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# Interactions of Vesicular-Arbuscular Mycorrhizal Fungi, Phosphorus, and *Heterodera glycines* on Soybean[1]

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*Abstract:* Effects of vesicular-arbuscular mycorrhizal (VAM) fungi and soil phosphorus (P) fertility on parasitism of soybean cultivars Bragg and Wright by soybean cyst nematode (SCN) were investigated in field microplot and greenhouse experiments. VAM fungi increased height of both cultivars and yield of Wright in microplot studies in 1986 and 1987. Conversely, yield of mycorrhizal and nonmycorrhizal plants of both cultivars was suppressed by SCN. Soil population densities of SCN were unaffected by VAM fungi in 1986 but were greater in microplots infested with VAM fungi than in control microplots in 1987. Growth of Wright soybean was stimulated by VAM fungi and suppressed by SCN in greenhouse experiments. The effect of VAM fungi on SCN varied with time. Numbers of SCN in roots and soil were decreased by VAM fungi by as much as 73% at the highest SCN inoculum level through 49 days after planting. Later, however, SCN numbers were usually comparable on mycorrhizal and nonmycorrhizal plants. Soil P fertility generally had no effect on SCN. Results of a split-root experiment indicated that VAM fungal suppression of SCN was not systemic.

*Key words:* endomycorrhizae, *Glycine max*, *Heterodera glycines*, interaction, microplot, phosphorus, soybean, soybean cyst nematode, vesicular-arbuscular mycorrhizal fungi.

A major factor limiting soybean (*Glycine max* (L.) Merr.) production in the United States is parasitism by the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe. In 1987 yield suppression due to SCN in 16 soybean producing states in the South was estimated to be 419 million kg worth $84.5 million (20). This was the greatest loss due to any single plant pathogen on soybean in this area, and it represented approximately 20% of the overall disease-induced yield suppression.

Vesicular-arbuscular mycorrhizal (VAM) fungi, obligate symbionts on soybean roots, generally have a stimulating effect on plant growth increasing shoot, root, and seed weights as well as pod and seed numbers (8,23). Plant growth stimulation induced by VAM fungi is believed to be due to increased host uptake of soil phosphorus (P), primarily, but also of other minerals (19,23). Mycorrhizal soybeans also exhibit greater drought tolerance than nonmycorrhizal plants (5,24).

In addition to stimulation of plant growth, VAM fungi may suppress plant-parasitic nematodes on soybean. Although research on this phenomenon has been conducted primarily with the root-knot nematode, *Meloidogyne incognita* (9,16,26), observations have been made of apparent antagonism of SCN by VAM fungi. In a survey of soybean fields in Florida (25), high population densities of SCN were usually inversely related with abundant VAM fungal spores and mycelia. More recently, chlamydospores of the VAM fungus, *Glomus fasciculatum*, were detected within SCN cysts (12). Spores were also observed in SCN eggs, and egg infection occurred in greenhouse experiments (12). Structures of VAM fungi have also been recovered from cysts of other *Heterodera* species (13,29).

Resistant cultivars and nematicides limit SCN activity. Environmental concerns (28), withdrawal of registrations (2), and high costs have limited nematicide usage on soybean. Furthermore, overuse of host resistance for management of SCN results in natural selection of races of SCN capable of reproducing on resistant cultivars (22,27,30). Consequently, development of environmentally safe and effective alter-

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natives for SCN management is needed. The use of VAM fungi for suppression of nematode parasitism represents one possible alternative. The objective of the microplot and greenhouse experiments described herein was to determine the influence of VAM fungi and soil P fertility on development and reproduction of SCN and the growth and yield of SCN-infected soybeans.

## Materials and Methods

*General procedures:* Race 3 of SCN was propagated on soybean cultivar Lee in greenhouse cultures. Eggs were extracted from cysts collected from roots of approximately 60-day-old plants (4). Inoculum of VAM fungi consisted of chopped roots and soil from 1-year-old greenhouse cultures on Bahia grass, *Paspalum notatum* Flugge. All VAM fungal treatments received a mixture of inoculum of *Gigaspora margarita* Becker & Hall, *Glomus etunicatum* Becker & Gerdemann, *Glomus macrocarpum* Tul. & Tul., and *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. Inoculum was applied at a rate of 2.0 g/500 $cm^3$ soil for microplot studies and 6.5 g/500 $cm^3$ soil for all greenhouse experiments. To standardize soil microflora, nonmycorrhizal treatments received a filtrate of the VAM fungal inoculum prepared by suspending an equivalent volume of inoculum in water and passing the water through Whatman #1 filter paper and a 25-$\mu$m-pore sieve. Filtrate was applied to all nonmycorrhizal experimental units of microplot, greenhouse, and split-root studies.

*Field microplot experiments:* Sixty-four microplots at the University of Georgia Plant Sciences Farm near Athens were used for experiments in 1986 and 1987. Microplots consisted of 75-cm-d cylinders constructed from 91-cm-wide sheets of fiberglass buried 66 cm deep in an Appling coarse sandy loam (Typic Hapludult, clayey, kaolinitic, thermic, 73% sand, 12% silt, 15% clay; pH 6.2). Results of initial soil analysis were as follows: P = 19, K = 157, Ca = 552, Mg = 80, Zn = 4 kg/ha. Three to four weeks before planting, soil within and between microplots was fumigated with methyl bromide at 0.12–0.19 kg/$m^2$. The soil was aerated for at least 2 weeks before infestation with microorganisms or planting. Trifluralin ($\alpha\alpha\alpha$-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; 0.56 kg a.i./ha) was applied preplant for weed control, and toxaphene (chlorinated camphene, 1.75 liter/ha) was applied outside the microplots after planting to control insects.

