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SHORT NOTE

G. S. Pattinson · D. I. Warton
R. Misman · P. A. McGee

The fungicides Terrazole and Terraclor and the nematicide Fenamiphos have little effect on root colonisation by *Glomus mosseae* and growth of cotton seedlings

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Abstract The effect of three pesticides on the initiation and early development of arbuscular mycorrhiza in cotton was examined in experiments under controlled conditions. The fungicides Terrazole and Terraclor initially inhibited mycorrhizal infection of roots of cotton. The inhibition disappeared after 4 weeks, and neither fungicide had a lasting effect. The nematicide Fenamiphos slightly increased shoot dry weight at 6–10 weeks from planting and had no effect on mycorrhizal infection. We conclude that these pesticides have no sustained, detrimental effect on mycorrhizal infection or growth of cotton seedlings when applied at recommended rates.

Key words Arbuscular mycorrhiza · Initiation of infection · Mites · Pesticides

Introduction

In agricultural systems, arbuscular mycorrhizas may be reduced in plants by cultural practices (Evans and Miller 1988, 1990; Fairchild and Miller 1988, 1990; Jasper et al. 1989; McGonigle et al. 1990), including crop rotation (Black and Tinker 1979), and the application of pesticides (Trappe et al. 1984) and perhaps fertilisers (Johnson and Pfleger 1992), with a consequent reduction of plant growth rates. Cultural practices may indirectly affect crops by reducing the fitness of the symbiont. Arbuscular mycorrhizal fungi readily colonise seedlings of cotton (*Gossypium hirsutum* L.) growing in the field. It is thought that cotton plants are dependent on mycorrhiza for maximum rates of growth and production of lint (Rich and Bird 1974) and an understanding of the effects of cultural practices on the mycorrhizal association is thus of economic importance. A range of pesticides are commonly used in the production of cotton in Australia. While all pesticides may affect mycorrhizas, it is more likely that fungicides, or pesticides applied as a soil drench, such as nematicides, have the greatest potential effect on the association (Trappe et al. 1984). The fungicidal effect on mycorrhizal fungi varies from detrimental to beneficial and results using the same fungicide have been contradictory (see Trappe et al. 1984 for examples). The reasons for these differences are unclear and without further knowledge of the mechanisms underlying the interaction, data concerning pesticide effects must be viewed cautiously.

In Australia, Terrazole (active ingredient 5-ethozy-3-trichloromethyl-1,2,4-thiadiazole: Uniroyal Chemical Pty. Ltd.) is used mainly as a fungicide to control seedling root diseases in turf and ornamentals. Terraclor (active ingredient pentachloronitrobenzene, PCNB: Uniroyal Chemical Pty. Ltd.) is used in the cotton industry to control root diseases and can be applied as a seed dressing or as a soil drench. Both fungicides may have detrimental effects on mycorrhizal fungi. The application of Ethazole (Terrazole) as a soil drench at the time of inoculation of sudan grass with *G. fasciculatus* had no effect on the level of mycorrhizal infection of the plants or the quantity of spores produced by the fungus. When Ethazole was applied 45 days after inoculation, the length of mycorrhizal hyphae per centimetre of root and number of spores produced by the fungus were significantly increased, but the number of vesicles per centimetre of root was significantly lower than in the controls (Menge et al. 1979). The application of PCNB as a soil drench at the time of inoculation of sudan grass with *G. fasciculatus* resulted in a significant decline in the length of hyphae per centimetre of root and the number of spores, but not in the number of vesicles per centimetre of root. At 45 days, the application of PCNB resulted in a significant reduction in

G. S. Pattinson (✉) · D. I. Warton · P. A. McGee
School of Biological Sciences A12, University of Sydney, 2006 NSW, Australia
Fax: +61-2-9351-4771; e-mail: fbiol@bio.usyd.edu.au

R. Misman
Faculty of Biology, General Soedirman University, Purwokerto, Central Java, Indonesia

hyphal length, number of vesicles and number of spores produced by the fungus (Menge et al. 1979). Gnekow and Marschner (1989) found application of PCNB to oats significantly reduced the length of roots infected by mycorrhizal fungi and the uptake of phosphate into shoots.

