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RESEARCH ARTICLE

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Influence of the insecticide dimethoate on arbuscular mycorrhizal colonization and growth in soybean plants

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Summary Application to the soil of the insecticide dimethoate had no effect on the growth of soybean colonized by the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* and by the indigenous AM fungus. The application of the recommended concentration of dimethoate decreased the percentage of colonization of soybean by the indigenous AM population, but no significant effect was observed on the colonization of soybean inoculated with *G. mosseae*. The insecticide did not affect the germination of *G. mosseae* spores; however, 0.5 mg/l of dimethoate increased the germination of *Gigaspora roseae* and 5 mg/l of dimethoate decreased the germination of *Scutellospora castaneae* spores.

Key words *Gigaspora roseae* · *Glomus mosseae* · *Glycine max* · *Scutellospora castaneae* · Dimethoate

Introduction

Arbuscular mycorrhizal (AM) symbioses are widespread throughout the plant kingdom. They benefit the host plant primarily by increasing the capability of the root system to absorb and translocate phosphorus through an extensive network of external hyphae [2].

The use of pesticides to control diseases is a fundamental component of soybean crop production. However, despite the frequent application of pesticides, information about their effect on plant growth and on nontarget microorganisms such as AM fungi is limited [12]. Some insecticides have not effect on AM symbiosis or to enhace it. While, high doses of other insecticides decreased plant root colonization [16, 17]. Dimethoate is an organic-phosphorated, systemic insecticide used to control several soybean pests such as *Nezara viridula* and *Piezodorus guildinii* [9].

The present study was designed to determine the influence of dimethoate on the formation and functioning of AM in soybean plants.

Materials and methods

Influence of dimethoate on soybean plants The experiments were performed in 300-ml pots of soil collected from the province of Buenos Aires. Half of the soil (Argiudol type), pH 5.4, 2.28% C containing (ppm) 331 N, 9.5 P and 3.2 Ca, was steam-sterilized and mixed 1:1 (v/v) with sterilized quartz sand. The mixture was brought to field capacity (140 ml) with an aqueous suspension of dimethoate (dioxydimethyldithiophosphorylacetic acid N-monomethyl amide) from Camani SA (38% active ingredient) at a concentration of 0.012, 0.12 and 12 g/l equivalent to 0.38, 3.8 and 38 kg/Ha (agronomical doses). Soybean plants (*Glycine max* cv. Nidera) were grown in a greenhouse with a day/night cycle of 25/19°C and 50% relative humidity. Plants were watered from below.The sporocarps and spores were isolated by the wet-sieving technique [4] and identified as *Glomus mosseae* (Nicol. & Gerd.) according to Gerdemann and Trappe [5]. One hundred *G. mosseae* spores were inoculated to alfalfa plant pots. These plants were cultured for 4 months until their roots became well colonized (80% root length) and new *G. mosseae* spores were developed (41 spores/g of soil). The AM inoculum consisted of 5 g of rhizosphere soil from the alfalfa plant pot cultures which contained spores, mycelium and colonized root fragments. Half of the sterilized and nonsterilized pots were inoculated with *G. mosseae*. Uninoculated plants were given filtered leachings from the inoculum soil. Soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of mycorrhizal plants was added to the AM uninoculated treatment. The filtrate contained common soil microorganisms, but no propagules of

Table 1 Effect of dimethoate on the percentage of root length colonization and on the shoot, root and total dry weight (mg) of soybean (*Glycine max*) grown in nonsterilized soil and in sterilized soil inoculated or uninoculated with *Glomus mosseae*

Amount of dimethoate applied (g/l)	Root length colonization		Shoot dry weight (g)		Root dry weight (g)		Total dry weight (g)	
	M+	M–	M+	M–	M+	M–	M+	M–
Sterilized								
0	12.3	0	0.70	0.71	0.23	0.25	0.93	0.96
0.012	15.3	0	0.86	0.70	0.24	0.25	1.10	0.95
0.12	11.2	0	0.83	0.86	0.23	0.22	1.06	1.08
1.2	17.4	0	0.79	0.88	0.24	0.36	1.03	1.24
Nonsterilized								
0	25.6	27.2	0.72	0.75	0.28	0.24	1.00	0.99
0.012	13.2	30.6	0.81	0.67	0.20	0.21	1.01	0.88
0.12	16.9	23.9	0.86	0.71	0.23	0.22	1.09	0.93
1.2	14.5	9.8 *	0.77	0.77	0.24	0.18	1.01	0.96

Each figure is the mean of eight replications.
* = Significantly different at the 0.05 level.
M+ = Inoculated with *G. mosseae*, M– = Noninoculated with *G. mosseae.*

G. mosseae. Pots inoculated or not with the endophyte and without insecticides were used as controls.

Harvest was carried out 8 weeks after sowing. Part of the root system was cleared and stained [13], and the percentage of root colonization was measured [6]. There were 8 replicates per treatment.