Treatments were assigned randomly to four replicate blocks in a complete factorial arrangement. In 1986 the following four experimental factors were used, each with two levels: soybean cultivar—Bragg and Wright; soil P fertility—low and high (as recommended by the University of Georgia Extension Service for soybean); VAM fungi—present or absent; and SCN initial population density (Pi)—0 and 125 eggs/100 $cm^3$ soil. Both soybean cultivars are susceptible to SCN race 3, but Bragg is intolerant and Wright is moderately tolerant (4). Microplot soil was fertilized with superphosphate, $CaHPO_4$, to a projected level of 33.6 kg/ha total P for low P and 78.5 kg/ha for high P treatments. The P fertilizer was applied to the microplot soil surface and manually incorporated to a depth of 22 cm throughout. *Heterodera glycines* inoculum consisted of a suspension of eggs adjusted to the proper density in 1,600 ml water and manually incorporated into the soil to a depth of 22 cm throughout. Control plots received 1,600 ml water. Inoculum of VAM fungi was similarly incorporated into the soil, except it was applied in a 20-cm-wide band directly under the seed row within each microplot. Following incorporation of fertilizer and inoculum, 45 seeds of the appropriate soybean cultivar were planted in a single row in the center of each microplot. To simulate field planting, seeds of the same cultivar planted within each microplot were planted in the row between the microplots and parallel with the microplot seed rows on either side 95 cm apart as border rows. All seeds were inoculated with a commercial preparation of *Bradyrhizobium japonicum* (Kirchner) Jordan in the seed furrow. The field was sprin-

kle irrigated after planting and periodically throughout the growing season. Seedlings were thinned to 20 per microplot 14 days after planting (DAP).

Six to eight soil cores (2.5 cm d, 20 cm deep) were taken from near the center row of each microplot 60, 90, and 120 DAP. Soil cores from each microplot were combined, and a 250-$cm^3$ subsample was processed for extraction of cysts and second-stage juveniles (J2) by elutriation (6). Eggs were extracted from cysts (4), and J2 were separated from sediments by centrifugal flotation (15). A reproductive factor, Pf/Pi, was calculated at each sampling date where Pf = soil J2 and egg population densities combined. Near harvest, plant height and number were determined. Maturity date, when 95% of pods had attained mature color, was also recorded. At maturity, seeds were mechanically harvested from plants within microplots and dried to a seed moisture content of 13%. Total seed yield (g/microplot) and seed size (g/100 seed) were determined. Yield of microplots containing fewer than 20 plants at harvest was calculated using average yield per plant to reflect total yield for 20 plants.

The 1987 experiment was similar to the one in 1986 except that only one soybean cultivar, Wright, was planted and four SCN Pi—0, 7, 42, and 252 eggs/100 $cm^3$ soil—were used. The P fertility and VAM fungi levels were not changed. In addition, a second row of 20 seeds was planted within each microplot parallel to the center seed row. Inoculum of VAM fungi was incorporated into the soil directly under the seed row before planting. After 14 days, plants were carefully removed with root systems intact. Five root systems per microplot were randomly selected, stained (7), and observed microscopically to determine nematode penetration. All other plant and nematode response variables measured were identical to those in 1986.

*Greenhouse experiments:* Two greenhouse experiments were conducted on the basis of results of the 1987 microplot study. Soil obtained from the microplot site was mixed 1:1 (v/v) with washed river sand and fumigated with methyl bromide. Half of the soil, designated high P, was amended with $CaHPO_4$ at rates of 38 kg/ha in the first study and 78 kg/ha when the experiment was repeated. The fertilizer was incorporated into the soil with a motor-driven mixer before fumigation. Low P soil received no fertilization in either experiment. Soil was placed in 4,000-$cm^3$ plastic pots (20 cm d). Pots infested with VAM fungi in the first experiment were filled in one-third increments, and 25 g VAM fungal inoculum mix was added in two layers, 5 and 10 cm below the soil surface. The VAM inoculum was incorporated throughout the soil in a mixer when the experiment was repeated. Inoculum of SCN was applied immediately before planting in 2.5-cm-deep, circular furrows at Pi of 0, 7, 42, and 252 eggs/100 $cm^3$ soil. Four Wright soybeans were planted in each pot, *B. japonicum* inoculum was applied, and pots were watered immediately. Pots were watered as needed thereafter and seedlings were thinned to one per pot 10–14 DAP. Each of the six blocks in both experiments contained a complete factorial arrangement of the same treatments used in the 1987 microplot study. In addition, enough pots of each treatment were included in each block to allow for destructive sampling at 49 and 98 DAP during the first experiment and 35, 77, and 105 DAP when the experiment was repeated. After each sampling date, remaining pots were fertilized with 100 ml of complete nutrient solution (18) minus P.

