Interaction of Pratylenchus brachyurus and Gigaspora margarita on Cotton

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Abstract: An endomycorrhizal fungus, *Gigaspora margarita,* was more effective in stimulating the growth of cotton *(Gossypium hirsutum)* 'Coker 201' at a low fertility level (1.77 gm 10-10-10 N-P-K/pot) than doubling the fertility rate for nonmycorrhizal plants. *Gigaspora margarita* alone stimulated shoot growth (height, weight, and flower production by 96%, 553%, and 760%, respectively) and root growth (385%) over that of nonmycorrhizal controls at low fertility. Plant development was also stimulated by *G. margarita* at the high fertility level (3.54 gm 10-10-10 N-P-K/pot), but the magnitude of the increase was not as great as that at the low fertility level. Although cotton was a suitable host for *Pratylenchus brachyurus,* plant development was not retarded by this nematode at either fertility level. In concomitant culture, mycorrhizal-induced plant growth, and sporulation of the endomycorrhizal fungus were not affected by *P. brachyurus.* Reproduction of *P. brachyurus* was similar on mycorrhizal and nonmycorrhizal cotton. *Key Words: Gossypium hirsutum,* endomycorrhizal fungus.

Little is known about the interaction of plant-parasitic nematodes and endomycorrhizal root symbionts even though they commonly occur together in the roots or rhizosphere of the same plant. We previously reported that when root-knot-nematode-resistant and susceptible cotton, *Gossypium hirsutum* L., cultivars were jointly inoculated with *Gigaspora margarita* Becker and Hall and *Meloidogyne incognita* (Kofoid & White) Chitwood, the beneficial effect of the endomycorrhizal development offset the nematode damage to the susceptible cultivar (8). Endomycorrhizal stimulation of growth of susceptible and resistant cultivars was unaffected by the parasitic activities of the root-knot nematode. Sporulation of the mycorrhizal fungus was highest in the concomitant culture, whereas the larger root systems on the mycorrhizal cotton plants increased nematode reproduction only on the susceptible cultivar. Reproduction of *M. incognita* increased on mycorrhizal soybean, although this interaction varied with different *Endogone* species and soybean cultivars (12). Fox and Spasoff (2) reported that *Heterodera solanacearum* and *E. gigantea* mutually suppressed the reproduction of the other on tobacco.

The plant-parasitic nematodes used in previous studies were sedentary endoparasites. Except for a few observations, interactions of endomycorrhizal fungi with plant-parasitic nematodes with other types of feeding habits have not been investigated. A field study showed that soil fumigation which controlled *Pratylenchus brachyurus* (Godfrey) Filip. & Schuur.-Stekh. and other plant-parasitic nematodes increased endomycorrhizal synthesis in cotton roots (1). Ruehle (9) observed that structures of an endomycorrhizal fungus deteriorated in lesions formed by *P. brachyurus* on feeder roots of *Liriodendron tulipifera* L.

This paper reports on the interaction of a migratory endoparasitic nematode *(P. brachyurus)* with an endomycorrhizal fungus *(G. margarita)* on cotton at two different fertility levels.

MATERIALS AND METHODS

Five-day-old 'Coker 201' cotton seedlings, germinated in vermiculite, were transplanted singly into 20-cm plastic pots containing methyl-bromide-fumigated soil. The soil was a Marlboro loamy sand obtained from a field that had been out of

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agricultural production for several years. Prior to potting, the soil was analyzed by the University of Georgia Soil and Plant Testing Laboratory, Cooperative Extension Service. The soil had a pH of 4.7, and contained 2.3% organic matter, 6 mhos x 10^{-5} soluble salts, and the following elements: NO_3N 50, P 14, K 28, Ca 108, Mg 35, Zn 8, Mn 30, and B 0.8 μg/ml. The soil was then mixed with vermiculite and washed river sand (4:1:1 v/v/v) and the pH was adjusted to 6.3 with hydrated lime. Soil mixtures with low or high fertility levels, equivalent to 560 and 1,120 kg 10-10-10 N-P-K/ha, were prepared during mixing by adding 1.77 gm or 3.54 gm 10-10-10 N-P-K/pot, respectively (8).

Pratylenchus brachyurus was propagated on *Glycine max* (L.) Merr. 'Lee' grown in pots and extracted from roots placed in a modified Seinhorst mist chamber (11). Fourteen days after transplanting, 5,000 nematodes were added to the soil around the base of each designated plant.

