Increased Endomycorrhizal Infection of Maize and Soybeans After Soil Treatment and Metalaxyl

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ABSTRACT

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Soil incorporation of metalaxyl increased the vesicular-arbuscular mycorrhizal (VAM) infection of maize roots from 57 to 72% after 30 days in greenhouse experiments. Infestation of soil with inoculum of *Glomus fasciculatum* also increased VAM infection frequency but had no additional effect in combination with metalaxyl. In a 90-day experiment with maize, metalaxyl was associated with increased VAM infection after 30 and 60 days, but after 90 days, the differences in VAM infection between metalaxyl and control treatments were insignificant. Spore production by VAM fungi was not significantly affected. Metalaxyl soil treatment was also related to an increase in VAM infection of soybean roots.

The importance of vesicular-arbuscular mycorrhizal (VAM) associations for growth and mineral nutrition of plants has been demonstrated thoroughly (4,5,9). Many fungicides (eg, dicloran, captan, benomyl, dazomet, and PCNB), when added to soil, have decreased VAM infection of many different plant species (3,7,10). Thiram, PCNB, dicloran, chloronitropropane, and captan at 25, 50, and 100 mg/kg soil reduced root infection of maize by VAM fungi (11). Ethoprop and captan at certain concentrations, however, have had no significant effect on VAM infection (10). Sutton and Sheppard (14) reported an increase in VAM infection on beans (*Phaseolus vulgaris* L.) treated with a 0.5-mM captan soil drench, and Bird et al (2) found an increase in VAM infection in cotton (*Gossypium hirsutum* L.) planted in soil fumigated with the nematicide dibromochloropropane.

Metalaxyl is an acylalanine systemic fungicide that moves acropetally (within the apoplast) in the plant. It exhibits highly specific fungitoxicity toward some Oomycetes. Nemec (10) observed that sour orange seedlings had more VAM infection when grown in metalaxyl-treated soil than in untreated natural soil for 8 mo. Metalaxyl treatment did not increase vesicle number or chlamydospore production (10). Metalaxyl, however, has a half-life of only 30–125 days in most soils (CIBA-Geigy Corp., Greensboro, NC, *personal communication*). The purpose of this study was to assess the immediate responses of VAM fungi to metalaxyl and to study possible mycorrhizal-metalaxyl interactions with agriculturally important herbaceous plants.

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Mention of specific products or companies does not imply endorsement over similar products or companies. All uses of pesticides must be registered by appropriate state or federal agencies before they can be recommended.

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MATERIALS AND METHODS

Inoculum. Endomycorrhizal fungi used were *Glomus fasciculatum* Gerd. & Trappe and *G. mosseae* Gerd. & Trappe. *G. fasciculatum* inoculum used in the maize experiments was produced on maize plants grown in an autoclaved sand culture in the greenhouse. *G. mosseae* inoculum, used in the soybean experiment, was produced on sorghum plants. Inoculum was prepared by removing most of the plant roots and blending the remaining sand, which contained numerous spores of the VAM fungus. The sand-spore mixture was used as inoculum at the rate of 100 cm^3/pot. The inoculum control was the filtrate from 100 cm^3 of inoculum sieved through a 400-mesh sieve to remove VAM spores and added to 100 cm^3 of autoclaved sand.

Soil. Soil used was Spillville loam (pH 6.5; 40% sand, 40% silt, and 20% clay; 20 μg available P/g soil) collected in an uncropped grass area at the Hinds Experimental Farm near Ames, IA. Soil was processed through a soil shredder, then screened and stored in a moist condition in covered (not airtight) containers. It was mixed with washed sand (2:1, soil:sand) before use. Soil weight is expressed as oven-dry weight (24 hr at 103 C in a mechanical convection oven).

Soil temperature chamber. Soil temperature chambers, which we designed and built, maintained the roots in the soil at 19 ± 1 C and allowed the tops to be exposed to greenhouse conditions (20–30 C with 16 hr of supplemental fluorescent light). The chamber used temperature-controlled air to regulate soil temperature and allowed free drainage from 19-cm-diameter black polyethylene pots supported on racks in the chamber. The chamber was used in the short-term maize and soybean experiment.

Metalaxyl and VAM infection of maize. Metalaxyl was tested in a 2 × 3 factorial experiment with four replicates arranged in a randomized complete block design (RCBD). The 3 kg of soil in each pot was either infested with *G. fasciculatum* or treated with the VAM sporefree filtrate in sand (control). Metalaxyl was added to the soil surface at 0, 1.4, and 2.9 mg a.i./kg soil and incorporated into the top 8 cm. Two seeds of a single-cross hybrid (A619 × A632) were planted per pot and seedlings were thinned to one plant per pot after emergence. The plants were watered once with 10 ml of a 10 g/L solution of NH_4NO_3 plus 300 ml of distilled water. Thereafter, plants were watered on alternate days with distilled water. The experiment was harvested 30 days after planting. Plant height and top dry weight were recorded.

Prolonged exposure of maize to metalaxyl. Treatments were metalaxyl applied at the rate of 2.9 mg/kg soil and an untreated control. Each pot (12-L capacity polyethylene) contained 12 kg of soil. Three replicates were employed in a completely random design arranged on the greenhouse bench. Four grams of NH_4NO_3 and 1 g KNO_3 were dissolved in 100 ml of distilled water and added to each pot at the beginning of the experiment. Two maize seeds were planted in each pot and plants were watered on alternate days to near field capacity. Plants were thinned to one per pot after emergence. Root samples were collected 30 and 60 days after planting, and at the same time, metalaxyl was reapplied (2.9 mg/kg soil). After 90 days, plants were harvested and root and soil samples collected.

