Reduction of the Effects of Pathogenic, Root-Infecting Fungi on Soybean by the Mycorrhizal Fungus, *Glomus mosseae*

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ABSTRACT

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Soybean growth responses to *Glomus mosseae* in combination with either *Macrophomina phaseolina, Rhizoctonia solani*, or *Fusarium solani* were studied using autoclaved and/or nonautoclaved field soil (91 ppm P and pH 6.8) in a greenhouse with air temperatures varying from 25–35 C. After 45 days, plants in autoclaved soil containing either *M. phaseolina, R. solani*, or *F. solani* had significantly less root weight, shoot weight, or plant height than control plants in soil not infested with either *G. mosseae* or the pathogens. Plants exposed to either *M. phaseolina, R. solani*, or *F. solani* in autoclaved and nonautoclaved soil had 20–30% and 10–16% less seed weight, respectively, than control plants. *G. mosseae* did not affect the incidence of infection of soybean by the pathogens but the pathogens significantly reduced (average 38%) root colonization by *G. mosseae* in autoclaved soil. The addition of *G. mosseae* to autoclaved soils with and without the pathogens significantly increased plant growth responses. Plants with *G. mosseae* had seed yields increased 50 and 15% in autoclaved and nonautoclaved soil, respectively, over that of comparable control plants without *G. mosseae*. Significant correlations occurred between the percentage of roots colonized by *G. mosseae* and root weight, shoot weight, and plant height. The correlation between seed weight and plant growth responses at 45 days was also significant. Although the incidence of infection by the pathogens was about the same in mycorrhizal and nonmycorrhizal plants, plants colonized by *G. mosseae* appeared to tolerate this infection by the pathogens better than did nonmycorrhizal plants.

Additional key words: *Glycine max*, microorganism interaction, vesicular-arbuscular mycorrhizae.

Vesicular-arbuscular (VA) mycorrhizal fungi are of great value in promoting phosphorus uptake, plant growth, and yield of cultivated crops (1,5,8,9,15,17). Furthermore, VA mycorrhizae can influence the severity of disease in several plant host-pathogen combinations (19). The interactions involving VA mycorrhizae and root-infecting fungi seem to vary with the species of mycorrhizal fungi and with plant cultivars (19).

There are several reports of effects on plant growth and seed yield of soybean (*Glycine max* (L.) Merr.) by VA mycorrhizal fungi (1,14,15,18). The effect of mycorrhizal fungi on *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kuhn or *Fusarium solani* (Mart.) App. and Wr. emend. Snyd. and Hans. in the

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rhizosphere of soybean is not known. This study was undertaken to evaluate the effect of *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe on infection of soybean by *M. phaseolina, R. solani*, and *F. solani* and on resultant plant growth responses and seed yield.

MATERIALS AND METHODS

The isolates of the pathogenic fungi used in this study, the procedures for inoculum production, storage, and the quantitative population estimates of the organisms as well as the greenhouse conditions for the experiments were those described by Zambolim et al (21).

Glomus mosseae was isolated from soybean in Florida and was maintained in pot culture in a greenhouse. The isolate was chosen for its rapid colonization of soybean roots in preliminary tests with other species of VA mycorrhizal fungi.

The chlamydospores of *G. mosseae* were produced on roots of soybean and bahiagrass (*Paspalum notatum* Flugge) plants grown in autoclaved field soil in 15-cm-diameter pots in a greenhouse. After 4 mo, the chlamydospores were extracted from the soil by using the wet-sieving and decanting method (4), followed by high-speed centrifugation in a 1.3 M sucrose solution for 90 sec. The chlamydospores were then collected on a 62.8 μm (240-mesh) sieve, backwashed into Ringer's solution, and stored in a refrigerator at 5 C. Numbers of spores of *G. mosseae* in each treatment were estimated by extracting spores, as described above, from a 25-g subsample of a composite soil sample of three 2.5-cm-diameter cores from each of ten replicates. The number of spores in each subsample were counted in 15-cm-diameter petri dishes under a dissecting microscope (×40).

Arredondo fine sand collected from a soybean field was used throughout this study. After all of the collected soil was thoroughly mixed in a cement mixer, a portion of it was stored at 4–6 C without any treatment, and part was autoclaved twice for 4 hr at 24-hr intervals at 121 C. The nutrient contents of the soil (pH 6.8) were 91.6 ppm P, 42 ppm K, 450 ppm Ca, 108 ppm Mg, 27 ppm NO_3, 384 ppm Al, 13.6 ppm Fe, and 80 ppm Zn.