Azygospores of *G. margarita* were increased on *Sorghum vulgare* cv. *roxburghii* (Stapf) Haines in pot culture and extracted from the soil by using a centrifugal-flotation method (5). Plants designated to become mycorrhizal were inoculated with 250 spores/seedling in 25 ml of water during transplanting. A 25-ml aliquant of spore suspension filtrate, previously passed through Whatman #1 filter paper, was added to the soil in each pot in all non-mycorrhizal treatments to standardize microflora.

Treatments consisted of inoculations with *P. brachyurus* or *G. margarita,* inoculations with both, and appropriate controls (Table 1). All treatments were evaluated at two fertility levels. Treatments were replicated 10 times and arranged in a randomized complete-block design on a greenhouse bench. The entire test was repeated once.

Seventy-seven days after transplanting, shoot height, fresh shoot and root weights, and number of flowers were recorded. Roots were stained with acid fuchsin, cleared with chloral hydrate (6), and examined under a stereoscopic microscope for nematodes and mycorrhizal development. The McBryde method (6) used for staining mycorrhizal roots infected with nematodes was superior to other procedures (1) which stain the symbiont but destroy the nematodes. Azygospore and nematode populations in the soil were determined by the centrifugal-flotation method (5). Roots of plants inoculated with *P. brachyurus* were incubated in the mist chamber for 29 days, and nematodes were collected and counted every 3-8 days.

RESULTS AND DISCUSSION

Although the higher fertility level enhanced the growth and reproduction of cotton, plant development was most pronounced when plants were inoculated with *G. margarita* (Fig. 1-A, B). Shoot height and weight of mycorrhizal plants were increased 96% and 553%, respectively, over

TABLE 1. Influence of single and combined inoculations with *Gigaspora margarita* and *Pratylenchus brachyurus* on cotton growth and reproduction, and sporulation of the mycorrhizal fungus.

Treatment			Shoot height cm	Shoot weight gm	Root weight gm	No. flowers/ plant	Azygospores/ 100 cm^3 soil (in 1000's)
Mycorrhizal fungus	Nematode	Fertility[x]					
None	None	Low	23.7 c[y]	8.5 d	5.7 c	0.0 d	
None	None	High	31.2 b	21.6 c	10.0 c	3.2 c	
G. margarita	None	Low	46.5 a	55.5 ab	26.1 b	7.6 b	3.1 a
G. margarita	None	High	49.1 a	62.2 a	39.1 a	11.5 a	2.9 a
None	*P. brachyurus*	Low	22.7 c	7.7 d	4.3 c	0.1 d	
None	*P. brachyurus*	High	30.1 b	18.3 c	8.3 c	1.6 cd	
G. margarita	*P. brachyurus*	Low	44.1 a	50.5 b	27.7 b	7.9 b	3.8 a
G. margarita	*P. brachyurus*	High	49.2 a	60.3 a	32.9 ab	11.5 a	2.6 a

[x]Low = 1.77 gm 10-10-10 N-P-K/pot; high = 3.54 gm 10-10-10 N-P-K/pot.
[y]Column means followed by the same letter are not different according to Duncan's multiple range test ($P = 0.05$).

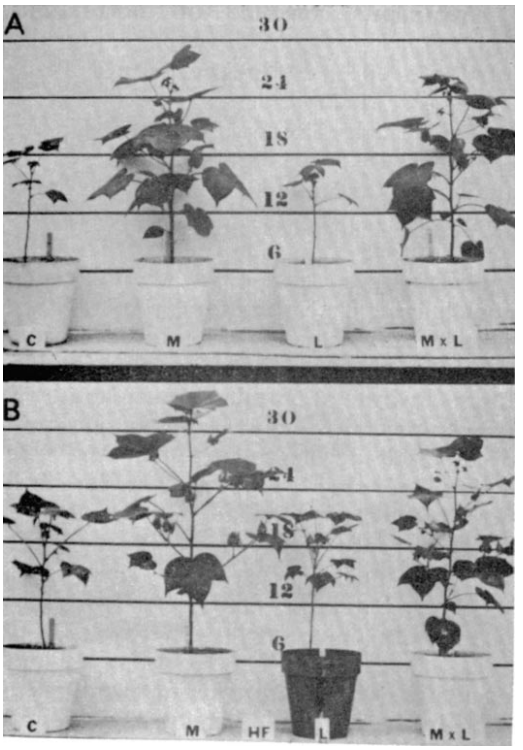

FIG. 1-(A-B). Influence of *Gigaspora margarita* and *Pratylenchus brachyurus*, singly and combined, on growth of cotton at two different fertility levels (1.77 or 3.54 gm 10-10-10 N-P-K/pot). *A)* Low fertility; left to right, C = check, M = *G. margarita*, L = *P. brachyurus*, and MxL = combined inoculation. *B)* High fertility; same treatments as in A.

