Interactions Between a Vesicular-Arbuscular Mycorrhizal Fungus and Root-Knot Nematode on Soybean

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ABSTRACT

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In greenhouse tests, roots of cultivar Pickett soybean plants were inoculated with chlamydospores of *Glomus macrocarpus* and/or eggs of *Meloidogyne incognita* by using an aluminum foil funnel technique. Soybeans were inoculated with one organism 10 days prior to the second organism or with both organisms simultaneously either at or 10 days after planting. After 14 wk, plants which were infected with both organisms had significantly fewer galls per gram of root, greater root weights, and higher yields than did those infected with the nematode alone. Chlamydospore production and percentage of mycorrhizal roots were not significantly different in the presence or absence of nematodes. Atypical hyphae, vesicles, and arbuscules of the fungus were observed in galled tissue and 57% of the galls examined were not associated with mycorrhizal roots. With the level of nematode inoculum used in these tests, the presence of *G. macrocarpus* reduced the number of galls produced by *M. incognita*; the presence of the nematode affected mycorrhizal development in the immediate area of the gall, but had little effect on the mean percentage of mycorrhizal roots or the number of chlamydospores produced by the fungus.

Additional key words: endomycorrhizal fungus, *Glycine max*.

Vesicular-arbuscular (VA) mycorrhizal fungi influence the nutritional status and physiology of host plants, and increase or decrease the susceptibility of the host to pathogens (12,17). Root-knot nematodes also alter the physiology of roots and induce galling (3,8). Previous studies on interactions between VA mycorrhizal fungi and sedentary endoparasitic nematodes indicate that interactions vary with the host, fungus, and nematode involved, and may also be influenced by inoculum densities (1,2,5,10,16,19,20).

Schenck et al (19) observed that differences in the interactions between VA mycorrhizal fungi and root-knot nematodes on soybeans (*Glycine max* [L.] Merr.) depended on the species of fungus involved and on the susceptibility of the cultivar to the nematode. With plants of the root-knot nematode-susceptible cultivar, Pickett, yield and root weight were increased when plants were inoculated with *Glomus macrocarpus* var. *macrocarpus* (Nicol. & Gerd.) Gerd. & Trappe, but fewer larvae of *Meloidogyne incognita* (Kofoid & White) Chitwood were recovered from plants colonized by this fungus compared to other species of mycorrhizal fungi. A similar reduction in numbers of larvae was observed on a root-knot nematode-resistant cultivar, Forrest, inoculated with *Gigaspora heterogama* (Nicol. & Gerd.) Gerd. & Trappe (19).

This paper reports further observations on the interaction between *G. macrocarpus* and *M. incognita* on Pickett soybean. Experiments were designed to determine the effects of root colonization by the fungus or nematode on subsequent root colonization by either organism. In addition, nematode galls, fungus colonization and sporulation on soybean roots were observed periodically.

MATERIALS AND METHODS

Soybean seeds of cultivar Pickett were planted in aluminum foil funnels containing soil previously infested with either the fungus or the nematode. The funnels were similar to those described by Gerdemann (7) and were constructed by firmly molding aluminum foil around a plastic funnel with a diameter of 10 cm and a length of 12 cm. The plastic funnel was then removed, the base of the aluminum foil funnel was covered with cheesecloth, and the apparatus supported by an inverted styrofoam cup (47 ml) (Fig. 1). Upon removal of the foil, the root mass was transplanted with minimal disturbance of the root system. Prior to planting, seeds were surface disinfested in 0.53% NaOCl for 3 min, rinsed with deionized water, and incubated in plastic petri dishes on moist filter paper for 30–40 hr at 27 C. Each funnel held ~150 g of a sandy soil (92.9% sand, 4.4% silt, 2.7% clay; 1.1% organic matter; Ca, 360

