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Influence of selected pesticides on *Glomus* species and their infection in citrus*

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Summary Four sterol inhibiting fungicides, two general biocides, and three nonfumigant nematicides were tested for their activity against the mycorrhizal fungi, *Glomus intraradices* or *G. mosseae*. Of the four fungicides, propiconazole was the most inhibitory and triforine the least. These fungicides act systemically, and directly on the fungus in soil. One of the biocides, methylenebis-thiocyanate was toxic to *G. mosseae*, the other Bis-bromoacetoxy-2-Butene was not. All nematicides, aldicarb, fenamiphos and fensulfothion, had little or no inhibitory effect on the fungi.

Introduction

The benefits provided by vesicular-arbuscular mycorrhizal (VAM) fungi for plant growth raises concern for their survival and preservation in soils. Their conservation in soils covers many aspects of agricultural crop production and management programs for soils. This concern includes maintaining populations after strip-mining in western range lands[10,14], modifying populations through crop rotation[12,15], preventing reduction in infection through excessive use of fertilizers high in phosphorus and nitrogen[3,4]; the reduction of these fungi in eroded soils[5], and inhibition of infection and reduced populations caused by pesticides[2,9,17].

The reduction of VAM fungi in agricultural soils following pesticide applications is probably the most serious mismanagement practice of those known. It is also one that can be rectified through use of safer pesticides. More than 32 fungicides, 8 fumigants and nematicides, and 14 herbicides have been evaluated in various tests with these fungi (ref. 9 and Nemec, unpublished information) and many of them have been found to be safe. This body of information needs to be continually expanded to serve as a basis for recommending alternative pesticides in situations where mycorrhizal associations are critical.

Materials and methods

Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe was tested with the fungicides triforine and propiconazole; the nematicides, phenamiphos and aldicarb; and the general biocides, methylenebis-thiocyanate and Bis-bromoacetoxy-2-Butene. The nematicide-insecticides, fenamiphos and fensulfothion, were tested with *G. intraradices* (Schenck and Smith), as well as the fungicides plifenate and triadimefon. All pesticides were tested at three rates each.

Chlamydospores of the *Glomus* species were produced on either citrus or sudangrass

* This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation by the U.S. Department of Agriculture nor does it imply registration under FIFRA.

(*Sorghum bicolor* var. *sudanese* (Piper) Stapf.). Astatula fine sand subsoil (hyperthermic, uncoated typic quartzipsamments, 94–97% sand, 0.25% organic matter) low in phosphorus (< 9–12 ppm P) was steam-pasteurized and mixed with pot culture inoculum of 700 spores per 15 cm clay pot in all tests, except the aldicarb-fenamiphos test in which 500 spores per pot were used. Chlamydospores and soil were mixed in a cement mixer; plifenate and triadimefon were applied in a spray to the soil during mixing; fenamiphos, fensulfothion and aldicarb were applied as granules to the soil during mixing; and the remaining pesticides were applied as drenches to the soil after it had been added to pots.

Treated chlamydospore-amended soil was put in seven replicate pots per treatment on a greenhouse bench. All pesticides were applied only once to the potting soil, and each experiment was terminated after 5 to 8 months. Inoculated but untreated soil, untreated soil, untreated soil amended with 210 ppm phosphorus, and peticide-treated but uninoculated soil served as controls.

Four sour orange (*Citrus aurantium* L.) seedlings were grown in each pot. All seedlings were fertilized monthly with a 12–0–6 solution containing urea as the nitrogen source and minor elements as prescribed by Hoagland and Arnon[6]. At harvest, plant height, top fresh weight, or fresh root weight were determined. Height was reported as mean individual plant height, and top and root weight were determined by pooling all plants per pot.