that of the nonmycorrhizal control plants at low fertility (Table 1). Mycorrhizal development increased root growth by 358%, whereas flower production was increased 760%. Growth increases were also noted for mycorrhizal plants at the high fertility level, although the stimulation was not as great as for plants growing at the low fertility level. Shoot height and weight, root weight, and flower production were increased 57%, 188%, 291%, and 260%, respectively, on mycorrhizal plants at the high fertility level (Table 1). Doubling the fertility rate for nonmycorrhizal check plants, however, resulted in increases of only 32% and 154% for shoot height and weight, respectively. Root weight and flower production on these plants were increased 75% and 320%, respectively. Consequently, greater increases in growth and reproduction of cotton occurred when plants were inoculated with a mycorrhizal symbiont than when the soil fertility level was doubled. The stimulation in growth induced by the fungus symbiont was similar to previous results obtained with different cotton cultivars (8) and other crops (3).

Sasser (10), in a review of nematode diseases of cotton, indicated that, although cotton is a suitable host for certain *Pratylenchus* spp., there is insufficient evidence to suggest that any of these nematodes cause appreciable damage to this crop. In our work *P. brachyurus* did not affect the growth of mycorrhizal or nonmycorrhizal plants, regardless of fertility level (Fig. 1-A, B; Table 1). *Meloidogyne incognita* also had little effect on the mycorrhizal-induced growth of *M. incognita*-susceptible and -resistant cotton cultivars (8). Root-knot nematode damage to the susceptible cultivar, however, was offset by mycorrhizal synthesis.

Sporulation of *G. margarita* was not affected by *P. brachyurus* (Table 1). Stained and cleared root sections of mycorrhizal plants showed that the cortical tissue was extensively colonized with arbuscules and coiled hyphae of *G. margarita*. Nematodes were rarely found in cortical root tissue extensively colonized by the symbiont, but instead were primarily present in nonmycorrhizal roots. Ruehle (9) reported that when *P. brachyurus* produced large lesions on mycorrhizal feeder roots of *Liriodendron tulipifera* seedlings and collapsed the cortical cells, the fungal structures within these cells deteriorated. Although structures of an endomycorrhizal fungus were not observed in *M. incognita*-galled tissue on cotton roots, azygospore production was greatest on cotton roots inoculated with both organisms; however, this increase was variable (8). Schenck et al. (12) showed *M. incognita* decreased spore production of *Endogone calspora* on root-knot-susceptible Ransom soybean but increased azygospores on nematode-resistant Forest at a low level of nematode inoculum.

Extraction of *P. brachyurus* from the cotton roots in the mist chamber during a 29-day period resulted in two peaks of nematode recovery (Fig. 2). If the roots had been extracted for only 7 days, a period of time often reported for *Pratylenchus* studies (4, 13), only 30% and 40% of the nematode population would have been recovered from the mycorrhizal and nonmycorrhizal roots.

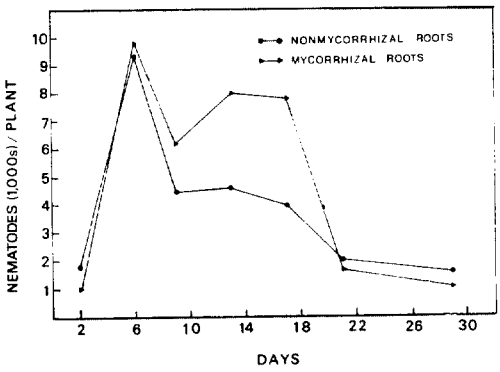

FIG. 2. Extraction of *Pratylenchus brachyurus* from mycorrhizal and nonmycorrhizal cotton roots in a mist chamber at different time intervals.

respectively, and this amount would have given us a conservative total population estimate in the roots. Most of the adult female nematodes were recovered by 9 days. Nematodes recovered at 13 and 17 days consisted primarily of juvenile stages which hatched from eggs laid in the root tissue during the study and which, therefore, contributed to the total population. The soil counts of *P. brachyurus* were very low and are not reported herein.