Plant parasitic nematodes are a major problem in many cotton growing regions of the world, where severe stunting of the cotton plant may result from heavy infestation. The root-knot nematode *Meloidogyne incognita* infects cotton plants in many countries (Heald and Orr 1984) and the nematode is widespread in Australia (Bird 1978). It is, therefore, timely to examine the effect of a nematicide commonly used in Australia, Fenamiphos (Bayer Australia Ltd.), on the mycorrhizal association in cotton plants. Fenamiphos has been shown to reduce the shoot weight of *Citrus aurantium* and the quantity of spores produced by *G. mosseae* when applied at 5.6 kg ha^{-1} (Nemec 1985), but not at higher or lower rates. Similar trends were observed when the fungus *G. intraradices* was used.

In this study, the effect of the fungicides Terrazole and Terraclor on the growth of seedlings of cotton and the initiation of arbuscular mycorrhizas by *G. mosseae* was examined. In the mycorrhizal association, primary causal effects may obscure interactions between host and fungus. For instance, if the fungus is affected by a pesticide, the plant may suffer from nutrient deficiency, unrelated to the direct effect of the pesticide on the plant (Trappe et al. 1984). Appropriate controls are needed to determine whether the pesticide affects the plant, the mycorrhizal fungus or both. For this, mycorrhizal and non-mycorrhizal plants should have similar growth rates and this requires provision of additional P.

Materials and methods

The soil mixture used in all experiments consisted of a grey, self-mulching clay collected from the Australian Cotton Research Institute, Narrabri, NSW, Australia, blended with coarse river sand, one part clay to four parts sand, autoclaved for 70 min at 121 °C.

The mycorrhizal inoculum consisted of soil, roots, hyphae and spores from pot cultures of *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe (isolate NBR 4.1 from Narrabri, McGee unpublished), grown in association with *Allium porrum*. In all experiments, inoculated plants were grown in one part pot culture mixed with nine parts of the soil mixture. Non-mycorrhizal seedlings were grown in the soil mixture only.

Seedlings were grown in "book leaf" pots (Yates Pty. Ltd.) which consist of three rectangular cells, each 7×5×25 cm (l×w×h). The two outer cells of each of the book leaf pots were filled with approximately 2 kg of moist soil mixture. The middle cell was left empty to prevent cross contamination of treatments. Two pregerminated, surface-sterilised seeds of cotton, cultivar CS50, weighing 0.8–1 g were sown in each outer cell of the pots and thinned to one after emergence. The pots were watered daily with deionised water.

Experiment 1

The experiment comprised a 2×3 factorial of mycorrhizal status (with or without the addition of mycorrhizal inoculum) and pesticide application (Terrazole, Terraclor, no pesticide). Treatments were replicated five times. Book leaf pots were half filled with the soil mixture, then topped up either with the inoculum or the soil mixture, to which Terrazole or Terraclor had been added or not. The required quantity of Terrazole (0.6 kg ha^{-1}) and Terraclor (3.75 kg ha^{-1}), based on the pot surface area, was bulked up with 150 g of cellulose to aid in mixing the fungicide through the soil. Cellulose (150 g) was incorporated into the treatment lacking fungicides. The pots were watered and placed in a growth room with a day length of 13 h at a PAR of 700 μmol m^{-2} sec^{-1} at 25 °C. The night temperature was set at 20 °C. After 1 week, the pots were sown with pregerminated cotton seeds. Nutrient solution containing 12.25 mM KH_2PO_4, 10 mM KNO_3, 3 mM $Ca(NO_3)_2$, 2 mM $MgSO_4.7H_2O$ and 1 ml l^{-1} of micronutrients solution was applied at a rate of 20 ml per cell once a week. A preliminary experiment showed that P applied at 12.25 mM gave matched mycorrhizal and non-mycorrhizal cotton plants as far as growth and% P were concerned, without adversely affecting mycorrhizal development under the experimental conditions. The plants were destructively harvested after 2, 4, and 6 weeks. The soil was rinsed from the roots and the shoots and roots weighed. A subsample of roots was cleared and stained (Phillips and Hayman 1970) and the total length of roots and mycorrhizas determined by the grid intersect method (Giovanetti and Mosse 1980). The remaining roots and shoots were dried at 70 °C for 24 h and weighed. The P concentration in leaves minus petioles was determined using the ammonium molybdate method (Allen et al. 1974).