Chlamydospore numbers were determined by wet sieving 25 g of soil from each pot at the termination of the tests. Vesicle production and hyphal development in fibrous roots were determined in selected tests. Roots from each plant in each pot were pooled and chopped into 1 cm long pieces. They were stained with hot acid fuchsin in lactophenol, destained in lactophenol, and 30 pieces per pot were rated for the presence of vesicles and hyphae on a scale of 0–3. For vesicles, 0 indicated none, 1 = 1–50, 2 = 51–100, and 3 = more than 100. For hyphal development, 0 = none; 1 = light, 2 = moderate; and 3 indicated extensive development.

Results

Triforine at all rates had no significant effect on mycorrhizal plant growth nor on spore production; in contrast, propiconazole at all rates retarded growth of inoculated plants and production of chlamydospores (Table 1).

Only one of the general biocides, methylenebis-thiocyanate, inhibited mycorrhizae synthesis by *G. mosseae*. This inhibition was not overcome by its potential to stimulate plant growth as exhibited in the noninoculated treatments (Table 1). The low and medium rates of Bis-bromoacetoxy-2-Butene stimulated the plant growth response due to *G. mosseae* (Table 1).

Aldicarb had no depressing effect on the symbiosis between the host and *G. mosseae* (Table 2). Fenamiphos had a slight depressive effect on *G. mosseae* induced plant growth (Table 2); and similar results occurred with the medium rate in the test with *G. intraradices*. A similar effect appeared to occur with the medium and high rates of fensulfothion (Table 3).

The sterol-inhibiting compounds plifenate and triadimefon both caused a slight depression of mycorrhizal root development at the low and medium rates, but all rates significantly reduced infection (Table 3).

Discussion

Propiconazole, the most inhibitory of the fungicides, is a broad-spectrum, systemic triazole derivative effective in controlling plant pathogens in the ascomycetes, basidiomycets and deuteromycetes. Its effect on VAM probably would cause stunting effects on mycorrhizae-sensitive plants in the field. Triadimefon in this study was inhibitory to the fungus and the development of roots; and triforine had no significant effect on the fungus and on plant

Table 1. Growth of *Citrus aurantium* and chlamydospore production by *Glomus mosseae* in inoculated soils, and growth of *C. aurantium* in noninoculated soils after soil treatment with triforine, propiconazole, methylenebis-thiocyanate and Bis-bromoacetoxy-2-Butene

		G. mosseae treatments			Noninoculated treatments	
Fungicides and biocides	Rate (kg/ha)	Top wt (g)	Root wt (g)	Chlamydospores no./25 g	Top wt (g)	Root wt (g)
Test No. 1						
Triforine	0.56	12.9^{ns}	12.3^{ns}	177^{ns}	6.0^{ns}	9.4***
Triforine	1.12	10.4^{ns}	11.5^{ns}	114^{ns}	6.1^{ns}	8.8^{ns}
Triforine	1.68	9.2^{ns}	11.1^{ns}	62^{ns}	7.7^{ns}	10.6^{ns}
Propiconazole	0.56	5.4***	8.1*	0***	6.2^{ns}	10.0**
Propiconazole	1.12	5.7***	9.0^{ns}	8***	6.2*	9.1*
Propiconazole	1.68	5.2***	8.8*	5.7***	5.2^{ns}	8.5*
Control, inoculated		11.6	11.1	66.3	–	–
Control, noninoculated		–	–	–	5.4	7.5
Test No.2						
Methylenebis-thiocyanate	2.8	3.9***	6.7***	0***	15.9*	26.8^{ns}
Methylenebis-thiocyanate	5.6	4.3***	6.7***	2**	18.9***	28.5*
Methylenebis-thiocyanate	11.2	4.4***	8.2***	1**	19.0***	32.6**
Bis-bromoacetoxy-2-Butene	2.8	12.3^{ns}	19.0*	96*	11.9^{ns}	23.4^{ns}
Bis-bromoacetoxy-2-Butene	5.6	14.2*	21.4**	115***	11.7^{ns}	22.2^{ns}
Bis-bromoacetoxy-2-Butene	11.2	7.9^{ns}	11.7^{ns}	53^{ns}	10.7^{ns}	20.2^{ns}
Control, inoculated		9.6	13.8	53	–	–
Control, noninoculated		–	–	–	10.7	19.5

All treatments consisted of 7-pot replicates, 4 seedlings per pot. Dunnett's trest used to compared *G. mosseae* treatment means with inoculated control, and noninoculated treatment means with the noninoculated control, $*P = 0.05$, $**P = 0.01$, and $***P = 0.001$, and ns = not significant. All noninoculated treatments and the noninoculated control of test 2 were grown in sand amended with 210 ppm phosphorus.