Shoot dry weight, root fresh weight, and stem diameter at the soil line were determined at each sampling date. Shoots were dried at 70 C for 72 hours before weighing. In the second experiment, the phenological stage of the plant at time of sampling also was recorded (11). Randomly selected 0.5-g root samples, with nodules removed, were stained (7) and nematodes within root tissue were enumerated. Root samples also were stained (17) to confirm VAM fungal colonization. Adult females and cysts were removed from the root surface with a high pressure water spray, collected on a 250-$\mu$m-pore sieve, and counted. Eggs were

subsequently extracted and counted. Fecundity was calculated as the number of eggs per cyst on each root system. Eggs and J2 were extracted from 500-cm³ soil samples as was done in the microplot experiments.

*Split-root greenhouse experiment:* Wright soybeans were germinated and grown for 10 days in vermiculite in the greenhouse. The lower 70% of each root was excised, and the roots were split longitudinally with a scalpel. Seedlings were transplanted into double-compartment pots consisting of two 200-cm³ paper cups fastened together. After 9 days, seedlings with half-root systems of uniform size were selected and transferred to double pots constructed from 900-cm³ polypropylene freezer containers fastened together. Double pots were filled with the same fumigated, low P soil mix used in the greenhouse experiments. Treatments, replicated six times, consisted of soil infested with VAM fungi and SCN in various combinations, either together in the same compartment or in separate compartments of the double pots. A randomized complete block design was used. Inoculum of VAM fungi was mixed into appropriate soils throughout at the rate used in the greenhouse experiments and *B. japonicum* inoculum was applied to the soil surfaces of all pots. *Heterodera glycines* inoculum was applied in a 2.5-cm-deep, semi-circular trench around appropriate half-root systems, and soil in all compartments was covered with a 2-cm-deep layer of fumigated sand. Pots were watered immediately and then as needed thereafter. The experiment was terminated 35 days after infestation with SCN and VAM fungi. Following determination of half-root fresh weights, females and cysts were removed from roots and counted, then eggs were extracted and enumerated. Random root samples were collected and stained and nematodes were counted. Egg and J2 population densities were determined from 500-cm³ soil samples.

*Statistical analyses:* Data were initially subjected to analysis of variance. *Heterodera glycines* Pi effects were also characterized by linear regression analysis. Differences between treatments in the split-root experiment were further separated by a protected Fisher's least significant difference (LSD) test.

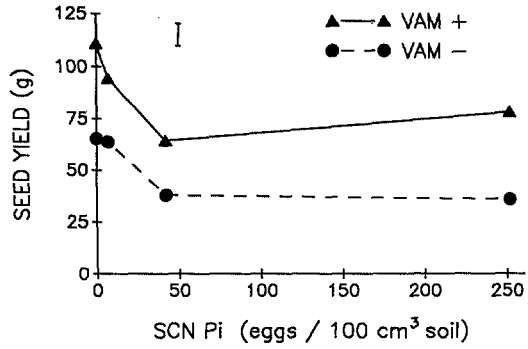


FIG. 1. Seed yield of Wright soybeans with (VAM +) and without (VAM −) vesicular-arbuscular mycorrhizal fungi as influenced by *Heterodera glycines* (SCN) initial population densities (Pi) in microplots near Athens, Georgia, in 1987. Bar represents standard error of the means.

## RESULTS

*Microplot experiments:* Vesicular-arbuscular mycorrhizal fungi had the greatest effect on overall plant growth of all experimental factors examined. Plant height of both cultivars (Table 1) and yield of Wright (Table 2) were increased ($P < 0.01$) by VAM fungi in 1986. Wright soybean yielded 48% more in microplots infested with VAM fungi than in microplots not infested with VAM fungi. In 1987 VAM fungi affected growth and yield of Wright soybeans in an identical manner (Table 3, Fig. 1). The maturity dates of both soybean cultivars planted in 1986 were unaffected by VAM fungi. In 1987, however, mycorrhizal plants matured 1 day earlier ($P < 0.05$) than nonmycorrhizal plants (Table 3). Overall, plants matured later in 1986 than in 1987.

Soybean cyst nematodes adversely affected plant growth. Yield of Bragg in 1986 and Wright in both years was suppressed ($P < 0.01$) by SCN parasitism. Overall yield suppression in 1986 was 43% for Bragg and 39% for Wright, relative to the uninfested control microplots (Table 2). In 1987 yield suppression of Wright was 35% at the

TABLE 1. Main effects of *Heterodera glycines* (SCN) initial population density (Pi), vesicular-arbuscular mycorrhizal (VAM) fungi, and soil phosphorus (P) fertility on height and seed size of Bragg and Wright soybeans in microplots near Athens, Georgia, in 1986.

| | | Bragg | | Wright | |
|---|---|---|---|---|---|
| | Level† | Plant height (cm) | Seed size (g/100 seed) | Plant height (cm) | Seed size (g/100 seed) |
| SCN Pi | 0 | 77 a | 13.7 b | 67 a | 13.3 a |
| | 125 | 78 a | 14.8 a | 68 a | 12.9 a |
| VAM | − | 70 b | 14.0 a | 56 b | 12.8 a |
| | + | 84 a | 14.5 a | 79 a | 13.4 a |
| P | Low | 80 a | 14.8 a | 70 a | 12.9 a |
| | High | 75 b | 13.7 b | 65 a | 13.3 a |

Numbers in columns within experimental factor not followed by the same letter are significantly different according to analysis of variance ($P < 0.05$).