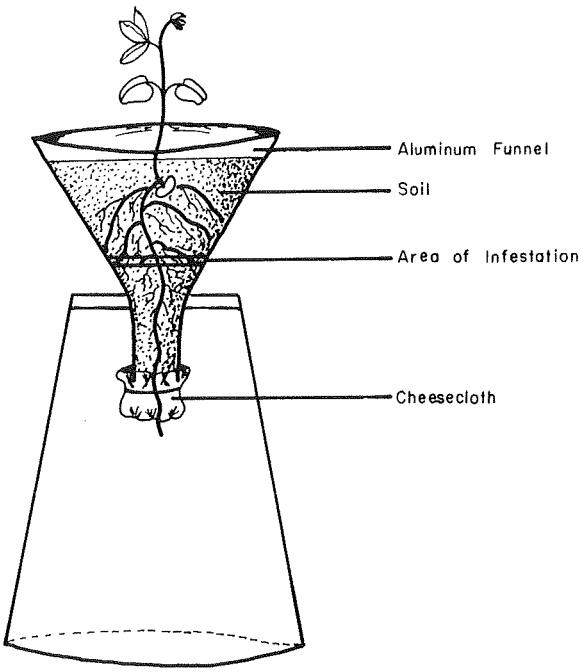

Fig. 1. Aluminum foil funnel used for initial growth of soybean seedlings inoculated to test interactions with *Glomus macrocarpus*, *Meloidogyne incognita*, or both.

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μg/g; Mg, 61 μg/g; P, 4 μg/g; and K, 70 μg/g) autoclaved twice at 120 C on two consecutive days for 4 hr. The initial soil pH of 5.3 was adjusted to 6.2–6.5 by the addition of $Ca(OH)_2$ to autoclaved soil (21). Chlamydospores used in all tests were produced in soybean pot culture (11) and stored until use at 4–6 C in soil dried to approximately 2% moisture content. A single egg mass isolate of *M. incognita*, originally obtained in 1974 by D. W. Dickson, University of Florida, from a tobacco field in Gilchrist County, Florida, was maintained on tomato (*Lycopersicon esculentum* Mill. 'Rutgers'). Eggs were collected as described by Hussey and Barker (9). Necks of the funnels were filled with 50 g of soil onto which either 25 chlamydospores of *G. macrocarpus* suspended in Ringer's solution (6) or 100 *M. incognita* eggs suspended in deionized water, or both, were poured. Suspensions from which the inocula were removed were added to the controls to establish similar microflora in all treatments. After covering the inoculum with 50 g of soil, a pregerminated and surface disinfested seed was placed in each funnel and covered with an additional 50 g of soil. After 10 days in the greenhouse, the plants with soil and roots intact were removed from the funnels and placed in funnel-shaped depressions made in the soil in 15-cm diameter plastic pots. Prior to transplanting, either 25 *G. macrocarpus* chlamydospores in Ringer's solution or 100 *M. incognita* eggs in deionized water, or both, were poured around the sides of the funnel-shaped depression on the soil. Suspensions from which the inocula had been removed by filtration were added similarly to the controls. The seven treatments included in this study were: mycorrhizal fungus added at planting, nematode added at planting, nematode and mycorrhizal fungus added together at planting, nematode and mycorrhizal fungus added together at transplanting, nematode added at planting and mycorrhizal fungus added at transplanting, mycorrhizal fungus added at planting and nematode added at transplanting, and neither organism.

The experimental materials were maintained in a greenhouse with air temperature ranging from 22–28 C. This test was conducted twice with four or five replications. Commercial *Rhizobium* sp. inoculum was applied with the seed in the second test.

Shoot and root fresh weight, number of nodules per plant, percentage of roots colonized by the fungus, number of galls per plant, and the number of chlamydospores per 100 cm^3 of soil were determined 3, 5, and 8 wk after planting. Four soil cores were removed from each pot with a no. 13 cork borer, and 100 cm^3 of this soil was assayed for fungal chlamydospores and nematode larvae. Chlamydospores were collected on a 105-μm sieve and counted under a dissecting microscope (× 30). Nematode larvae were collected from the same soil sample on a 44-μm sieve and were extracted by centrifugation. Roots were washed, blotted dry, and weighed, and the number of galls were counted. Approximately 2.5–3.0 g of roots were randomly selected from each plant, cleared by heating in 10% KOH, and stained with 0.05% Trypan blue (13). These roots were dispersed in 15 ml of water in a 7-cm petri dish and observed under a dissecting microscope (× 10–70). Vertical and horizontal scans were made of the entire root sample, and mycorrhizal and nonmycorrhizal portions of roots visible in each field of vision were counted. The mean percentage of mycorrhizal roots of each plant was estimated from these numbers.