Table 2. Growth of *Citrus aurantium* and chlamydospore production by *Glomus mosseae* in inoculated soils and growth of *C. aurantium* in noninoculated soils after soil treatment with fenamiphos and aldicarb

		G. mosseae treatments				Noninoculated treatments		
Nematicides	Rate (kg/ha)	Plant ht (cm)	Top wt (g)	Root wt (g)	Chlamydospores no./25 g	Plant ht (cm)	Top wt (g)	Root wt (g)
Fenamiphos	5.6	10.6*	8.8*	13.5^{ns}	142*	6.3^{ns}	3.9^{ns}	5.9^{ns}
Fenamiphos	11.2	12.1*	13.3^{ns}	20.1^{ns}	251^{ns}	5.7^{ns}	3.1^{ns}	5.7^{ns}
Fenamiphos	22.4	17.8^{ns}	17.4^{ns}	22.2^{ns}	384^{ns}	5.9^{ns}	3.2^{ns}	5.0*
Aldicarb	5.6	15.7^{ns}	15.7^{ns}	20.3^{ns}	230^{ns}	6.1^{ns}	3.5^{ns}	5.7^{ns}
Aldicarb	11.2	15.3^{ns}	13.5^{ns}	19.9^{ns}	408^{ns}	6.1^{ns}	3.3^{ns}	4.8*
Aldicarb	22.4	14.3^{ns}	14.5^{ns}	18.7^{ns}	373^{ns}	6.8^{ns}	3.8^{ns}	5.4^{ns}
Control, inoculated		15.1	14.4	17.7	461	–	–	–
Control, noninoculated		–	–	–	0**	6.2	3.2	7.5

All treatments consisted of 7-pot replicates, 4 plants per pot. Dunnett's test used to compare *G. mosseae* treatment means with inoculated control, and noninoculated treatment means with the noninoculated control. $*P = 0.05$, $**P = 0.01$, and ns = Not significant.

Table 3. Growth of *Citrus aurantium*, and hyphal development, vesicle formation, percentage of infection and chlamydospore production by *Glomus intraradices* in inoculated soils, and growth of *C. aurantium* in noninoculated soils after soil treatment with fenamiphos, fensulfothion, plifenate and triadimefon

