

Interactions of Vesicular-Arbuscular Mycorrhizal Fungi, Root-Knot Nematode, and Phosphorus Fertilization on Soybean

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ABSTRACT

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The effects of two vesicular-arbuscular mycorrhizal (VAM) fungi, *Meloidogyne incognita*, and phosphorus (P) fertilization (0, 25, 50, and 150 $\mu\text{g/g}$ of soil) on soybean growth and yield were determined in a greenhouse study. Maximum growth and yield occurred at P fertilization rates of 50–150 $\mu\text{g/g}$, regardless of whether plants were uninoculated or were inoculated with *Gigaspora margarita*, *Glomus etunicatum*, or *M. incognita* or coinoculated with either endophyte and the nematode. Growth and yield were stimulated by mycorrhizae at the lowest P rate. The performance of non-VAM and VAM plants was little affected by *M. incognita* at any P rate; therefore, tolerance to the nematode could not be evaluated. Egg production on VAM plants (eggs per root system and eggs per gram of root) was suppressed at the lowest P rate as well as by increased P fertilization, indicating the induced resistance is possibly due to improved P nutrition in the host. *M. incognita* had no effect on root colonization by VAM fungi, but at the lowest P rates, the nematode stimulated sporulation by *G. margarita* and suppressed sporulation by *G. etunicatum*.

Additional keyword: endomycorrhizae

The root systems of many crops may be colonized simultaneously by plant-parasitic nematodes and vesicular-arbuscular mycorrhizal (VAM) fungi, the activities of which have a pronounced effect on crop production and the life cycles of the microorganisms. Generally, vesicular-arbuscular mycorrhizae tend to make plants more tolerant to nematode attack, but no consistent trend has been reported in the colonization patterns and reproduction of the microorganisms (6). The interaction results are invariably

determined by specific endophyte-nematode-plant combinations (12).

Common coinhabitants on soybean (*Glycine max* (L.) Merr.) are *Meloidogyne incognita* (Kofoid & White) Chitwood and species of the VAM fungi *Acaulospora*, *Gigaspora*, and *Glomus* (11). Two studies evaluating effects of vesicular-arbuscular mycorrhizae on root-knot nematode infecting soybean have been reported (8,12). The reaction of plants mycorrhizal with any of three VAM fungi and coinoculated with *M. incognita* was affected by the fungal symbiont, nematode inoculum level, and cultivar resistance to the nematode (12). The endophytes generally stimulated plant growth and nematode reproduction (juveniles per volume of soil); however, the density of juveniles varied with each VAM fungus. Subsequent

research with the root-knot-susceptible soybean cultivar Pickett showed that root weight and yield were greater in plants inoculated with *Glomus macrocarpum* and challenged with *M. incognita* than in nonmycorrhizal plants attacked by the nematode (8). Mycorrhizal plants also had significantly fewer galls per gram of root than nonmycorrhizal plants. Neither the development of mycorrhizae nor sporulation was affected by the nematode.

Most challenge studies with plant-parasitic nematodes have been carried out in soils or mixes deficient in phosphorus (P), selected to promote maximum colonization by VAM fungi (6,13). Smith (13) discussed the importance of comparing nematode parasitism on mycorrhizal and nonmycorrhizal plants of similar size and nutrient status, to determine whether the mechanism of tolerance or resistance to plant-parasitic nematodes is due to some change in the host other than improved P nutrition. The role of P fertilization in interactions of VAM fungi and *Meloidogyne* has been studied in diverse crops, such as alfalfa (3), clover (1), cotton (14,15), onion (10), tamarillo (2), and tomato (1,16). Improved P nutrition usually increased tolerance to nematode attack; however, these plants had greater nematode populations per plant and per gram of root (13). There have been no similar studies of soybean. Therefore, our objective was to evaluate the effects of VAM fungi and *M. incognita* on soybean growth and yield and on microorganism activities at four P fertilization rates, ranging from suboptimum to optimum for plant growth.

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MATERIALS AND METHODS

Soil preparation. The tests were conducted in a Dothan loamy sand (thermic plinthic paleudults consisting of 74% sand, 14% silt, and 12% clay), which had not been in crop production for the last 20 yr. Analyses of soil samples were performed by the University of Georgia Soil and Plant Testing Laboratory, Cooperative Extension Service, with the following results: pH 4.9; mineral contents (per gram) of 3 µg of P, 34 µg of K, 252 µg of Ca, 54 µg of Mg, 2 µg of Zn, 63 µg of Mn, and 0.2 µg of B; 2.1% organic matter; and soluble salts with conductivity of 8×10^{-5} mhos. The soil was screened to remove large debris and blended with washed river sand (1:1, v/v) in a soil mixer. During mixing, Ca(OH)₂ was added to adjust the pH to 6.2, and CaHPO₄ (Dikal 18%, Smith-Douglas, Norfolk, VA) was added at various concentrations to establish four actual rates of added P: 0, 25, 50, and 150 µg/g of mix. The mixes were fumigated with methyl bromide (Dowfume MC-2, Dow Chemical Co., Midland, MI) at a rate of 1.36 kg/800 L of mix for 48 hr under a polyethylene film and vented for approximately 5 days prior to planting. The mixes were placed in 4-L plastic pots and maintained on a greenhouse bench.