In another experiment, seeds were planted in soil previously infested with either 25 chlamydospores of *G. macrocarpus* or 100 *M. incognita* eggs, or both, as previously described. The four treatments included: nematode alone, mycorrhizal fungus alone, nematode and mycorrhizal fungus together, and controls. After 10 days, the aluminum foil was removed and the soil and root mass was transferred to 15-cm diameter plastic pots which contained approximately 1.5 kg of soil. This experiment was conducted twice with four or five replications in a randomized complete block. Shoot and root fresh weight, percentage of the root system which was mycorrhizal, number of galls per plant, number of chlamydospores per 100 cm^3 of soil, and number of *Rhizobium* sp. nodules per plant were recorded 8, 10, 12, and 14 wk after initiation of the experiment. Dry seed weight was determined after 14 wk. Nodulation occurred from the natural infestation of *Rhizobium* sp. already present in the greenhouse. Treatment means in all tests were compared according to Duncan's multiple range test.

To determine the relationship of nematode galls to the position of the mycorrhizal fungus, galls occurring in mycorrhizal roots were removed and examined under a compound microscope (× 400). Observations were made on the development of the VA mycorrhizal fungus in and near 582 galls. The positions of the galls on the roots in relation to the location of the fungus were classified as follows: galling not directly associated with the fungus; galling with the fungus above or below, but not within, the hyperplastic tissue; the gall with the fungus above and below, but not within, the hyperplastic tissue.

RESULTS

Soybean seedlings grew well in the funnels. The cotyledons emerged 5–6 days after planting when the primary root was 10–12 cm long and had grown out of the bottom of the funnel and had become desiccated. After desiccation of the primary root, lateral root development increased on the radicle in the area immediately above the neck of the funnel. *Glomus macrocarpus* colonized at least one site in all root systems examined 10 days after planting. The time required for maximum penetration by *M. incognita* was 9 days after planting. Gall formation on roots was observed 11 and 13 days after planting.

There were no significant differences among treatments in fresh root weight, number of nodules per plant, number of galls per plant, percentage of mycorrhizal roots, or number of chlamydospores produced by the fungus 3, 5, and 8 wk after planting. *M. incognita* induced an average of seven galls per plant in all treatments and larvae were not recovered from the soil. Plants inoculated with nematodes alone had significantly lower fresh root weights and more galls 14 wk after planting than did plants associated with the other three treatments (Table 1). There were fewer galls per gram of root on the mycorrhizal plants than on the nonmycorrhizal plants on all dates examined; however, these differences were not significant until 12 and 14 wk after planting (Fig. 2). Nematode larvae were not recovered from the soil.

The number of chlamydospores per plant and the percentage of the root system colonized by *G. macrocarpus* were not significantly

TABLE 1. Effect of the vesicular-arbuscular mycorrhizal fungus, *Glomus macrocarpus*, and the root-knot nematode, *Meloidogyne incognita*, on the fresh root weight, number of galls and dry seed weight of soybean[w]

Inoculation[x]	Fresh root weight (g) at:				Galls per plant (no.) at:				Dry seed wt (g)
	8 wk[yz]	10 wk	12 wk	14 wk	8 wk	10 wk	12 wk	14 wk	14 wk
Control	9.1 a	11.9 a	13.0 a	13.4 a	...	...	...	...	2.2 c
M. incognita	7.5 a	11.3 a	10.3 a	11.2 b	101.7 a	154.0 a	318.7 a	428.9 a	1.8 c
G. macrocarpus	9.3 a	14.0 a	14.8 a	16.4 a	...	...	...	...	3.5 a
G. macrocarpus + *M. incognita*	9.6 a	13.9 a	11.8 a	15.8 a	57.6 a	88.2 a	99.5 b	254.4 b	3.0 b

[w] Each number represents the mean of all replications.
[x] Plants were inoculated in aluminum foil funnels with chlamydospores of *G. macrocarpus* and/or eggs of *M. incognita*.
[y] Plants were examined 8, 10, 12, and 14 wk after planting.
[z] Numbers in the same vertical column followed by the same letter do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

different in the presence or absence of nematodes. After 14 wk, colonization averaged 49% for plants inoculated with the fungus alone, and 48% for plants inoculated with both the nematode and the fungus. The number of chlamydospores produced apparently increased exponentially (Fig. 3).