† Pi = 0 or 125 eggs/100 cm³ soil. Level of VAM fungi: − = uninfested; + = infested. Soil P fertility: low = 33.6 kg/ha; high = 78.5 kg/ha total soil P.

highest SCN Pi, relative to the control (Fig. 1). On the average, Bragg matured more than 2 days later ($P < 0.01$) in SCN-infested microplots than in control microplots in 1986, but Wright was unaffected (data not shown). However, the relationship between maturity date of Wright and SCN Pi was linear ($P < 0.01$) in 1987 (Table 3). No interactions for plant growth response variables were detected between SCN and VAM treatments in either year.

The effects of soil P fertility on plant growth were variable. Bragg soybeans grown in high P microplots in 1986 were 6% shorter ($P < 0.05$) and produced seeds 7% smaller ($P < 0.05$) than those grown under low P fertility (Table 1), but no differences were detected in yield (Table 2). Growth of Wright was unaffected by P fertility treatments in 1986; however, plant height and seed size were 8 and 5% greater ($P < 0.05$), respectively, in high P than in low P microplots in 1987 (Table 3), although yields were comparable.

The response of SCN to the experimental factors varied greatly between the 2 years. Penetration of J2 after 14 days in 1987 was 14, 97, and 384/g fresh root for Pi 7, 42, and 252 eggs/100 cm³ soil, respectively. The relationship between SCN Pi and J2 penetration was linear ($P < 0.01$) but neither P fertility nor VAM fungi affected penetration. Soil population densities of J2 peaked at 90 DAP in both years, and no differences in densities were detected between treatments at any sampling date in 1986 (data not shown). In 1987, however, soil J2 densities increased linear-

TABLE 2. Influence of *Heterodera glycines* (SCN) initial population density (Pi)†, vesicular-arbuscular mycorrhizal (VAM) fungi, and soil phosphorus (P) fertility on Bragg and Wright soybean yield (g/microplot) in microplots near Athens, Georgia, in 1986.

| | | Seed yield | | | | | |
|---|---|---|---|---|---|---|---|
| | | Bragg | | | Wright | | |
| | Level‡ | Pi = 0 | Pi = 125 | Mean | Pi = 0 | Pi = 125 | Mean |
| VAM | − | 169 | 75 | 122 a | 151 | 87 | 119 b |
| | + | 190 | 130 | 160 a | 216 | 136 | 176 a |
| P | Low | 150 | 95 | 122 a | 160 | 117 | 138 a |
| | High | 208 | 110 | 159 a | 206 | 106 | 156 a |
| Mean | | 179 x | 102 y | | 183 x | 111 y | |

Numbers in columns within VAM and P experimental factor means and in row for SCN Pi means not followed by the same letter are significantly different according to analysis of variance ($P < 0.05$).

† Pi = 0 or 125 eggs/100 cm³ soil.

‡ Level of VAM fungi: − = uninfested; + = infested. Soil P fertility: low = 33.6 kg/ha; high = 78.5 kg/ha total soil P.

TABLE 3. Main effects of *Heterodera glycines* (SCN) initial population density (Pi), vesicular-arbuscular mycorrhizal (VAM) fungi, and soil phosphorus (P) fertility on growth of Wright soybeans in microplots near Athens, Georgia, in 1987.

| | Level† | Plant height (cm) | Seed size (g/100 seed) | Maturity date (da) |
|---|---|---|---|---|
| SCN Pi | 0 | 74 | 10.9 | 150 |
| | 7 | 70 | 10.9 | 150 |
| | 42 | 63 | 10.6 | 151 |
| | 252 | 65 | 11.0 | 152 |
| Linear model‡ | | NS | ND | ** |
| VAM | − | 56 b | 10.8 a | 152 a |
| | + | 80 a | 10.9 a | 151 b |
| P | Low | 66 b | 10.6 b | 151 a |
| | High | 71 a | 11.1 a | 151 a |

Numbers in columns within experimental factors not followed by the same letter are significantly different according to analysis of variance ($P < 0.05$).

† Pi = eggs/100 cm³ soil. Level of VAM fungi: − = uninfested; + = infested. Soil P fertility: low = 33.6 kg/ha; high = 78.5 kg/ha total soil P.

‡ Results of linear regression: ** significant at $P < 0.01$; NS = nonsignificant regression although significant difference detected with analysis of variance ($P < 0.05$); ND = nonsignificant regression and no significant difference detected by analysis of variance.

ly with increasing SCN Pi at 60 ($P < 0.01$) and 90 DAP ($P < 0.05$) in microplots with VAM fungi but only at 60 DAP ($P < 0.01$) for microplots without VAM fungi (Table 4). Egg population densities were highest for both cultivars at 120 DAP in 1986 but peaked at 90 DAP on Wright soybeans in 1987. There were no treatment main effects or interactions detected for egg densities at any sampling date in 1986 (data not shown). A linear increase of egg densities in response to SCN Pi was not detected until 90 DAP ($P < 0.01$) in 1987 and persisted through 120 DAP ($P < 0.05$) only in microplots infested with VAM fungi. There was a SCN Pi × VAM interaction ($P < 0.05$) for egg densities at 90 DAP in 1987. In general, SCN population densities were greater in microplots infested with VAM fungi in 1987. No other interactions were detected among the three experimental factors in relation to SCN soil population densities. The SCN reproductive factor was not affected by any experimental factor in 1986 but was inversely related to SCN Pi in 1987 (Table 4).