Metalaxyl and VAM infection of soybeans. This experiment was conducted using procedures similar to the 30-day maize experiment. The VAM fungus was *G. mosseae*. Three seeds of Corsoy

Table 1. Percent vesicular-arbuscular mycorrhizal infection of maize roots 30 days after the addition of *Glomus fasciculatum* inoculum and metalaxyl

Metalaxyl rate (mg/kg)	*G. fasciculatum*	
	Absent	Present
0.0	57[a]	75
1.4	62	70
2.9	72	73

[a] All values are means of four replicates.

Table 2. Percent vesicular-arbuscular mycorrhizal infection of soybean roots 30 days after the addition of *Glomus mosseae* inoculum and metalaxyl

Metalaxyl rate (mg/kg)	*G. mosseae*	
	Absent	Present
0.00	26[a]	25
0.92	25	36
2.40	33	34

[a] All values are means of five replicates.

soybeans were planted in each pot. Metalaxyl rates were 0, 0.92, and 2.4 mg a.i./kg soil. Five replicates were used and pots were arranged in a RCBD. The experiment was harvested after 30 days.

Assay for mycorrhizal infection. A random sample of living roots <1 mm in diameter was collected from the soil and cut into 1-cm segments. The roots were stained by the method of Phillips and Hayman (12). Fifty root segments from each sample were placed in lactophenol on glass slides, covered with coverglasses, and examined microscopically for VAM fungal structures. Infections by VAM fungi were expressed as the percentage of 1-cm root segments that were mycorrhizal. Estimates of spore production were made by direct counts of VAM spores extracted from a 100-cm^3 soil sample by using a centrifugal-flotation technique (6).

Statistics. All analysis of variance (13) computations were performed using SAS 76 programs (1) and the facilities of the Iowa State University Computation Center. A 5% significance level ($P = 0.05$) was used in all the tests.

RESULTS

Maize. All plants showed uniformly mild phosphorus deficiency symptoms. Treatments did not have a significant effect on top dry weight or plant height. Infections of roots by VAM fungi were significantly different among the treatments (Table 1). Inoculations with *G. fasciculatum* significantly increased VAM infection in the absence of metalaxyl. Metalaxyl increased the infection rate of VAM fungi only in the uninoculated controls. There were no significant interactions between inoculum and metalaxyl.

Prolonged metalaxyl exposure. Metalaxyl at 2.9 mg/kg significantly increased VAM infection of maize roots from 35% in the controls to 53% in the metalaxyl treatments at the end of 30 days. At 60 days after planting, the control plant roots had a mean VAM infection frequency of 41% and the metalaxyl-treated plants were significantly higher at 63%. At the end of the experiment, the controls had a mean VAM infection of 74% and treated plants a mean of 79%. VAM chlamydospore concentration in the soil at the end of the experiment was 2.8/cm^3 for the control and 3/cm^3 for the metalaxyl-treated soil. There were no significant differences between control and metalaxyl treatments for either percentage VAM infection or spore production at the end of the experiment.

Soybeans. Results from the soybean experiment differed only slightly from the initial maize experiment. All plants appeared healthy. Addition of metalaxyl at 2.4 mg/kg increased infection of soybean roots by VAM fungi, and where inoculum of *G. mosseae* was also applied, both rates of metalaxyl significantly increased VAM infection (Table 2). In treatments without metalaxyl, fewer plants per pot germinated and survived until harvest than in pots with metalaxyl treatments. Plant height was not affected by the treatments, but both the metalaxyl treatment and the mycorrhizal inoculum treatment significantly increased the average top dry weight compared with the uninoculated, no-metalaxyl treatment.

DISCUSSION

Most soil applications of pesticides, especially fungicides, have resulted in decreased VAM infections (3,7,8,11). Conversely, in our experiments, applications of metalaxyl at realistic usage rates resulted in increased VAM infections of maize and soybean roots, or at least, they did not reduce VAM fungal infections. These results agree somewhat with the results of Nemec (10), who found an increased VAM infection of sour orange roots 8 mo after metalaxyl was used and probably long after any significant amount of the chemical remained in the soil. During our 3-mo experiment, metalaxyl increased VAM infection of maize roots during the first 2 mo, but at 3 mo, differences among treatments were nil. Failure to detect differences after 3 mo may be a reflection of the plant growth stage (maize plants were nearing maturity) or the root growth habits. The sour orange roots in Nemec's (10) experiment cannot be compared with the physiological stage of the maize root in our 3-mo experiment.

Two other chemicals that have been associated with increased VAM infection are dibromochloropropane (2) and captan (14). These compounds and metalaxyl have very little in common, and all probably decreased competition from other root-infecting microorganisms rather than affecting the VAM fungi directly.

Metalaxyl used as a soil treatment will probably not affect VAM infection adversely and may increase it. Effectiveness of mycorrhizal infection of plant roots may be enhanced with metalaxyl. Metalaxyl may also aid in production of VAM inoculum in pot culture by reducing the threat of certain contaminating plant pathogens. When benefits of metalaxyl soil treatments are assessed, they usually are in terms of disease control; however, one must not dismiss the beneficial interaction of metalaxyl with plants and VAM fungi as presented in this paper.

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