Inocula increase and inoculation. Spores of *Gigaspora margarita* Becker & Hall and *Glomus etunicatum* Becker & Gerd. were increased on *Sorghum bicolor* (L.) Moench. 'Shallu' in pot culture and extracted from the soil by a modified centrifugation-flotation procedure (7). Pots were half-filled with soil, and 500 spores of each endophyte in 25 ml of water were added to the appropriate pots. The pots were then filled, and four soybean seeds (cultivar Ransom) were sown in each. The plants were thinned to one per pot 7 days later. Each nonmycorrhizal plant received 25 ml of the filtrate from a spore suspension of both VAM fungi passed through Whatman no. 1 filter paper. A

commercial preparation of *Bradyrhizobium japonicum* (Kirch.) Jordan (200 mg per pot) was added at the soil surface and watered.

M. incognita was propagated on greenhouse-grown tomato (*Lycopersicon esculentum* Mill. 'Rutgers'), and eggs were collected in 0.5% NaOCl (5). To inoculate soybean plants, 10,000 eggs were poured into a shallow trench in the soil around each plant 10 days after planting.

Experimental design, plant maintenance, and collection of data. Treatments consisted of no inoculation, inoculation with one of the VAM fungi or *M. incognita*, and joint inoculations with a fungus and the nematode at each P level. Each treatment (one plant per pot) was replicated 10 times, and the experiment was arranged in a randomized complete block design. It was terminated 95 days after planting. The study was repeated, with similar results; therefore, data from a single test are reported.

Routine spraying was essential to control mites.

Plant growth data collected were the fresh weights of pods, roots, and shoots.

Samples for VAM assay were collected by placing a root system on a flat surface and cutting a transverse band, 5–10 mm wide, at a point 5–7 cm below the root crown. All cut root segments were collected; the weight of the sample was approximately 2.5% of that of the total root system. The roots were stained by the Phillips-Hayman clearing and staining technique (9), and visual estimates were made with a stereoscopic microscope according to the following index: 0 = 0%, 1 = less than 10%, 2 = 11–40%, 3 = 41–70%, 4 = 71–95%, and 5 = more than 95% mycorrhizal colonization.

Spore densities in the soil were determined by a modified centrifugation-flotation procedure (7).

The reproduction of *M. incognita* was measured by collecting and counting eggs from the roots remaining after the

samples had been taken for VAM assay (5).

Analysis of variance was used to analyze data, and significant means were separated by Duncan's multiple range test or Fisher's LSD.

RESULTS

Plant growth and yield responses. Increasing P fertilization up to 50–150 µg/g of soil improved soybean growth and pod production (Table 1). Mycorrhizae also stimulated plant growth and yield, but there was a significant interaction with P fertilization. The benefits of mycorrhizae occurred primarily in soil not fertilized with P. Both VAM fungi stimulated root growth and pod production equally well; however, *G. etunicatum* increased shoot growth to a greater degree than *G. margarita*. The suppression of plant growth and yield by *M. incognita* was inconsistent. Consequently, there was no statistically significant interaction of VAM fungi and *M. incognita* affecting the performance of dually inoculated plants.

Microorganism activities. Egg production by *M. incognita* was affected by mycorrhizae and P fertilizer. Egg production (eggs per root system and eggs per gram of root) was suppressed on nonmycorrhizal plants and plants mycorrhizal with *G. margarita* as P fertilization was increased; it was unaffected by P fertilization on plants mycorrhizal with *G. etunicatum* (Table 2). More eggs per root system and per gram of root were produced on nonmycorrhizal plants than on mycorrhizal plants, particularly in the soil not fertilized with P. At the higher P fertilization rates, the effect of mycorrhizae on egg production was inconsistent.

Increased P fertilization, particularly at the highest rate, suppressed root colonization by both endophytes (Table 3). However, *M. incognita* had a minimal effect on the development of mycorrhizae in roots.

