VAM infectivity of the soil may fluctuate (Plenchette et al. 1989), particularly in cultivated soils where cultural practices may have significant effects (Plenchette 1989). Of these practices, the use of fungicides to control pathogens is generally reported to be detrimental to VAM fungi (Menge 1982; Trappe et al. 1984). Some fungicides reduce the development of VAM in plant roots (Backman and Clark 1977; Boatman et al. 1978; El-Giahmi et al. 1976; Nemec 1980; Nesheim and Linn 1969). Fungicide application is in general less damaging for VAM fungi populations than soil fumigation (Menge 1982). However, in some cases mycorrhizal infection may be reduced and the beneficial effects of the symbiosis, such as increased phosphorus uptake, may be lost (Boatman et al. 1978).

In most reported experiments, fungicides were mixed with the soil or the substrate at high concentrations (El-Giahmi et al. 1976; Manjunath and Bagyaraj 1984; Nemec 1980, 1985) or applied as a soil drench (Baylis and Safir 1978; Menge et al. 1979; Nesheim and Linn 1969). Since the fungicides were applied to the soil before planting, the effects obtained mainly concerned mycorrhiza formation. Little information is available about the effects of fungicides on mycorrhizal development after foliar application. For most major crops, fungicides and fertilizers are applied together during the growing season when mycorrhizae are already well established. Therefore, in the present studies the effects on VAM association of some fungicides widely used in European agriculture were assessed as foliar sprays.

Materials and methods

Culture of endophyte

A strain of *Glomus intraradices* (Schenck and Smith 1982) was propagated on leeks growing on Terra-green (a calcined clay distributed by Oil-dri, Chicago, Ill., USA) and fed with Long Ashton solution (Hewitt 1966). This strain was used in previous studies (Jabaji-Hare and Kendrick 1987; Plenchette 1982). Terra-green replaced Turface previously used in the development of a method for inoculum production (Plenchette 1982). Terra-green and Turface are two brands of arcilitte (Wildon and O'Rourke 1964), of which Terra-green is more readily available on the European market than Turface.

Plant material

The experimental plants were leek (*Allium porrum* L. cv Olaf) and wheat (*Triticum aestivum* L. cv Arminda). Leek and wheat seeds were surface sterilized for 5 min in 10% sodium hypochlorite. Before planting out, leek seeds were sown in autoclaved vermiculite and grown for 4 weeks; wheat seeds were germinated in petri dishes for 3 days.

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The experiment was conducted in plastic pots (9 × 9 cm) containing 300 g Terra-green and one plant per pot. Each pot received 5 ml of Long Ashton solution weekly. The phosphorus content of this solution was lowered tenfold for wheat. After planting, pots were placed in a greenhouse in which artificial illumination (370 μmol s^{-1} m^{-2}) was given to maintain a 16-h day with a mean day/night temperature of 22° C/16° C.

Each pot was inoculated with 1 g of chopped leek roots infected with *G. intraradices*. The inoculum was surface sterilized for 5 min in 2% M chloramine T and 10% mercryl solution. The controls received 1 g of boiled leek roots. There were four replicates of each treatment.

Application of fungicides

Plants were grown for 6 weeks prior to the application of fungicides to ensure a well-developed mycorrhizal infection. Eleven fungicides commonly used in agriculture were tested at four concentrations of the active ingredient (Table 1). Concentration 2 corresponds to the recommended field rate; 1 and 3 are equivalent to half and double the recommended application, respectively. These fungicides were chosen for their different modes of action: fosetyl-aluminium works against oomycetes, although it has recently been shown to stimulate the development of VAM fungi; dinocap and thiram interfere with respiration; carbendazim, benomyl and thiophanate methyl interact with cell growth, division and development; propiconazole and triadimefon inhibit biosynthetic reactions; dichlofluanid, mancozeb and captan have a non-specific mode of action.

The fungicides were mixed with deionized water and applied to the foliage with a hand sprayer without covering the potting media. For each treatment, the eight pots (four mycorrhizal and four non-mycorrhizal) were assembled in a group and 7.2 ml fungicide solution was applied, corresponding to the recommended application of 300 l/ha. The pots were then randomly distributed in the greenhouse.

Measurements

Plants were harvested 6 weeks after fungicide application. The shoot dry weight (after drying at 65° C for 48 h) and the mycorrhizal infection of each plant were recorded. Roots were cleared in 10% KOH for 1 h at 90° C and stained for 15 min with acid fuschin (0.05% in lactoglycerol). The percentage of infected root length was evaluated using the gridline intersect method (Giovannetti and Mosse 1980). Analysis of variance was performed on all data for the percentage value of mycorrhizal infection after transformation ($\arcsin\sqrt{\%}$). Tissue phosphorus content was determined colorimetrically (Charlot 1966) for those treatments decreasing root infection.