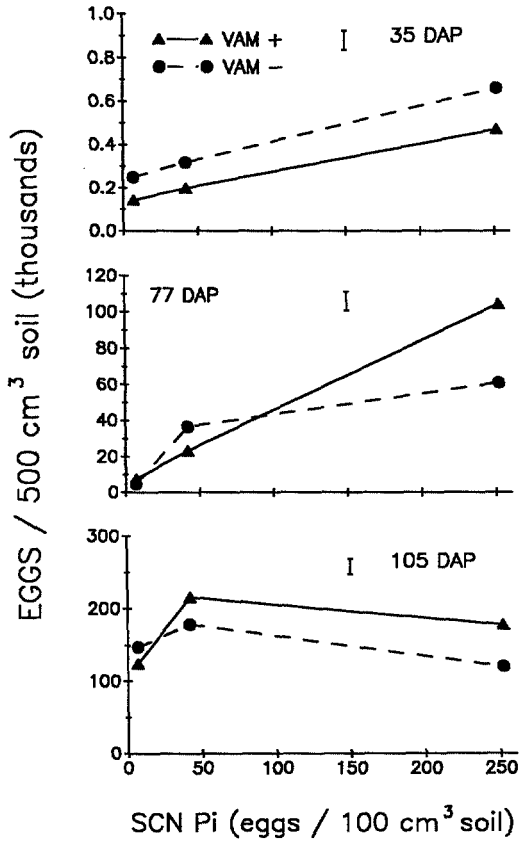


FIG. 2. Changes in soil population densities of *Heterodera glycines* (SCN) eggs in greenhouse pot cultures of Wright soybeans with (VAM +) and without (VAM −) vesicular-arbuscular mycorrhizal fungi as affected by initial SCN population density (Pi) at 35, 77, and 105 days after planting (DAP). Bars represent standard errors of the means.

*Greenhouse experiments:* Because results of the two greenhouse studies were consistent, unless otherwise stated data presented are from the second experiment in which three sampling dates were used.

Throughout the experiment VAM fungi stimulated Wright soybean growth. Mycorrhizal plants had larger roots ($P < 0.05$) 35 DAP and larger shoots ($P < 0.01$) at all three sampling dates than did nonmycorrhizal soybeans (Table 5). Shoot growth was also greater ($P < 0.05$) for high P soils than for low P soils at 77 and 105 DAP (data not shown). Suppression of plant growth due to SCN was first detected at 77 DAP. Shoot weight decreased linearly ($P < 0.01$) with increasing SCN Pi at 77 DAP

TABLE 4. Effects of *Heterodera glycines* (SCN) initial population density (Pi)† and vesicular-arbuscular mycorrhizal (VAM) fungi‡ on SCN egg and second-stage juvenile (J2) soil population densities and reproductive factors on Wright soybeans at 60, 90, and 120 days after planting (DAP) in microplots near Athens, Georgia, in 1987.

| | SCN/250 cm³ soil | | | | |
|---|---|---|---|---|---|
| | J2 | | Eggs (× 10³) | | |
| SCN Pi | VAM − | VAM + | VAM − | VAM + | RF§ |
| | | | 60 DAP | | |
| 7 | 10 | 16 | 23.9 | 33.0 | 1,624 |
| 42 | 4 | 45 | 10.4 | 39.5 | 238 |
| 252 | 34 | 119 | 44.7 | 20.7 | 52 |
| Linear model‖ | ** | ** | NS | NS | ** |
| Mean | 16 b | 60 a | 26.3 a | 31.0 a | |
| | | | 90 DAP | | |
| 7 | 159 | 184 | 24.3 | 22.1 | 1,337 |
| 42 | 223 | 694 | 18.7 | 41.4 | 290 |
| 252 | 465 | 1,053 | 45.1 | 83.7 | 103 |
| Linear model | NS | * | ** | ** | ** |
| Mean | 282 b | 643 a | 29.3 b | 49.1 a | |
| | | | 120 DAP | | |
| 7 | 186 | 201 | 16.2 | 17.0 | 962 |
| 42 | 206 | 230 | 18.7 | 26.9 | 219 |
| 252 | 280 | 301 | 22.1 | 47.0 | 55 |
| Linear model | NS | NS | NS | * | * |
| Mean | 224 a | 244 a | 19.0 b | 30.3 a | |

Numbers within the same row not followed by the same letter are significantly different according to analysis of variance ($P < 0.05$).

† Pi = eggs/100 cm³ soil.

‡ Level of VAM fungi: − = uninfested; + = infested.

§ Reproductive factor = Pf/Pi where Pf is soil J2 and egg population densities combined.

‖ Results of linear regression: * significant at $P < 0.05$, ** $P < 0.01$; NS = nonsignificant regression although significant difference detected by analysis of variance ($P < 0.05$).

regardless of the mycorrhizal status of the plants. However, root weight and stem diameter of only nonmycorrhizal plants were reduced ($P < 0.01$) by SCN parasitism at 77 DAP. All mycorrhizal and nonmycorrhizal plant growth response variables decreased linearly ($P < 0.01$) as SCN Pi increased at 105 DAP. There were interactions between SCN Pi and VAM treatments for stem diameter ($P < 0.01$) at 77 and 105 DAP and for shoot weight ($P < 0.05$) at 105 DAP. Interactions between SCN Pi and VAM treatments were not detected in the first experiment.