Sporulation by both VAM fungi was

Table 1. Effect of vesicular-arbuscular mycorrhizal fungi, soil phosphorus (P), and *Meloidogyne incognita* on soybean growth and yield

	P (µg/g of soil)	No mycorrhizae		<i>Gigaspora margarita</i>		<i>Glomus etunicatum</i>	
		No <i>M. incognita</i>	<i>M. incognita</i>	No <i>M. incognita</i>	<i>M. incognita</i>	No <i>M. incognita</i>	<i>M. incognita</i>
Shoot weight (g)	0	11.6 d ^z	6.6 d	37.8 b	31.2 c	49.5 a	39.0 b
	25	71.6 c	58.8 d	72.9 c	69.0 c	88.8 a	82.7 ab
	50	102.7 ab	85.6 b	100.9 ab	90.2 b	108.1 a	92.2 ab
	150	124.0 a	117.2 a	124.4 a	115.3 a	121.2 a	117.4 a
Root weight (g)	0	20.5 c	18.3 c	48.9 b	63.3 a	57.6 ab	61.0 a
	25	91.8 a	94.8 a	93.6 a	100.4 a	89.5 a	86.6 a
	50	116.0 ab	122.8 a	119.6 a	115.8 ab	96.3 b	95.5 b
	150	108.2 a	116.2 a	101.8 a	106.4 a	117.0 a	108.9 a
Pod weight (g)	0	0.22 c	0.18 c	0.86 a	0.40 bc	0.81 a	0.62 ab
	25	3.00 a	0.97 c	2.81 ab	1.52 bc	3.06 a	2.64 ab
	50	3.65 ab	2.43 b	2.90 ab	2.53 b	4.26 a	2.16 b
	150	1.40 ab	1.47 ab	1.85 ab	1.05 b	2.13 a	1.84 ab

^zValues in the same row followed by different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).

affected by P fertilization, by *M. incognita*, and by their interaction. Spore production by *G. margarita* increased with increasing P fertilization from 0 to 50 µg/g and decreased with the further increase from 50 to 150 µg/g (Table 3). However, sporulation by *G. etunicatum* alone and by either endophyte coinoculated with *M. incognita* decreased with increasing P fertilization. *M. incognita* tended to stimulate sporulation by *G. margarita* but suppressed spore production by *G. etunicatum* at the two lowest fertility levels.

DISCUSSION

Tolerance to attack by plant-parasitic nematodes, including *M. incognita* on soybean, is the most frequently reported benefit of VAM (6,8,13). Tolerance to *M. incognita* was not evident in our study, since the nematode did not consistently affect the growth of either nonmycorrhizal or mycorrhizal plants. A

statistical analysis of main effects indicated no significant interaction of VAM fungi and *M. incognita*.

Soybean resistance to *M. incognita*, expressed as suppressed egg production, was induced by either VAM fungus and by higher P fertilization rates in our study. Kellam and Schenck (8) reported fewer galls per root system in soybean mycorrhizal with *G. macrocarpum* than in nonmycorrhizal root systems, even though the latter were smaller. Higher P fertilization rates also decreased reproduction by root-knot nematode on bean (13); however, with other crops, it stimulated nematode activities, including egg production (1-3,14). Whereas our results indicate that soybean resistance to *M. incognita* is closely associated with improved P status of the host, other studies have concluded that mycorrhizae-influenced resistance to *Meloidogyne* spp. on alfalfa (3), clover (1), cotton (14,15), onion (10), tamarillo (2),

and tomato (1) may be due to some other factor involved in symbiosis and is likely independent of P nutrition.

Root colonization and sporulation by VAM fungi were inconsistently affected by *M. incognita* and P fertilization. Typically, both the development of mycorrhizae and fungal sporulation were suppressed by increasing P fertilization, as has been frequently reported (4). *M. incognita* had a minimal effect on root colonization but tended to suppress sporulation by *G. etunicatum* and stimulate sporulation by *G. margarita*. We are uncertain of the cause of this variable response, but it has been noted with other crops (6,13).

The increased tolerance to root-knot nematode in soybean associated with P fertilization needs to be studied in the field. Smith et al (15) concluded that tolerance to *M. incognita* on cotton, induced by P fertilization in the greenhouse, as reported by Hussey and Roncadori (6), did not occur in field microplots, because a very high rate of P fertilization may have limited zinc uptake.

Table 2. Influence of soil phosphorus and vesicular-arbuscular mycorrhizal (VAM) fungi on egg production by *Meloidogyne incognita* on soybean

VAM fungus	Phosphorus fertilization rate (µg/g) ^y			
	0	25	50	150
	Eggs per root system (× 10³)^z			
None	45.6 a	25.1 a	26.2 a	11.0 a
<i>Gigaspora margarita</i>	46.9 b	22.1 a	8.4 b	13.0 a
<i>Glomus etunicatum</i>	9.6 b	12.9 b	22.1 ab	20.7 a
	Eggs per gram of root (× 10³)^z			
None	2.7 a	0.30 a	0.24 a	0.09 b
<i>Gigaspora margarita</i>	0.5 b	0.22 a	0.07 a	0.12 b
<i>Glomus etunicatum</i>	0.4 b	0.15 a	0.26 a	0.21 a

^yThe rates are for amounts of P added to the soil. The initial rate of P in the soil was 3 µg/g.