Effect of dimethoate on spore germination The effect of the insecticide on germination of *G. mosseae*, *Scutellospora castanea* (BEG 13) and *Gigaspora roseae* (BEG 9) spores was tested in vitro on 1% sterile water agar (Difco-Bacto) plus 10 mM 2-(N-morpholin) ethane sulfonic acid (MES) to maintain the pH of the medium at 7 throughout the experiment. This buffer was previously shown not to affect germination of AM fungal spores in vitro [10]. The insecticide was added to the agar of each Petri dish at a concentration of 0.5 or 5 mg/l (agronomical doses) equivalent to that applied to each pot of the experiment described above. Five to eight surface-desinfected spores per plate were placed in each Petri dish [11]. Eight replications of each insecticide doses and controls were used. The plates were incubated at 25°C and spore germination was determined after 20 days of incubation for *G. mosseae*, 7 days for *S. castaneae* and 3 days for *G. roseae* under a light microscope.

Experimental design and statistics The experiment was designed as a 2 3 2 3 4 factorial, with soil sterilization, AM colonization and insecticide dose rate as factors. Pots were arranged in a completely random manner. The main effects were due to (i) inoculation with *G. mosseae* (M+) or its absence (M–); and (ii) insecticide application at the rates of 0, 0.1, 0.5 and 1 times the agronomical dosis. Data obtained for dry weight parameters were subjected to three-way analysis of variance, using insecticide concentration, inoculation with *G. mosseae* and sterilization as the factors. Data obtained for percentage colonization of root and germination of spores were arcsine-transformed and subjected to a two-way analysis of variance. The means were compared by Tukeys test at the 5% level.

Results and Discussion

The results from the three-way analysis of variance showed that there were significant interactions between concentration of the insecticide and inoculation with *G. mosseae* in the root, shoots and total dry weight of plants. Therefore, these interactions were analyzed further.

The kind of AM inoculum present in the soil can determine the efficiency of the fungus [8]. However, neither *G. mosseae* nor the indigenous AM fungi, were able to influence soybean plant growth regardless of the presence or absence of dimethoate. Relatively low percentages of root colonization were observed in control treatments without insecticide. However, low or undetectable levels have been found during vegetative growth in soybean plants, followed by a rapid increase during host reproduction [1]. The addition of the recommended concentration of dimethoate to plants cultivated in soil not sterilized and not inoculated with *G. mosseae* decreased the percentage of soybean root length mycorrhization. However, nonsignificant differences in the percentage of AM of plants colonized by *G. mosseae* were observed with all doses of insecticide used (Table 1).

Indigenous endophytes were as effective as *G. mosseae* in root colonization, but were more sensitive to the action of dimethoate. As has been found with other pesticides, variations in their effect upon mycorrhizal fungi may be attributed to the mycorrhizal species involved [3, 15]. These results indicate that some species of the indigenous fungi, like *S. castaneae*, were sensitive to the action of the insecticide (*Scutellospora* spp.

Table 2 Effect of dimethoate on the percentage of germination of *Glomus mosseae*, *Scutellospora castaneae* and *Gigaspora roseae* spores on water-agar

Amount of dimethoate applied (mg/l)	Percent spore germination		
	G. mosseae	*S. castaneae*	*G. roseae*
0	33	49	16
0.5	31	40	43*
5	26	22*	23

Each figure is the mean of eight replications.
* = Significantly different at the 0.05 level.

was observed in this soil, data not shown). In fact dimethoate did not affect the germination of *G. mosseae* spores. However, the application of 0.5 mg/l dimethoate increased the germination of *G. roseae*, and 5 mg/l of the insecticide decreased the germination of *S. castaneae* spores (Table 2). It is known that some organic acids and sugars inhibit spore germination of some species but stimulates hyphal growth of others [14]. This finding may explain the different effects of the same insecticide or group of insecticides on AM colonization of plant roots [7, 16].

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