Experiment 2

Soil with or without mycorrhizal inoculum was either mixed with Fenamiphos (25 kg ha^{-1}) in 150 g of cellulose or only cellulose. Each treatment was replicated eight times. Book leaf pots were soil amended with either Terrazole, Terraclor or left unamended (*Nil*), harvested after 2, 4 and 6 weeks

Table 1 Mean (±SD) percentage of total root length (%*AM*) infected or not by *Glomus mosseae* (±*AM*), shoot dry weight (*SDW*) and percentage P (%*P*) in dry leaves of cotton grown in

		% AM			SDW			% P		
		Terrazole	Terraclor	Nil	Terrazole	Terraclor	Nil	Terrazole	Terraclor	Nil
Week 2	+AM	4.4 ±2.4[a]	3.9 ±2.1[a]	11.8 ±3.9	0.20±0.02[a]	0.20±0.03	0.23±0.02	0.74±0.11	0.70±0.05	0.62±0.07
	−AM				0.19±0.02[a]	0.21±0.04	0.24±0.01	0.79±0.13	0.76±0.16	0.65±0.07
Week 4	+AM	29.13±8.00	25.61±9.52	30.32±8.81	0.16±0.02[a]	0.24±0.06	0.28±0.04	0.99±0.13[a]	0.70±0.10	0.64±0.08
	−AM				0.22±0.01	0.29±0.08	0.23±0.06	0.93±0.11	0.59±0.21	0.72±0.26
Week 6	+AM	52.64±5.93	54.51±9.22	53.73±3.94	0.34±0.09*	0.42±0.09	0.35±0.10	0.66±0.10	0.63±0.05	0.63±0.05
	−AM				0.37±0.04	0.43±0.15	0.48±0.06*	0.68±0.09	0.54±0.13	0.60±0.06

[a] Significant difference ($P<0.05$) between pesticide treatment and control
* Significant differences ($P<0.05$) between treatments with the same symbol

half filled with the soil mixture, then filled either with the inoculum mixture or the soil mixture, to which the nematicide was added. The pots were placed in the growth room, watered and left for 1 week prior to sowing with pregerminated cotton seeds. Nutrient solution was applied once a week. The plants were harvested after 1, 3, 6 and 10 weeks. The P concentration of the leaves was determined after 6 and 10 weeks.

Data were analysed using the Systat 5.04 programme (Systat Inc.). Means for each treatment were compared using analysis of variance (ANOVA) and Tukey's HSD test.

Results

Experiment 1

Terraclor had no observed effect on shoot dry weight or the P concentration in dry leaves after 2 weeks (Table 1). However, the application of Terraclor significantly reduced mycorrhizal infection of cotton plants examined at the 2-week harvest. There were no significant effects on shoot dry weight, mycorrhizal infection or uptake of P apparent after 4 and 6 weeks. Mycorrhizal infection did not influence shoot growth or P uptake of cotton plants harvested after 4 and 6 weeks.

After 2 weeks, the application of Terrazole had significantly reduced the shoot dry weight of both mycorrhizal and non-mycorrhizal cotton plants (Table 1). Treatment with Terrazole was associated with a significant reduction in mycorrhizal infection.

After 4 weeks, the shoot P concentration was significantly higher than the controls in mycorrhizal plants grown in soil amended with Terrazole but not in non-mycorrhizal plants (Table 1). Statistical differences between the shoot dry weights of the cotton plants were not apparent at 4 weeks due to interaction of the factors mycorrhizas and amendment with Terrazole in the 2-way ANOVA, except for a significant decrease in mycorrhizal plants grown in soil amended with Terrazole. Terrazole had no significant effect on the level of mycorrhizal infection.