Table 1. Fungicides and concentrations of active ingredients used

Commercial name	Active ingredient	Concentration (μg/ml)			
		0	1	2	3
Systemic					
Pelt 44	Thiophanate methyl	0	180	360	720
Aliette	Fosetyl-aluminium	0	800	1600	3200
Benlate	Benomyl	0	100	200	400
Tilt C	Propiconazole	0	312	625	1250
	+ carbendazim	0	350	700	1400
Tilt 125	Propiconazole	0	312	625	1250
Bayleton 5	Triadimefon	0	1	2	5
Non-systemic					
Dithane M45	Mancozeb	0	800	1600	3200
Orthocide 83RP	Captan	0	726	1452	2905
Euparene	Dichlofuanid	0	312	625	1250
Karathane	Dinocap	0	54	109	219
Trimpomol 80	Thiram	0	800	1600	3200

Results

Effects on shoot biomass

The fungicides had no significant effect on the shoot growth of mycorrhizal or non-mycorrhizal plants at any concentration tested. However, mycorrhizal inoculation led to a significant ($P<0.01$) growth stimulation of leek plants (average dry matter 1.98 and 0.30 g per plant for mycorrhizal and non-mycorrhizal leeks, respectively) but not of wheat.

Effect on mycorrhizal infection

The non-systemic fungicides did not reduce root colonization of leek (Table 2) at any concentration applied. Three systemic fungicides (benomyl, propiconazole alone or associated with carbendazim) had negative effects (Table 2), but this effect did not lead to a drastic reduction of infection. Moreover, the small reduction in root infection did not significantly alter plant growth.

In contrast, mycorrhizal wheat plants were susceptible to non-systemic fungicides (mancozeb, dichlofluanid, thiram) and among the systemics only benomyl significantly reduced root colonization at the highest concentration of the active ingredient tested (Table 2).

The correlation coefficients of the linear regression between the mycorrhizal infection percentage and the concentration of active ingredient were calculated (Table 3). For leek, a high negative correlation coefficient ($P<0.01$) was observed with benomyl and with propiconazole + carbendazim. For wheat, significant negative correlation coefficients were obtained with five fungicides, and only applications of fosetyl-aluminium were positively correlated with mycorrhizal infection.

Tissue-phosphorus content of shoots was determined for each treatment which reduced the VAM root infection. In all cases, mycorrhizal plants had a significantly (Newman-Keuls, $P<0.05$) higher phosphorus content than non-mycorrhizal plants (means of 821 and 611 μg/g, respectively, for wheat, and 2012 and 1391 μg/g, respectively, for leek) but the fungicides had no significant effect on tissue-phosphorus content.

Discussion

Some systemic fungicides such as benomyl and carbendazim were able to reduce VAM root colonisation, whereas other systemic fungicides (thiophanate methyl, propiconazole and triadimefon) appeared to have

Table 2. Effect of foliar sprays of various fungicides on mycorrhizal infection (*Glomus intraradices*) of leek and wheat plants. * Results followed by the same letter are not significantly different ($P<0.05$ Newman Keuls test)

	Concentration level[a]	Root infected length (%)	
		Leek	Wheat
Non-systemic			
Mancozeb	0	60.0a*	36.3a
	1	58.1a	31.0b
	2	54.2a	32.9b
	3	56.5a	32.1b
Captan	0	60.1a	37.5a
	1	59.1a	35.0a
	2	59.3a	34.7a
	3	60.9a	32.1a
Dichlofuanid	0	62.1a	44.6a
	1	60.5a	41.9a
	2	60.7a	40.4b
	3	62.9a	36.4b
Dinocap	0	55.3a	42.1a
	1	54.1a	38.8a
	2	52.3a	37.4a
	3	51.7a	39.4a
Thiram	0	53.3a	37.3a
	1	54.2a	30.2b
	2	55.7a	29.6b
	3	51.9a	26.3b
Systemic			
Thiophanate methyl	0	60.2a*	35.5a
	1	56.5a	33.1a
	2	57.1a	31.0a
	3	57.0a	31.9a
Fosetyl-aluminium	0	60.7a	42.3a
	1	57.1a	42.4a
	2	56.4a	44.1a
	3	57.0a	45.1a
Benomyl	0	62.6a	37.7a
	1	56.7b	37.1a
	2	54.2bc	36.7a
	3	52.7c	29.6b
Propiconazole	0	59.1a	38.5a
	1	51.4b	37.4a
	2	53.7b	36.1a
	3	57.6a	33.9a
Propiconazole + carbendazim	0	60.9a	40.1a
	1	60.0a	37.7a
	2	54.4b	38.6a
	3	48.8c	37.3a
Triadimefon	0	57.2a	41.4a
	1	55.2a	40.7a
	2	56.9a	39.5a
	3	55.5a	41.5a

[a] See Table 1

either no effect or a slight stimulatory effect (fosetyl-aluminium) on VAM development. In addition, the effect of the fungicides depended greatly on the plant species.