Soil P fertility and VAM fungi also influenced plant phenology (data not shown). Development of high P treatment plants averaged 0.5 vegetative stage or node more ($P < 0.05$) than plants grown in low P soil at 35 and 77 DAP. Mycorrhizal plants were nearly one full vegetative growth stage ahead ($P < 0.01$) of nonmycorrhizal plants at 35 DAP. Furthermore, approximately 40% of the nonmycorrhizal plants had initiated flowering at 77 DAP, but mycorrhizal plants were still vegetative. Mycorrhizal plants had more rapid and prolonged vegetative development than plants not inoculated with VAM fungi. Parasitism by SCN generally suppressed vegetative development and delayed flowering.

Soybean cyst nematode was greatly affected by VAM fungi but not soil P fertility. A P fertility main effect ($P < 0.05$) was detected only once. At 35 DAP, 31 and 44 cysts were produced per root for low and high P fertility treatments, respectively. The influence of VAM fungi on SCN changed with time. At 35 DAP, SCN reproduction and development were suppressed by VAM fungi. Fewer ($P < 0.05$) females and cysts and eggs were produced

TABLE 5. Influence of *Heterodera glycines* (SCN) initial population density (Pi)† and vesicular-arbuscular mycorrhizal (VAM) fungi‡ on growth of Wright soybeans in the greenhouse at 35, 77, and 105 days after planting (DAP).

| SCN Pi | Shoot dry weight (g) | | Root fresh weight (g) | | Stem diameter (mm) | |
|---|---|---|---|---|---|---|
| | VAM − | VAM + | VAM − | VAM + | VAM − | VAM + |
| | | | 35 DAP | | | |
| 0 | 1.7 | 2.3 | 11.7 | 13.1 | 4.3 | 4.1 |
| 7 | 2.0 | 2.3 | 11.1 | 11.1 | 4.1 | 4.0 |
| 42 | 2.0 | 2.5 | 10.7 | 12.0 | 4.6 | 4.3 |
| 252 | 1.9 | 2.4 | 10.2 | 12.0 | 4.4 | 4.2 |
| Linear model§ | NS | NS | NS | NS | NS | NS |
| Mean | 1.9 b | 2.4 a | 10.9 b | 12.1 a | 4.3 a | 4.2 a |
| | | | 77 DAP | | | |
| 0 | 13.5 | 15.4 | 41.4 | 43.7 | 7.2 | 6.6 |
| 7 | 14.3 | 15.2 | 49.8 | 40.9 | 8.0 | 6.5 |
| 42 | 8.5 | 13.1 | 36.3 | 41.4 | 6.3 | 6.7 |
| 252 | 8.3 | 11.6 | 34.9 | 34.8 | 6.6 | 6.2 |
| Linear model | ** | ** | * | NS | * | NS |
| Mean | 11.1 b | 13.8 a | 40.6 a | 40.2 a | 7.0 a | 6.5 b |
| | | | 105 DAP | | | |
| 0 | 27.5 | 27.3 | 62.8 | 55.9 | 10.3 | 8.6 |
| 7 | 14.3 | 23.5 | 54.9 | 52.9 | 8.4 | 7.9 |
| 42 | 9.1 | 15.8 | 44.0 | 45.3 | 6.8 | 7.2 |
| 252 | 6.1 | 12.1 | 33.7 | 35.9 | 6.4 | 6.6 |
| Linear model | ** | ** | ** | ** | ** | ** |
| Mean | 14.3 b | 19.7 a | 48.9 a | 47.5 a | 8.0 a | 7.5 b |

Numbers within the same row not followed by the same letter are significantly different according to analysis of variance ($P < 0.05$).

† Pi = eggs/100 cm³ soil.

‡ Level of VAM fungi: − = uninfested; + = infested.

§ Results of linear regression: * significant at $P < 0.05$, ** $P < 0.01$; NS = nonsignificant regression although significant difference detected by analysis of variance ($P < 0.05$).

on plants grown in soil infested with VAM fungi (Table 6). Soil nematode population densities and nematode numbers within roots were also reduced ($P < 0.05$) by VAM fungi at this sampling date. At all SCN Pi, egg population densities were lower ($P < 0.05$) in soils infested with VAM fungi than in uninfested soils 35 DAP (Fig. 2). Densities of J2 were affected similarly (data not shown). A SCN Pi × VAM fungi interaction ($P < 0.05$) occurred with J2 but not egg densities at 35 DAP. Soybean cyst nematode Pi had a nonlinear effect ($P < 0.01$) on fecundity with 31, 154, and 117 eggs produced per cyst for Pi 7, 42, and 252 eggs/100 cm³ soil, respectively.

At 77 DAP there was a linear increase ($P < 0.01$) in all SCN numbers with increasing SCN Pi (Table 6); however, no effect of VAM fungi on SCN numbers in roots and soil was detected. Vesicular-arbuscular mycorrhizal fungi did not affect any measure of SCN reproduction and development at this sampling date. Soybean cyst nematode Pi × VAM fungi interactions affected numbers of females and cysts ($P < 0.01$) produced on roots as well as soil J2 ($P < 0.01$) and egg ($P < 0.05$) population densities.