A random 2.0-g sample of clean soybean root sections taken from each of 10 plants per treatment was cleared and stained by the method of Phillips and Hayman (12). In each sample, the number of root pieces with either vesicles, arbuscules, or surface mycelium of the endomycorrhizal fungus were counted under a dissecting microscope (×40). The percentage of root colonization was obtained by dividing the total number of colonized root sections by the total number of root sections examined and multiplying the quotient by 100.

Soil was infested with known numbers of propagules of each pathogen and mixed thoroughly with an electric model N-50 Hobart mixer (Hobart Manufacturing Company, Troy, OH 4361). Water was added to the soil during the mixing process to give a final water content of 10% (w/w), a water potential of approximately −75 mbar.

The inoculum density of *G. mosseae* used throughout this investigation was 500 chlamydospores per 15-cm-diameter clay pot containing 2 kg of soil. In preliminary tests, this level consistently resulted in moderate root colonization. Chlamydospores were distributed in one layer 5.0 cm below the soil surface. Two soybean seeds of cultivar Hood after surface sterilization in 0.5% sodium hypochlorite and inoculation with *Rhizobium japonicum* were placed 2.5 cm above the mycorrhizal fungus inoculum.

Autoclaved soil was used in the first part of this study. The inoculum densities per gram of soil used were 40 sclerotia of *M. phaseolina*, one sclerotium of *R. solani*, and 3,000 chlamydospores of *F. solani*. There were 10 replicates of one plant per pot for each treatment. The treatments consisted of each pathogen and *G. mosseae* alone and *G. mosseae* in combination with each pathogen. Soybean plants from 10 replicates of each treatment were harvested at both 25 and 45 days after seeding to determine percentage of root infection by *M. phaseolina* and *F. solani* and disease index for *R. solani*, root colonization by *G. mosseae*, plant height, root and shoot fresh weights, and the population of each organism in the soil by methods previously described (21).

To determine if the effects obtained on 25- and 45-day-old plants would persist to plant maturity, a second test was conducted in autoclaved and nonautoclaved field soil. The effect of each pathogen alone and in combination with *G. mosseae* on soybean seed weight was determined. The inoculum densities for the autoclaved and nonautoclaved soil were the same as those above. Plants were harvested about 110 days after seeding, and in addition to seed weight, the percentage of root and stem infection by the pathogens, root colonization by *G. mosseae*, and the spore population of *G. mosseae* and other naturally occurring mycorrhizal fungi in the nonautoclaved soil was determined also. In this second test, no evaluations of soybean growth or development of the pathogens or mycorrhizal fungus in nonautoclaved soil were determined at 25 or 45 days. There were 10-pot replications of each treatment. Data from the first and second experiments were statistically analyzed separately by using standard analysis of variance. Duncan's multiple range test was used to evaluate treatment means.

RESULTS

Interactions of *M. phaseolina* × *G. mosseae*. *M. phaseolina* reduced all plant responses measured, but only shoot weight and plant height at 45 days were significantly lower than the noninoculated control (Table 1). When *G. mosseae* was added to soil infested with *M. phaseolina*, plant response and seed weight were comparable to the noninfested control. The greatest increase in plant growth was obtained in soil infested with *G. mosseae* alone. The correlations between the percentage of roots colonized by mycorrhiza and root weight ($r = 0.82$), shoot weight ($r = 0.88$), and plant height ($r = 0.87$) were statistically significant ($P < 0.05$).

G. mosseae did not alter significantly the percentage of roots infected or the number of sclerotia per gram of soil produced by *M. phaseolina*, but *M. phaseolina* significantly reduced the percentage of *G. mosseae* root colonization by 63% and chlamydospores produced from 8.3 to 3.3/g of soil.