^zValues in the same column followed by different letters are significantly different according to Fisher's LSD ($P = 0.05$).

Table 3. Influence of soil phosphorus and *Meloidogyne incognita* on root colonization and sporulation by vesicular-arbuscular mycorrhizal (VAM) fungi on soybean

VAM fungus	<i>M. incognita</i> ^x	Phosphorus fertilization rate (µg/g)			
		0	25	50	150
		Root colonization index^{y,z}			
<i>Gigaspora margarita</i>	0	4.3	4.4	3.4*	1.4
	+	5.0	4.0	3.5	2.0
<i>Glomus etunicatum</i>	0	3.7	3.3*	3.1	1.3
	+	3.5	2.5	3.0	1.5
		Spores per 100 cm³ of soil^t			
<i>Gigaspora margarita</i>	0	317*	618*	765	189*
	+	1,090	1,008	790	339
<i>Glomus etunicatum</i>	0	4,176*	2,658*	1,616	267
	+	2,108	1,680	1,065	288

^x0 = Treatment without *M. incognita*; + = treatment with *M. incognita*.

^y0 = 0%, 1 = less than 10%, 2 = 11-40%, 3 = 41-70%, 4 = 71-95%, and 5 = more than 95% of the root length colonized.

^z* = Significant difference between means for the individual VAM fungus in treatments with and without *M. incognita* according to Fisher's LSD ($P = 0.05$).

LITERATURE CITED

- Cooper, K. M., and Grandison, G. S. 1986. Interaction of vesicular-arbuscular mycorrhizal fungi with root knot nematode on cultivars of tomato and white clover susceptible to *Meloidogyne hapla*. Ann. Appl. Biol. 108:555-565.
- Cooper, K. M., and Grandison, G. S. 1987. Effect of vesicular-arbuscular mycorrhizal fungi on infection of tamarillo (*Cyphomandra betacea*) by *Meloidogyne incognita* in fumigated soil. Plant Dis. 71:1101-1106.
- Grandison, G. S., and Cooper, K. M. 1986. Interaction of vesicular-arbuscular mycorrhizae and cultivars of alfalfa susceptible and resistant to *Meloidogyne hapla*. J. Nematol. 18:141-149.
- Harley, J. L., and Smith, S. E. 1983. Mycorrhizal Symbiosis. Academic Press, London. 483 pp.
- Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57:1025-1028.
- Hussey, R. S., and Roncadori, R. W. 1982. Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. Plant Dis. 66:9-14.
- Jenkins, W. R. 1964. A rapid centrifugation-floitation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
- Kellam, M. K., and Schenck, N. C. 1980. Interactions between a vesicular-arbuscular mycorrhizal fungus and root-knot nematode on soybean. Phytopathology 70:293-296.
- Kormanik, P. P., and McGraw, A.-C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. Pages 37-45 in: Methods and Principles of Mycorrhizal Research. N. C. Schenck, ed. American Phytopathological Society, St. Paul, MN. 244 pp.
- MacGuidwin, A. E., Bird, G. W., and Safir, G. R. 1985. Influence of *Glomus fasciculatum* on *Meloidogyne hapla* infecting *Allium cepa*. J. Nematol. 17:389-395.
- Schenck, N. C., and Kinlock, R. A. 1974. Pathogenic fungi, parasitic nematodes, and endomycorrhizal fungi associated with soybean roots in Florida. Plant Dis. Rep. 58:169-173.
- Schenck, N. C., Kinlock, R. A., and Dickson, D. W. 1975. Interaction of endomycorrhizal fungi and root knot nematode on soybean. Pages 607-617 in: Endomycorrhizas. F. E. Sanders, B. Mosse, and P. B. Tinker, eds.

- Academic Press, New York. 626 pp.
13. Smith, G. S. 1987. Interactions of nematodes with mycorrhizal fungi. Pages 292-300 in: *Vistas on Nematology*. J. W. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD. 509 pp.
 14. Smith, G. S., Hussey, R. S., and Roncadori, R. W. 1986. Penetration and postinfection development of *Meloidogyne incognita* on cotton as affected by *Glomus intraradices* and phosphorus. *J. Nematol.* 18:429-435.
 15. Smith, G. S., Roncadori, R. W., and Hussey, R. S. 1986. Interaction of endomycorrhizal fungi, superphosphate, and *Meloidogyne incognita* on microplot and field studies. *J. Nematol.* 18:208-216.
 16. Thomson Cason, K. M., Hussey, R. S., and Roncadori, R. W. 1983. Interaction of vesicular-arbuscular mycorrhizal fungi and phosphorus with *Meloidogyne incognita* on tomato. *J. Nematol.* 15:410-417.