After 6 weeks, one replicate in the non-mycorrhizal treatment lacking Terrazole with double the mass of the other replicates was considered to be an outlier and removed from the analysis. Amendment of the soil with Terrazole did not affect mycorrhizal infection, shoot dry weight or uptake of P. However, shoot dry weight of nonmycorrhizal controls was significantly higher than mycorrhizal cotton plants grown in soil amended with Terrazole.

Experiment 2

Mycorrhizal infection was not significantly affected by amendment of soil with Fenamiphos at any harvest (Table 2).

Shoot dry weight of seedlings after 1 week was not affected by either mycorrhizal infection or Fenamiphos (Table 2). After 3 weeks, the shoot dry weight was significantly higher in seedlings grown in soil amended with Fenamiphos than the controls. Mycorrhizal and Fenamiphos effects were also apparent after 6 weeks. Plants grown in soil amended with Fenamiphos were larger than plants grown in unamended soil. After 10 weeks, non-mycorrhizal plants grown in soil amended with Fenamiphos were significantly larger than plants grown in unamended soil. Mycorrhizal infection had no significant effect on shoot dry weight.

Mycorrhizal infection significantly increased the concentration of P in leaves at 6 and 10 weeks (Table 2). Amendment of soil with Fenamiphos had no significant effect on leaf P concentration at either 6 or 10 weeks.

Discussion

Mycorrhizal and non-mycorrhizal cotton plants grown in the Narrabri-soil/sand mix with addition of P at 12.5 mM grew at approximately the same rate and had similar shoot dry weights at all harvests. This enabled examination of the effect of pesticides on the plant and the fungus. Previous studies demonstrated that pesti-

Table 2 Mean (±SD) percentage of total root length (%*AM*) infected or not by *Glomus mosseae* (±*AM*), shoot dry weight (*SDW*) and percentage P (%*P*) in dry leaves of cotton grown in soil amended with or lacking Fenamiphos (*Nil*), harvested after 1, 3, 6 or 10 weeks

		% AM		SDW		% P	
		Fenamiphos	Nil	Fenamiphos	Nil	Fenamiphos	Nil
Week 1	+AM	4.17×11.79	0.84± 1.35	0.05±0.01	0.05±0.01		
	−AM			0.06±0.01	0.06±0.01		
Week 3	+AM	29.33±13.32	24.33±13.44	0.47±0.05[a]	0.39±0.04		
	−AM			0.43±0.08[a]	0.34±0.04		
Week 6	+AM	51.89± 2.97	50.36± 9.50	1.44±0.13[a]	1.24±0.09	0.50±0.09[b]	0.43±0.05[b]
	−AM			1.58±0.15[a]	1.27±0.09	0.36±0.06	0.33±0.04
Week 10	+AM	44.60± 3.03	41.38± 4.69	2.76±0.24	2.48±0.14	0.47±0.08[b]	0.50±0.06[b]
	−AM			2.92±0.20[a]	2.55±0.25	0.36±0.05	0.37±0.05

[a] Significantly different ($P<0.05$) to control
[b] Significant difference ($P<0.05$) between +AM and −AM treatments

cides can affect AM but, due to lack of the appropriate controls, the effects of pesticides on the plant, fungus and the association could not be separated (Gnekow and Marschner 1989; Nemec 1980, 1985; Chambers et al. 1979; Menge et al. 1979).

Terraclor did not affect cotton growth or mycorrhizal infection to the extent previously reported; only a transient detrimental effect on mycorrhizal infection was observed. PCNB was shown previously to have a detrimental affect on shoot growth and mycorrhizal infection in a number of species (Gnekow and Marschner 1989; Saleh and Sikora 1988; Menge et al. 1979), but at applied PCNB rates much higher than those in this study.