For wheat plants, the phosphorus concentration of the nutrient solution was reduced tenfold in order to obtain a mycorrhizal effect since wheat has a lower mycorrhizal dependency than leek (Plenchette et al. 1983). However, a mycorrhizal growth response was not obtained. It should be noted, however, that wheat was harvested before grain ripeness and mycorrhizal effects on wheat are often only observed on grain yield (Trouvelot et al. 1982).

Table 3. Correlation coefficients between the concentration of active ingredient applied and percentage infected root length. * Significant ($P<0.05$); ** significant ($P<0.01$)

Fungicides	Correlation coefficient (r)	
	Leek	Wheat
Thiophanate methyl	−0.30	−0.40
Fosetyl-aluminium	−0.43	+0.51*
Benomyl	−0.72**	−0.80**
Propiconazole + carbendazim	−0.86**	−0.28
Propiconazole	−0.04	−0.53*
Triadimefon	−0.16	−0.02
Mancozeb	−0.44	−0.31
Captan	−0.11	−0.60*
Dichlofuanid	−0.19	−0.80**
Dinocap	−0.39	−0.31
Thiram	−0.19	−0.77**

Fosetyl-aluminium tended to stimulate mycorrhizal development (Table 3), which is in agreement with the results of Jabaji-Hare and Kendrick (1987). The other fungicides slightly reduced the mycorrhizal infection, with mean decreases of 5.5% for leek and 13% for wheat. This result reflects the higher mycorrhizal dependency of leek (Plenchette et al. 1983). As a consequence, it is important to note that the mycorrhizae of plants with low mycorrhizal dependency could be more altered than those of plants with high mycorrhizal dependency.

The translocation of systemic fungicides within the plant downwards into the roots has been reported to be negligible (Erwin 1973) except for fosetyl-aluminium (Jabaji-Hare and Kendrick 1985). It is interesting to note that benomyl and carbendazim both had a negative influence on mycorrhizal development on leek and have the same mode of action. These fungicides interfere with cell growth, cell division and development but their efficiency depends greatly on their retention in or on the roots. Similarly, the detrimental effects on wheat of three of the non-systemic fungicides (mancozeb, dichlofluanid and thiram) could be attributed to their non-specific reactions with plant cell components, particularly thiol groups.

Our data reveal the importance of the degree of mycorrhizal development at the time when fungicides are applied. Fungicide had little or no effect, even at high concentrations, when mycorrhizal infection was well established. These results are of practical importance since the strategy of pathogen control involves repeated spraying of fungicide during the growing season. In disease control of cereals, for example, four or five fungicide applications are necessary between tillage and flowering (Daguenet and Eschenbrenne 1988).

Nutrient deficiency, particularly of phosphorus, at the grain-filling stage can lead to a yield decrease of

more than 15% (Dupont 1983). Thus, it is very important to know whether fungicide application during the growing season significantly alters mycorrhizal infection and consequently the phosphorus nutrition of mycorrhizal plants.

Greenhouse experiments are often disturbed by disease due to pathogenic fungi. Our results indicate an array of fungicides which may be applied at concentrations having little effect on mycorrhizae.

VAM soil infectivity fluctuates in relation to agricultural practices (Plenchette 1989), amongst which fungicide application is often considered to be the most detrimental, leading to a decrease in indigenous fungal populations (Trappe et al. 1984). It has been found that fungicides, especially of the systemics type, applied as a seed treatment or soil drench have a pronounced inhibitory effect on the development of VAM (Jalali and Domsch 1975; Nesheim and Linn 1969; Sreenivasa and Bagyaraj 1989) and may act more drastically on soil infectivity than fungicide spraying. It is also possible that practices such as straw burial have a negative effect: the period between the last spraying and straw burial is sometimes short (4 weeks) and the fungicide remaining in or on the straw is directly incorporated into the soil. The fungicidal substances in the rhizosphere of young rootlets can prevent mycorrhizal formation (Menge 1982).