Fifty-seven percent of the root galls occurred in root sections not colonized by *G. macrocarpus*. In 29% of the galls, the fungus colonized the root above or below the gall, and in 14% of the galls the fungus colonized the root both above and below the gall. The 57:29:14 ratio was independent of date of sampling over the 6 wk period (χ^2, $P = 0.05$).

Approximately 40% of the galls were in mycorrhizal roots, and microscopic examination revealed several instances in which the fungus was located in galled tissue. In the galls it appeared that arbuscules, which were once continuous with the fungal structures above and below the gall, were decomposing within the gall. In a few instances the fungus had grown completely through a gall on the side of the stele opposite the nematode, but the fungus was never observed in a gall on all sides of the stele. Mycorrhizal structures contiguous to the galls were atypical, and characterized by numerous small round vesicles and lysing hyphae. Hyphae grew along the surface of many galls. Appressorium formation and penetration of the galled tissue by *G. macrocarpus* was never observed.

Dry seed weight produced by plants colonized by the fungus alone was significantly greater ($P = 0.05$) than in any of the other treatments. Plants with both the nematode and the fungus yielded less than plants with the fungus alone, but more than plants infected by the nematode alone and the controls (Table 1).

DISCUSSION

Soybeans grew well in the aluminum foil funnels and the method was useful for achieving rapid infection by *M. incognita* and *G. macrocarpus*. The funnels were easily removed to leave a soil-root mass which could be transplanted with minimal root system disturbance. Under the conditions of these experiments, initial infection of a host plant by either *G. macrocarpus* or *M. incognita* did not predispose it to later infection by either organism. It is possible that higher initial inoculum densities of the nematode might affect the initial colonization by the fungus. It is doubtful that increasing the initial inoculum level of the fungus would exert an effect on the number of nematodes that initially penetrate a soybean seedling because the nematode requires only a few hours to infect a root (3), while the fungus requires at least 10 days to become established in the roots.

Although mycorrhizal plants had larger root systems than did nonmycorrhizal plants, the total number of galls produced on each mycorrhizal plant was less than the number of galls produced on nonmycorrhizal plants. Fewer galls in mycorrhizal roots could result from a reduced ability of the nematode to penetrate a mycorrhizal root, or the presence of the fungus may influence the development of giant cells which could, in turn, interfere with development of the nematode. The results are compatible with those of Sikora and Schönbeck (20), who reported a significant reduction in the number of *M. incognia* larvae that were able to reach adulthood in plants with a well established mycorrhizal root system. Reduced numbers of migratory endoparasitic nematodes in mycorrhizal cotton also have been observed (10). Other workers, however, have reported greater numbers of root-knot larvae recovered from mycorrhizal plants than from nonmycorrhizal plants and related the increase to the increased size of the mycorrhizal root system (1,15).

Mycelia, arbuscules, and vesicles of *G. macrocarpus* were observed in hypertrophied cortical tissue within galls. No gall was observed to be colonized by the fungus on all sides of the stele. Galls were observed in which the fungus appeared to be disintegrating. This may indicate that physiological changes induced by the nematode affected the symbiotic relationship and resulted in a less favorable environment for the fungus. Disintegration of the fungus within the hyperplastic tissue may have occurred in some of the galls observed to have contiguous mycorrhizal development. Certain pathogenic soil fungi have been shown to readily invade galls produced by *M. incognita* (14). In contrast, the results of our study indicate that nematode- or *Rhizobium* sp.-galled soybean root tissue is relatively unfavorable for the development of *G. macrocarpus*. A similar effect was reported on grape in which VA mycorrhizae developed in close proximity to phylloxera galls, but not in the gall tissue (4). Hyphae and vesicles of VA mycorrhizal fungi in root-knot galls of tobacco and carrot were reported, but the numbers of galls or the spacial relationship of galls to mycorrhizal root areas were not reported (20). These contrasting results may be due to different host-fungus combinations or to the stage of development of the gall or the mycorrhizae at the time of observation.