Terrazole reduced mycorrhizal infection, uptake of P and growth of cotton plants during the first 4 weeks but this effect was not apparent after 6 weeks. Walker and Smith (1984) showed that the level of infection in the first 3 weeks of growth is directly proportional to the density of mycorrhizal propagules present in the soil. Although Terrazole may transiently reduce the germination of propagules, hyphal elongation or development of infection points during the initiation of mycorrhizas, the effect does not appear to influence spread of the fungus in the roots of cotton. Menge et al. (1979) applied 12.5 mg Ethazole (Terrazole) to a 500-ml pot filled with soil at the time of planting with mycorrhizal or non-mycorrhizal sudan grass seedling and 45 days later. The plants were harvested 105 days after sowing. Mycorrhizal infection and the production of mycorrhizal spores were unaffected by the application of Terrazole at the time of planting. When applied 45 days after sowing both mycorrhizal infection and the number of spores produced by the fungus were enhanced by Terrazole. Approximately 52 mg of Terrazole was applied to each pot of 2 kg soil in the present study but we observed no significant difference in mycorrhizal infection after 6 weeks. These conflicting results may be due to differences in the concentration of active pesticide used, the timing of application or the harvest time. The results of both experiments suggest that Terrazole applied prior to sowing has no effect on plant growth, mycorrhizal infection, or uptake of P after 6 weeks.

Fenamiphos had no significant effect on the mycorrhizal association, but other unexpected effects were observed. The positive growth response of cotton to the application of the nematicide may be a direct or indirect response. Plant growth increases following addition of nematicide have been reported previously and few attributed the increases to a direct effect on the plant. As Fenamiphos can completely inhibit nematode maturation (Sipes and Schmitt 1989; Johnson et al. 1986), it is not surprising that addition of Fenamiphos significantly increases plant yield in nematode-infested soils (Lawrence et al. 1990; Johnson et al. 1986; Abawi and Mai 1983). Increased yield of cabbages in Fenamiphos-treated plots has also been observed in field soils free of nematodes, in the field and greenhouse (Abawi and Mai 1983). These authors found no significant yield increase in Fenamiphos-treated plants grown in autoclaved soil and concluded that Fenamiphos controlled a "soil-borne biotic agent" in the field soil not present in autoclaved soil.

Fenamiphos is systemic within the plant (Loffredo et al. 1991) and has been demonstrated to significantly reduce populations of above-ground arthropods, such as thrips and leaf hoppers (Lentz et al. 1983). The possibility that systemic insecticidal activity of Fenamiphos may have increased yield was discounted by Abawi and Mai (1983). Although we had difficulties controlling aphid and thrip infestation, the growth room was free of foliar arthropods except mites. Mites were observed on plant leaves and symptoms of mite infestation were noted during the experiment. Three-month-old cotton plants present in the growth room at the initiation of the experiment had obvious damage and could have been a source of mites. Thus it is possible that Fenamiphos reduced mite infestation of seedlings, resulting in faster growth rates of plants in Fenamiphos-treated soil.

This report highlights some of the problems associated with studying the effects of pesticides on mycorrhizal associations. The rate of application of a given pesticide should be determined with care; the rate should be related to the potential field use of the pesticide and at least one of the concentrations applied should be the recommended rate. The length of the experiment and the number of harvests are also important. Harvests within the first 3 weeks indicate pesticide effects on the propagules of the mycorrhizal fungus, their germination and elongation, and on the initiation of infection. Later harvests indicate pesticide effects on development of infection and formation of fungal propagules. Thus, the use of appropriate controls allows determination of pesticide effects on individual components of the mycorrhizal association. Furthermore, the apparent interaction between Fenamiphos and mites shows that non-target organisms may be relevant for interpretation of data. This problem was recognised previously (Trappe et al. 1984) and is particularly important in field studies.

The application of Terrazole, Terraclor and Fenamiphos probably have no long-term detrimental effects on mycorrhizal infection and growth of cotton seedlings. The use of Fenamiphos may give additional protection against foliar arthropods due to its systemic action.

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