		G. intraradices treatments						Noninoculated treatments	
Nematicides	Rate (kg/ha)	Top wt (g)	Root wt (g)	Vesicles	Hyphae	Infection (%)	Chlamydospores no./25g	Top wt (g)	Root wt (g)
Test No. 1									
Fenamiphos	5.6	14.9^{ns}	8.6^{ns}	0.22^{ns}	0.13^{ns}	17.1^{ns}	256^{ns}	14.4^{ns}	8.8^{ns}
Fenamiphos	11.2	10.0*	9.3^{ns}	0.01**	0.01**	1.4**	508*	18.4^{ns}	11.3^{ns}
Fenamiphos	22.4	13.4^{ns}	10.0^{ns}	0.13^{ns}	0.07^{ns}	10.9^{ns}	341^{ns}	14.9^{ns}	8.7^{ns}
Fensulfothion	11.2	13.1^{ns}	9.1^{ns}	0.02**	0.00**	2.3**	801^{ns}	16.5^{ns}	10.4^{ns}
Fensulfothion	22.4	10.6**	9.2^{ns}	0.22^{ns}	0.14^{ns}	16.1^{ns}	430^{ns}	18.4^{ns}	11.3^{ns}
Fensulfothion	33.6	10.3**	8.3^{ns}	0.10^{ns}	0.05^{ns}	9.0^{ns}	569*	15.6^{ns}	11.8^{ns}
Control, inoculated		14.1	9.4	0.29	0.22	23.3	206	–	–
Control, plus phosphorus noninoculated		–	–	0.00**	0.00**	0.00**	0***	16.6	10.1
Control, low phosphorus noninoculated		5.1***	5.9*	0.00**	0.00**	0.00**	2***	–	–
Test No. 2									
Plifenate	4	10.8^{ns}	8.3*	0.03**	0.01***	2.4***	391^{ns}	5.1^{ns}	8.2^{ns}
Plifenate	8	11.1^{ns}	8.8*	0.13*	0.04***	9.5***	373^{ns}	5.5^{ns}	7.9^{ns}
Plifenate	12	9.9^{ns}	9.9^{ns}	0.01**	0.004***	1.4***	618^{ns}	7.2^{ns}	8.7^{ns}
Triadimefon	4	8.9^{ns}	7.8**	0.01**	0.00***	1.4***	373^{ns}	5.1^{ns}	6.4*
Triadimefon	8	9.3^{ns}	8.1*	0.08*	0.16**	8.5***	270^{ns}	4.6***	6.4^{ns}
Triadimefon	12	9.6^{ns}	8.8^{ns}	0.04**	0.02***	2.8***	246^{ns}	5.1^{ns}	6.8^{ns}
Control, inoculated		13.3	11.6	0.33	0.39	44.3	473	–	–
Control, noninoculated		–	–	–	–	–	–	5.4	7.5

All treatments consisted of 7-pot replicates, 4 seedlings per pot. Vesicle and hyphae ratings range from 0–3. Dunnett's test used to compare *G. intraradices* treatment means with the inoculated control and noninoculated treatment means with the noninoculated control. $*P = 0.05$, $**P = 0.01$, $***P = 0.001$, and ns = not significant. Noninoculated treatment soil and phosphorus-amended control soil of test No. 1 were amended with 210 ppm phosphorus.

development, perhaps because it may have been partially bound to the soil surface. Both triforine and triadimefon applied as foliar sprays to mycorrhizal wheat reduced the number of chlamydospores produced in soil by 50% or more[7] and triadimefon inhibited infection by *Glomus* spores soaked in the fungicide or when mixed in inoculum-amended soil[16]. These sterol-inhibiting fungicides are known to inhibit the biosynthesis of ergosterol[8] in fungi, and this is the suggested mechanism of action in mycorrhizal species. Campestrol comprised 96% of the sterol isolated from *G. mosseae* chlamydospores, thus the sterol-inhibiting characteristics of these fungicides may be nonspecific[11]. The action of plifenate on the plant and fungus was very similar to the results obtained with triadimefon.

Of the two general biocides tested, only methylenebis-thiocyanate exhibited biocidal activity to *G. mosseae*. This chemical is commonly used as a microbiocide in industrial processing waters and has been shown to be an effective, broad-spectrum soil fungicide in irrigation water[13].

Aldicarb in this study had no inhibiting effect on the symbiont and host. Fenamiphos

effects were irregular in both experiments in which it was used. Slight inhibition occurred at medium and low rates, and this type of activity is difficult to explain. Fensulfothion at medium and high rates had a significant effect on top weight, but no effect on the fungus. It is possible that was due to an early temporary fungistatic effect and that infection occurred later in the course of the test. Of these nonfumigant systemic nematicides, information on their interaction with VAM is provided by only a few other studies. In an aldicarb plus oxamyl treatment and fenamiphos plus oxamyl treatment, the number of Glomus chlamydospores did not differ from those of the control[1]. Also, in pot studies with onion, aldicarb caused only a slight decrease in percentage of root length infected, but in field tests it had no effect on the fungus[16].

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