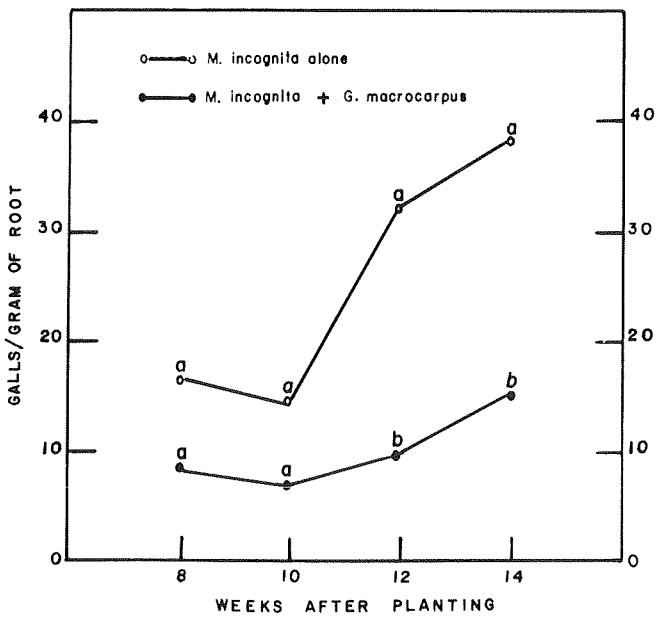

Fig. 2. Number of galls per gram of root tissue of soybean plants inoculated with *Meloidogyne incognita* alone or with both *M. incognita* and *Glomus macrocarpus*. Values represent averages from two tests. Values on the same date with the same letter do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

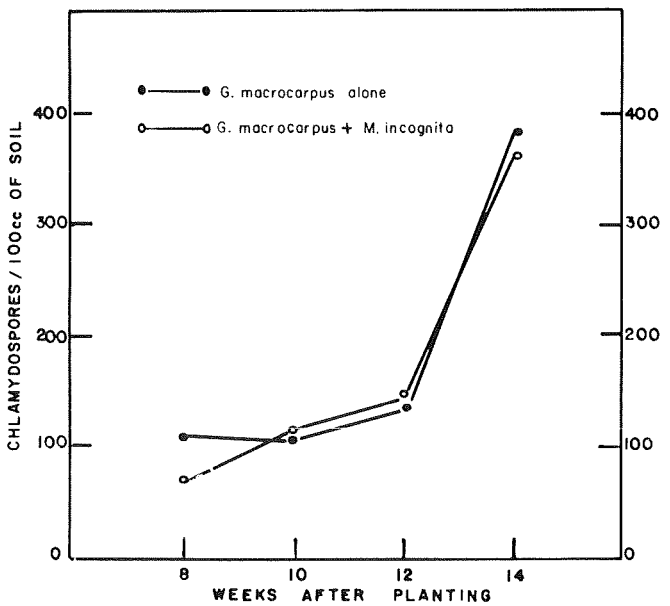

Fig. 3. Number of chlamydospores per 100 cm^3 of soil produced on soybean plants inoculated with *Glomus macrocarpus* or *G. macrocarpus* and *Meloidogyne incognita*.

Field observations relating the incidence of VA mycorrhizal fungi to the incidence of endoparasitic nematodes indicated that high populations of either organism result in low populations of the other (2,15,18). Results of this study indicate that *G. macrocarpus* and *M. incognita* are mutually inhibitory. Fewer galls were produced on highly mycorrhizal plants, but the galls were unsuitable colonization sites for the fungus; therefore, in situations with high populations of root-knot nematodes, galling might be a limiting factor on the development of *G. macrocarpus* on soybean.

The influence of both *G. macrocarpus* and *M. incognita* on the host was reflected in the yield data. Dually inoculated plants had intermediate yields, which indicated that the beneficial effect of the fungus did not completely compensate for the damage caused by the nematode.

Since interactions between VA mycorrhizal fungi and root-knot nematodes vary with the host plant cultivar (19,20), different fungus-host cultivar- nematode combinations should be evaluated, and various inoculum densities should be employed to determine if there is a population threshold of the pathogen, above which VA mycorrhizal fungi can no longer function as antagonists.

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