At the final sampling date, SCN Pi did not affect most nematode response variables. Soybean cyst nematode Pi had a nonlinear effect ($P < 0.05$) on soil egg population densities (Table 6). Similarly, fecundity was affected ($P < 0.01$) by SCN Pi but not linearly. Eggs per cyst for SCN Pi 7, 42, and 252 eggs/100 cm³ soil were 79, 67, and 66, respectively. The only nematode response variable affected by VAM fungi at 105 DAP was the production of females and cysts. Greater ($P < 0.05$) numbers of females and cysts were

TABLE 6. Effect of *Heterodera glycines* (SCN) initial population density (Pi)† and vesicular-arbuscular mycorrhizal (VAM) fungi‡ on SCN penetration and development in Wright soybean roots in the greenhouse at 35, 77, and 105 days after planting (DAP).

| | SCN/g fresh root weight | | | | | |
|---|---|---|---|---|---|---|
| | In roots | | Females and cysts | | Eggs ($\times 10^3$) | |
| SCN Pi | VAM − | VAM + | VAM − | VAM + | VAM − | VAM + |
| | | | 35 DAP | | | |
| 7 | 4 | 2 | 0.4 | 0.4 | 0.02 | 0.02 |
| 42 | 11 | 9 | 2.6 | 2.1 | 0.34 | 0.33 |
| 252 | 83 | 28 | 10.1 | 5.4 | 1.28 | 0.55 |
| Linear model§ | ** | * | ** | ** | ** | ** |
| Mean | 33 a | 13 b | 4.4 a | 2.6 b | 0.55 a | 0.30 b |
| | | | 77 DAP | | | |
| 7 | 150 | 308 | 7.0 | 6.7 | 0.5 | 0.5 |
| 42 | 631 | 800 | 47.4 | 40.5 | 4.1 | 3.0 |
| 252 | 1,146 | 1,467 | 66.5 | 109.7 | 5.3 | 6.8 |
| Linear model | ** | ** | ** | ** | ** | ** |
| Mean | 642 a | 858 a | 40.3 a | 52.3 a | 3.3 a | 3.4 a |
| | | | 105 DAP | | | |
| 7 | | | 71.9 | 60.5 | 5.3 | 5.9 |
| 42 | | | 38.1 | 89.6 | 2.9 | 5.4 |
| 252 | | | 50.6 | 94.0 | 3.8 | 5.3 |
| Linear model | | | NS | ND | NS | ND |
| Mean | | | 53.5 b | 81.4 a | 4.0 a | 5.5 a |

Numbers within the same row not followed by the same letter are significantly different according to analysis of variance ($P < 0.05$).

† Pi = eggs/100 $cm^3$ soil.

‡ Level of VAM fungi: − = uninfested; + = infested.

§ Results of linear regression: * significant at $P < 0.05$, ** $P < 0.01$; NS = nonsignificant regression although significant difference detected by analysis of variance ($P < 0.05$); ND = nonsignificant regression and no significant difference detected by analysis of variance.

recovered from mycorrhizal roots than from nonmycorrhizal roots. A SCN Pi × VAM fungi interaction ($P < 0.05$) affected soil J2 densities (data not shown) at 105 DAP, although densities did not increase with SCN Pi regardless of the mycorrhizal status of the plants. Nematode numbers within stained root samples could not be accurately counted at 105 DAP because of large numbers of nematodes in close proximity to one another.

*Split-root experiment:* For discussion purposes, half-roots and compartments of split-pots are designated A and B (Table 7). The growth of A half-roots were generally affected similarly by VAM fungi and SCN (Table 7). Only A half-roots of 0.5 SCN | 0.5 SCN plants were smaller ($P = 0.05$) than the A half-roots of all other treatments. A and B half-roots of SCN | VAM and SCN | SCN plants were of comparable size.

No differences were detected in nematode numbers within roots or egg production per gram root. However, production of females and cysts was greater ($P = 0.05$) on A half-roots of plants also inculated with VAM fungi on the same or opposite half-root than on A half-roots of plants not inoculated with VAM fungi. Although SCN soil egg densities were unaffected by VAM fungi, differences in J2 densities were detected. Soil J2 densities in A compartments of the SCN + VAM | − treatment were similar to the SCN | VAM treatment and less ($P = 0.05$) than for all non-VAM fungi treatments.

## DISCUSSION

Throughout our experiments, VAM fungi had a beneficial effect on plant growth. This effect, which has been reported previously on soybean (8,23), included increases in growth of both cultivars and in yield of Wright. The stimulatory

TABLE 7. Influence of vesicular-arbuscular mycorrhizal (VAM) fungi on half-root growth and *Heterodera glycines* (SCN) reproduction on split-roof Wright soybean plants 35 days after planting in the greenhouse.

| Treatment† A \| B | Half-root weight (g) | | SCN/g root | | | SCN/500 cm³ soil | |
|---|---|---|---|---|---|---|---|
| | A | B | Juveniles and males | Females and cysts | Eggs (× 10³) | Juveniles | Eggs (× 10³) |
| SCN + VAM \| – | 4.4 a | 2.1 d | 262 a | 25 a | 6.9 a | 223 c | 2.9 a |
| SCN \| VAM | 3.5 a | 3.7 ab | 274 a | 24 a | 10.1 a | 293 bc | 3.7 a |
| SCN \| – | 4.3 a | 2.8 cd | 382 a | 11 b | 6.4 a | 683 ab | 4.9 a |
| SCN \| SCN | 3.9 a | 4.0 a | 556 a | 14 b | 6.1 a | 720 a | 4.3 a |
| 0.5 SCN \| 0.5 SCN | 2.5 b | 3.1 bc | 398 a | 9 b | 3.6 a | 617 abc | 4.1 a |
| VAM \| – | 3.9 a | 2.7 cd | | | | | |

Numbers in columns not followed by the same letter are significantly different according to a Fisher's protected LSD test ($P = 0.05$).