TABLE 1. Effect of *Glomus mosseae* and *Macrophomina phaseolina* alone or in combination on soybean plant growth at 45 days and seed yield at 110 days after planting

Treatments[y]	Autoclaved soil							Non-autoclaved soil
	Root weight (g)	Shoot weight (g)	Plant height (cm)	Roots with *M. phaseolina* (%)	Roots with *G. mosseae* (%)	Propagules of *M. phaseolina* (no. per gram soil)	Seed weight per plant (mg)	Seed weight per plant (mg)
Uninoculated (control)	10.5 bc[z]	13.3 b	52.0 b	0.0	0.0	0 b	310 b	225 ab
M. phaseolina	9.4 c	7.5 c	36.3 c	50.5 a	0.0	20 a	247 b	188 b
G. mosseae	12.8 a	14.7 a	57.0 a	0.0	37.0 a	0 b	524 a	285 a
G. mosseae + *M. phaseolina*	10.8 b	12.9 b	50.5 b	48.0 a	24.8 b	18 a	329 b	242 ab

[y] Inoculum densities were 40×10^3 sclerotia of *M. phaseolina* per kilogram of soil and 500 chlamydospores of *G. mosseae* per 15-cm-diameter clay pot (2,000 g/of soil).

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

During 110 days in nonautoclaved soil, *M. phaseolina* significantly reduced the percentage of roots colonized by the indigenous mycorrhizal fungi from 49.1 to 30.1% and the number of chlamydospores of *G. etunicatum* from 3.1 to 1.8/g of soil, but did not affect the number of spores formed by *Gigaspora margarita* in nonautoclaved soil.

In autoclaved soil, *G. mosseae* alone significantly increased seed weight over the control by 69%. In nonautoclaved soil, seed weight was significantly higher with *G. mosseae* alone than with *M. phaseolina* alone. Although differences were not significant, seed weight averaged 27% higher with *G. mosseae* than in the uninoculated control plants.

Interactions of *R. solani* × *G. mosseae*. *R. solani* reduced root weight and plant height at 25 and 45 days significantly lower than the uninoculated control (Table 2). When *G. mosseae* was added to autoclaved soil infested with *R. solani*, plant growth was comparable to or significantly greater than the control. The greatest increase in plant growth was obtained with soil infested with *G. mosseae* alone. The correlations between the percentage of roots colonized by mycorrhiza and root weight ($r = 0.87$), shoot weight ($r = 0.84$), and plant height ($r = 0.92$) were statistically significant ($P < 0.05$). *G. mosseae* did not significantly reduce the disease index rating of *R. solani*, but *R. solani* significantly reduced the percentage of root colonization by *G. mosseae* in autoclaved soil.

In autoclaved soil, *G. mosseae* alone significantly increased seed weight over the control (66%), but plants grown in soil with *R. solani* and *G. mosseae* only had 39% greater seed weight than the controls. Seed weight was significantly reduced (30%) by *R. solani* alone compared to the uninoculated controls. Differences in seed yield among the treatments in nonautoclaved soil were not significant.

Interactions of *F. solani* × *G. mosseae*. *F. solani* alone significantly reduced all measured plant responses below those in the uninoculated control (Table 3). When *G. mosseae* was added to soil infested with *F. solani*, plant height and root weight were significantly greater than the control. The greatest increase in plant growth was obtained with soil infested with *G. mosseae* alone. The correlations between the percentage of roots colonized by mycorrhiza and root weight ($r = 0.78$), shoot weight ($r = 0.76$), and plant height ($r = 0.70$) were statistically significant ($P < 0.05$). *G. mosseae* did not influence either the percentage of roots infected by *F. solani* or the number of propagules of this fungus recovered per gram of soil at 45 or 110 days. However, in both autoclaved and nonautoclaved soil containing *F. solani*, the percentage of mycorrhizal roots were reduced significantly, and in nonautoclaved soil the number of spores of *G. mosseae* (from 104 to 2.0/100 g of soil) and *Gigaspora margarita* (from 66 to 15/100 g of soil) were reduced, but not the numbers of spores of *G. etunicatum*.

In autoclaved and nonautoclaved soil containing *G. mosseae* in combination with *F. solani*, seed weight was significantly increased over plants inoculated with *F. solani* alone (Table 3). Seed weight was significantly correlated with root weight and plant height at 45 days both in autoclaved and nonautoclaved soil. Thus, the evaluation of soybean growth, based on root weight and plant height at 45 days predicted the performance of the treatments in relation to seed yield.