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References

Backman PA, Clark M (1977) Effect of carbofuran and other pesticides on vesicular-arbuscular mycorrhizae in peanuts. Nematropica 7:13–17

Baylis JE, Safir GR (1978) Effect of benomyl on soybean endomycorrhizae. Phytopathology 68:1810–1812

Boatman N, Paget D, Hayman DS, Mosse B (1978) Effects of systemic fungicides on vesicular-arbuscular mycorrhizal infection and plant phosphate uptake. Trans Br Mycol Soc 70:443–450

Charlot G (1966) Les méthodes de la chimie analytique. Masson, Paris

Daguenet P, Eschenbrenne P (1988) Les fongicides des céréales. Perspect Agric 124:33–178

Dupont F (1983) L'intensification en Europe. Cultivar 167:63–67

El-Giahmi AA, Nicolson TH, Daft MJ (1976) Effects of fungal toxicants on mycorrhizal maize. Trans Br Mycol Soc 67:172–173

Erwin C (1973) Systemic fungicides: disease control, translocation and mode of action. Annu Rev Phytopathol 11:389–422

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring VA mycorrhizal infection in roots. New Phytol 84:489–500

Hewitt EJ (1966) Sand and water culture methods used in the study of plant nutrition. (Tech Comm 22, 2nd edn, revised) Commonwealth Agricultural Bureau, London

Jabaji-Hare SH, Kendrick WB (1985) Effects of phosetyl-Al on root exudation and on composition of extract of mycorrhizal and non-mycorrhizal leek roots. Can J Plant Pathol 7:118–126

Jabaji-Hare SH, Kendrick WB (1987) Response of an endomycorrhizal fungus in *Allium porrum* L. to different concentrations of the systemic fungicides, Metalaxyl (Ridomil®) and Fosetyl-al (Aliette®). Soil Biol Biochem 19:95–99

Jalali BI, Domsch KH (1975) Effect of systemic fungitoxicants on the development of endotrophic mycorrhiza. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, New York London, pp 619–626

Manjunath A, Bagyaraj DJ (1984) Effect of fungicides on mycorrhizal colonization and growth of onion. Plant Soil 80:147–150

Menge JA (1982) Effect of soil fumigants and fungicides on vesicular-arbuscular fungi. Phytopathology 72:1125–1132

Menge JA, Johnson ELV, Minassian V (1979) Effect of heat treatment and three pesticides upon the growth and reproduction of the mycorrhizal fungus *Glomus fasciculatus*. New Phytol 82:473–480

Nemec S (1980) Effect of 11 fungicides on endomycorrhizal development in sour orange seedlings. Can J Bot 58:522–526

Nemec S (1985) Infuence of selected pesticides on *Glomus* species and their infection in Citrus. Plant Soil 84:133–137

Nesheim ON, Linn B (1969) Deleterious effect of certain fungitoxicants on the formation of mycorrhizae on corn by *Endogone fasciculatus* and on corn root development. Phytopathology 59:297–300

Plenchette C (1982) Recherches sur les endomycorhizes à vésicules et arbuscules. Influence de la plante hôte, du champignon et du phosphore sur l'expression de la symbiose endomycorhizienne. PhD thesis, Université Laval, Québec

Plenchette C (1989) Mycorrhizal soil infectivity of Deherain's plots. C R Acad Agric Fr 75:21–27

Plenchette C, Fortin JA, Furlan V (1983) Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant Soil 70:199–209

Plenchette C, Perrin R, Duvert P (1989) The concept of soil infectivity and a method for its determination as applied to endomycorrhizas. Can J Bot 67:112–115

Schenck NC, Smith GS (1982) Additional new and unreported species of mycorrhizal fungi (endogonaceae) from Florida. Mycologia 74:77–92

Smith SE (1980) Mycorrhiza of autotrophic higher plants. Biol Rev Camb Phil Soc 55:475–500

Sreenivasa MN, Bagyaraj DJ (1989) Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum. Plant Soil 119:127–132

Trappe JM, Molina R, Castellano M (1984) Reaction of mycorrhizal fungi and mycorrhiza formation to pesticides. Annu Rev Phytopathol 22:331–359

Trouvelot A, Gianinazzi-Pearson V, Gianinazzi S (1982) Les endomycorhizes en agriculture; recherches sur le blé. In: Les mycorhizes: biologie et utilisation. (Les colloques de l'INRA, 13) INRA, Dijon, pp 559–561

Wildon CE, O'Rourke FLS (1964) The effect of Arcillite in media for pot plants. Research report. Business serials from the Michigan Stage University, Mich, no 16