† Treatments for A half and B half of root systems; SCN = 200 eggs/100 cm³ soil; 0.5 SCN = 100 eggs/100 cm³ soil; VAM = soil infested with VAM fungi; – = soil not infested with SCN or VAM fungi.

effect of VAM fungi on soybean growth apparently resulted from increased P nutrition of mycorrhizal plants. High soil P fertility resulted in increases in plant growth similar to those elicited by the VAM fungi, although this response was not observed consistently.

Soybean cyst nematode parasitism resulted in decreased growth of both cultivars in field and greenhouse experiments. The suppression of yield in microplots at relatively low SCN population densities emphasizes the high potential for economic loss when soybeans are grown in SCN-infested fields. Wright soybeans colonized by VAM fungi were more tolerant, or less sensitive (10), to SCN than nonmycorrhizal plants, but only in greenhouse experiments. In these studies, growth of Wright soybeans was suppressed with increasing levels of SCN parasitism regardless of the mycorrhizal status of the plants. Significant SCN × VAM fungi interactions indicated that growth suppression was less when plants were colonized by VAM fungi. However, tolerance to plant-parasitic nematodes, as measured by soybean yield, can be evaluated effectively only in field environments.

Suppression of SCN reproduction and development by VAM fungi was detected in greenhouse and split-root experiments. The antagonism, however, was transient. In contrast, VAM fungi suppressed *M. incognita* on soybean at 95 DAP or later (9,16,26), but they had no effect on nematode numbers through 70 DAP (16). Early suppression of SCN by VAM fungi in our experiments was due to some biological phenomenon unrelated to increased P nutrition of mycorrhizal plants. High P fertility treatments generally had no effect on nematode numbers. Nematode suppression might be the result of competition for plant nutrients between the VAM fungi and SCN. Vesicular-arbuscular mycorrhizal fungi are very dependent on host photosynthates during the early stages of root colonization, when fungal structures are formed throughout much of the root cortex and before production of extraradical hyphae and spores (14). Under conditions of poor soil fertility, colonization of host roots by VAM fungi can actually suppress plant growth (1,3). This period of intense VAM fungal biosynthesis coincides with the establishment of feeding sites by initial generations of SCN. Insufficient nutrients for early SCN generations could result in decreased nematode reproduction. Metabolites of VAM fungal biosynthesis also could have adversely affected nematode development.

The lack of long-term suppression of SCN by VAM fungi might be the result of differences in the carrying capacities of mycorrhizal and nonmycorrhizal soybeans later in the experiment. Carrying capacity determines the maximum population density or saturation level of an organism that a system is capable of supporting (21) and is particularly applicable to obligately par-

asitic plant nematodes. By 77 DAP, nonmycorrhizal root systems were visibly discolored and somewhat senesced, whereas mycorrhizal roots appeared to be healthy and actively growing. Consequently, although root weights were similar, there might not have been comparable amounts of healthy tissue present on mycorrhizal and nonmycorrhizal root systems for SCN development and reproduction. The carrying capacity of any host–pathogen system should be reached first at the highest inoculum level where disease pressure is greatest. In our study, differences in nematode numbers on mycorrhizal and nonmycorrhizal plants at 77 DAP were apparent only at the highest Pi. By 105 DAP, the carrying capacities of both mycorrhizal and nonmycorrhizal root systems at all SCN Pi apparently were reached. The development of maximum final SCN population densities after only 105 days, as observed in our greenhouse experiments, was most likely caused by root confinement. However, mycorrhizal and nonmycorrhizal root systems probably would have more comparable carrying capacities in field environments. If so, suppression of SCN by VAM fungi may be less transient under field conditions.

Results of the split-root experiment indicate that VAM fungal suppression of SCN at 35 DAP was not systemic. Soil J2 population densities were lowest when VAM fungi were coinoculated on the same half-root. Variability in soil J2 densities precluded detection of significant differences between treatments with SCN and VAM fungi on separate half-roots and treatments not inoculated with VAM fungi.

Soybean cyst nematode population densities were greater in microplots infested with VAM fungi than in those microplots that were not infested. The differences in SCN densities detected in 1987 microplots were, most likely, a reflection of the growth of mycorrhizal and nonmycorrhizal Wright soybeans under severe drought stress. In that dry year, the VAM fungi probably enhanced water uptake of the plants, resulting in larger root systems. This could not be established as the basis for the increase in SCN population densities associated with VAM fungi because root growth could not be monitored easily in microplots.

In our experiments, lack of consistent suppression of SCN by VAM fungi in the greenhouse and in microplot studies may have been caused by environmental conditions. Nematode suppression by VAM fungi must occur consistently under field conditions in all environments for this phenomenon to be of practical importance to soybean production. If VAM fungal suppression of SCN can be documented in natural soils, cultural practices could be employed to promote the increase of these common soil-inhabiting fungi resulting in increased soybean yields and decreased levels of SCN parasitism and accompanying yield suppression.

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