DISCUSSION

The three pathogens differed in major effects on plant response. Of the growth parameters measured, *M. phaseolina* significantly reduced shoot weight and plant height, *R. solani* significantly reduced root weight and plant height, while *F. solani* significantly reduced all three. Orellana et al (11) also observed a reduction of root and top weights of soybean at high inoculum densities of *R. solani*. The slightly higher disease index for *R. solani* at 25

TABLE 2. Effect of *Glomus mosseae* and *Rhizoctonia solani* alone or in combination on soybean plant growth at 25 and 45 days and seed yield at 110 days after planting

	Autoclaved soil									Non-autoclaved soil
	Root weight (g)		Shoot weight (g)		Plant height (cm)		Disease index *R. solani*[y]	Roots with *G. mosseae* (%)	Seed weight per plant (mg after 110 days)	Seed weight per plant (mg after 110 days)
Treatments[x]	25	45	25	45	25	45	45	45		
Uninoculated (control)	3.5 bc[z]	7.1 a	3.9 b	5.9 b	27.2 b	44.0 c	0.0 b	0.0 c	283 c	307 a
R. solani	2.6 c	5.9 b	3.5 b	5.7 b	24.4 c	35.5 d	2.8 a	0.0 c	197 d	277 a
G. mosseae	4.7 a	7.1 a	4.7 a	7.7 a	31.0 a	55.7 a	0.0 b	33.0 a	469 a	323 a
G. mosseae + *R. solani*	3.0 b	7.0 a	4.6 a	6.5 b	28.6 ab	48.4 b	2.4 a	30.6 b	392 b	317 a

[x] Inoculum densities were 1,000 and 2,000 sclerotia of *R. solani* per kilogram of autoclaved and nonautoclaved soil, respectively, and 500 chlamydospores of *G. mosseae* per 15-cm-diameter clay pot (2,000 g of soil).
[y] Index based on a scale of 0 to 4 with 0 = no symptoms on the hypocotyl and 4 = hypocotyl girdled, plant dead.
[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

TABLE 3. Effect of *Glomus mosseae* and *Fusarium solani* alone or in combination on soybean plant growth at 45 days and seed yield at 110 days after planting

	Autoclaved soil					Non-autoclaved soil
Treatments[y]	Root weight (g)	Shoot weight (g)	Plant height (cm)	Roots with *F. solani* (%)	Roots with *G. mosseae* (%)	Seed weight per plant (mg after 110 days)	Seed weight per plant (mg after 110 days)
Uninoculated (control)	6.1 c[z]	7.9 b	40.3 b	0 b	0 c	283 ab	296 ab
F. solani	3.7 d	4.0 c	35.4 c	55 a	0 c	228 b	252 b
G. mosseae	13.9 a	13.0 a	46.4 a	0 b	57 a	318 a	345 a
G. mosseae + *F. solani*	9.3b	8.1 b	44.4 a	52 a	44 b	322 a	335 a

[y] Inoculum densities were $3,000 \times 10^3$ chlamydospores of *F. solani* per kilogram of soil and 500 chlamydospores of *G. mosseae* per 15-cm-diameter clay pot (2,000 g of soil).
[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

compared to 45 days after planting, suggests that plants become resistant as they get older. This observation was reported by others (3,6). For *M. phaseolina* and *F. solani* the most striking reduction in plant growth response occurred at 45 days. These findings confirm other reports of damage by these fungi either under greenhouse conditions or in the field (1,16,20).

F. solani did not cause discoloration of the vascular system in the roots, but produced small brown lesions on the root surfaces and occasionally rot occurred at the base of the stem. The main effects of infection by *F. solani* on soybean in this study and in a study by Cheng (2) were root and shoot growth reduction.

When *G. mosseae* was present in combination with any of the pathogens, soybean growth increased in autoclaved soil compared to that obtained with plants inoculated with the pathogens alone. Seed yield responses to the presence of *G. mosseae* or the pathogens were consistent in both autoclaved and nonautoclaved soils, ie, compared to the uninoculated controls, *G. mosseae* consistently increased average yields, the pathogens consistently decreased average yields, and when *G. mosseae* was combined with the pathogens the average yields were always greater than the uninoculated controls. Thus, the effect of *G. mosseae* on soybean compensated for the effect of the pathogens without affecting the incidence of the pathogens in the host. This compensatory effect of *G. mosseae* may simply be due to increased nutrient uptake, especially phosphorus (P) as suggested by others (5,7,8,10) for many plant responses to mycorrhizal fungi. In this study, however, the P level was sufficient (92 ppm) to support normal soybean growth without additional fertilizer. Powell (13) also reported a beneficial effect from mycorrhizal fungi in soil with higher P levels than those used in this study.

The presence of the pathogens and *G. mosseae* together resulted in reduced root colonization by *G. mosseae* and seed weight as compared to that with *G. mosseae* alone. This is the first report of the presence of *M. phaseolina* or *R. solani* resulting in a significant reduction in root colonization by *G. mosseae*.

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