Reproduction of *P. brachyurus* may have been affected by synthesis of *G. margarita* in the cotton roots. Nematode counts/gm of root were lower in mycorrhizal plants than in roots of plants inoculated with only *P. brachyurus* (Fig. 3). The total number of nematodes/plant did not increase in concomitant culture

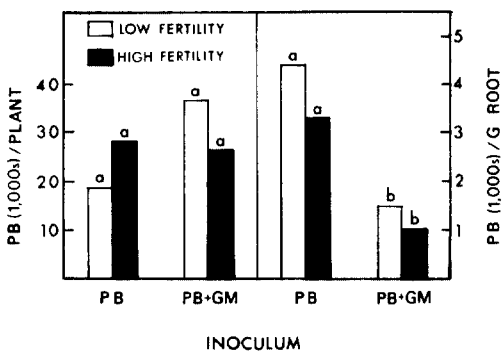

FIG. 3. Reproduction of *Pratylenchus brachyurus* (PB) on cotton when inoculated alone or combined with *Gigaspora margarita* (GM) at two fertility levels (1.77 or 3.54 gm 10-10-10 N-P-K/pot). Graph values followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test ($P = 0.05$).

even though the root systems were 291-385% larger on the mycorrhizal plants. The low nematode counts per gram of root in mycorrhizal plants may be simply a dilution effect from the larger root systems. However, the association of few *P. brachyurus* with cortical tissue extensively colonized by the symbiont suggests that this tissue might be an unfavorable food source for this nematode. Furthermore, arbuscules of *G. margarita* can occupy most of the interior volume of the cortical cells; therefore, the suggestion of a competition for space by both microorganisms cannot be dismissed. Additional studies are needed to determine whether the equilibrium density for *P. brachyurus* is different on mycorrhizal plants and nonmycorrhizal plants with comparable root volume. Interaction of *Endogone gigantea* with *Heterodera solanacearum* on tobacco also had a detrimental effect on nematode reproduction (2). Populations of *Pratylenchus* species have been reported to increase in interactions with the fungus pathogen *Verticillium* (7), and in our study with *M. incognita,* the larger root system on mycorrhizal cotton plants resulted in a significant increase in nematode reproduction (8).

Interactions of plant-parasitic nematodes and endomycorrhizal fungi have been shown to be either antagonistic or stimulatory. Since these organisms are ubiquitous in the rhizosphere of most agronomic plants under field conditions, determining the type of interaction from their association is important in understanding the overall effect of each organism on plant vigor, and the effect of the interaction on the nematode population. The latter effect is important in understanding the population dynamics of nematodes.

LITERATURE CITED

1. BIRD, G. W., J. R. RICH, and S. U. GLOVER. 1974. Increased endomycorrhizae of cotton roots in soil treated with nematicides. Phytopathology 64:48-51.
2. FOX, J. A., and L. SPASOFF. 1972. Interaction of Heterodera solanacearum and Endogone gigantea on tobacco. J. Nematol 4:224-225 (Abstr.).
3. GERDEMANN, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathology 6:397-418.
4. HACKNEY, R. W., and O. J. DICKERSON. 1975. Marigold, castor bean, and chrysanthe-

mum as controls of Meloidogyne incognita and Pratylenchus alleni. J. Nematol. 7:84-90.

5. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
6. MCBRYDE, M. C. 1936. A method of demonstrating rust hyphae and haustoria in unsectioned leaf tissue. Am. J. Bot. 23:686-689.
7. POWELL, N. T. 1971. Interactions between nematodes and fungi in disease complexes. Annu. Rev. Phytopathology 9:253-274.
8. RONCADORI, R. W., and R. S. HUSSEY. 1977. Interaction of the endomycorrhizal fungus Gigaspora margarita and root-knot nematode on cotton. Phytopathology 67:1507-1511.
9. RUEHLE, J. L. 1973. Nematodes and forest trees: types of damage to tree roots. Annu. Rev. Phytopathol. 11:99-118.
10. SASSER, J. N. 1972. Nematode diseases of cotton. Pages 187-214 *in* J. M. Webster, ed. Economic Nematology, Academic Press, New York. 563 p.
11. SEINHORST, J. W. 1950. De betekenis van de toestand van de grond voor het optreden van de aantasting door het stengelaaltje (Ditylenchus dipsaci Kuhn Filipjev). Tijdschr. PlZiekt. 56:289-348.
12. SCHENCK, N. C., R. A. KINLOCH, and D. W. DICKSON. 1975. Interaction of endomycorrhizal fungi and root-knot nematode on soybean. Pages 607-617 *in* F. E. Sanders, B. Mosse, and P. B. Tinker, eds. Endomycorrhizas, Proceedings of a symposium held at the University of Leeds, 22-25 July 1974. Academic Press, New York.
13. WILLIS, C. B. 1976. Effects of potassium fertilization and Pratylenchus penetrans on yield and potassium content of red clover and alfalfa. J. Nematol. 8:116-121.