Interaction of the Endomycorrhizal Fungus Gigaspora margarita and Root-Knot Nematode on Cotton

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ABSTRACT

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Inoculation of cotton (*Gossypium hirsutum* 'McNair 511' and 'Stoneville 213') with azygospores of *Gigaspora margarita* significantly increased vegetative growth and square production (flower bud initials and flowers) over nonmycorrhizal plants in greenhouse studies. Growth stimulation and plant development were greater at a low fertility rate (250 μg/g 10-10-10 NPK) than at a high fertility level (500 μg/g). Shoot weight and square production were reduced by *Meloidogyne incognita* in the nematode-susceptible Stoneville 213 cultivar, but not in the resistant McNair 511 cultivar. In joint inoculations with the endophyte and *M. incognita*, development of endomycorrhizae nullified the stunting caused by the nematode. Mycorrhizal stimulation of plant development, however, was affected little by root-knot nematode activities. Azygospore production was greatest in joint culture on McNair 511 at the high fertility rate and on Stoneville 213 at the low fertility level. Nematode egg production per gram of root was not affected by *G. margarita*, but total eggs per plant was increased on mycorrhizal Stoneville 213 since their root systems were considerably larger than those of the controls.

Additional key words: *Gossypium hirsutum*, *Meloidogyne incognita*.

The activities of root- or rhizosphere-inhabiting microorganisms exert a significant effect upon plant health. In cotton culture, both vesicular-arbuscular (VA) mycorrhizal fungi and the sedentary endoparasitic nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, (2, 9, 12) commonly infest the soil and colonize roots; each exerting a characteristic but opposite effect upon plant vigor. The obligately symbiotic endomycorrhizal fungi can stimulate plant development (9); whereas the root knot nematode is a pathogen that suppresses plant growth (12). Although both microorganisms frequently occur simultaneously in the roots or rhizosphere of the same cotton plant, the effects of their combined activities are not well understood.

Along with improving plant vigor, endomycorrhizal fungi may interact with root pathogens having a varied effect upon each member in the plant-pathogen relationship. Dehne and Schonbeck (4) demonstrated that tomato plants with VA mycorrhizae formed by *Glomus mosseae* produced less severe Fusarium wilt symptoms than nonmycorrhizal plants. Other beneficial symbiotic effects were reported by Baltruschat and Schonbeck (1), who noted that chlamydospore production by the root rot pathogen, *Thielaviopsis basicola*, was negatively correlated with VA mycorrhizal synthesis in tobacco. Mutual antagonism expressed as population reductions occurred between *Gigaspora gigantea* (*Endogone gigantea*) and *Heterodera solanacearum* on tobacco (5). In contrast, VA mycorrhiza development predisposed a susceptible soybean cultivar to Phytophthora root and stem rot (10). *Glomus mosseae*, however, had no significant effect upon *Pythium ultimum* or *Phytophthora megasperma* var. *sojae* on soybean (3). The extreme diversity of interactions between VA mycorrhizae and a root pathogen was illustrated by Schenck et al. (14). The results of their comprehensive greenhouse study, which involved soybean inoculations with *M. incognita* and three different VA mycorrhizal fungi, indicated that the effects of a given interaction were strongly influenced by nematode inoculum level, cultivar resistance to the nematode, and the specific fungal symbiont. Although VA mycorrhizae usually stimulated plant development and *M. incognita* suppressed root weight, seed yield, and fungal spore production, certain symbionts impaired nematode reproduction. They indicated that each mycorrhizal fungus-plant-nematode combination may be unique and generalizations should not be applied to other such systems without additional study.

Since field studies have shown a negative correlation between a mixed population of plant - parasitic nematodes, including *M. incognita*, and VA mycorrhizae on cotton (2, 9), we designed a greenhouse experiment to determine (i) the individual effects of the VA mycorrhizal fungus, *Gigaspora margarita*, Becker and Hall and *M. incognita* on cotton plant (*Gossypium hirsutum* L.) development, (ii) the effect of concomitant cultures of the two soil microorganisms on cotton development and their population dynamics, and (iii) the effects of fertility level and cotton cultivar on these systems.

MATERIALS AND METHODS

Cultivar selection and soil preparation.—The cotton cultivars, McNair 511 (nematode-resistant) and Stoneville 213 (nematode-susceptible), were chosen for their distinctive reactions to root-knot nematode attack. Untreated seed were germinated in rolled germination paper at 30 C for 30 hr and those with 4- to 6-mm-long radicles were used. Tests were conducted in a Marlboro loamy-sand soil which had no known recent history of use in agricultural production. The soil was analyzed by the University of Georgia Soil and Plant Testing Laboratory, Cooperative Extension Service. Prior to mixing, the results were: pH 4.7, NO_3-N 50, P 14, K 28, Ca 108, Mg 35, Zn 8, Mn 30, and B 0.8 μg/g (ppm), 2.3% organic matter, and 6×10^{-5} mhos soluble salts. The soil was screened to remove large debris and blended with No. 2 Vermiculite and washed river sand (4:1:1, v/v) in a soil mixer. During this operation, hydrated lime was added to adjust the pH to 6.3 and two fertility levels were established. A high fertility level of 500 μg/g of 10-10-10 (N = 10%, P = 4.3%, and K = 8.2%) fertilizer to approach the nutrient conditions satisfactory for cotton culture was compared with a lower rate of 250 μg/g. The soil mixes were fumigated with methyl bromide (Dowfume MC-2) at a rate of 1.36 kg/800 liters of mix for 48 hr under a plastic tarp and vented for 5 days prior to planting. The mixes were placed in 4-liter plastic pots and maintained on a greenhouse bench.

Inoculum increase and inoculation.—Azygospores of a single-spored isolate of *G. margarita*, obtained from a cotton soil, were increased on *Sorghum vulgare* '*roxburghii*' (Stapf) Haines in pot culture. Spores were extracted from the soil using a modified centrifugal-flotation technique (8). The inoculum concentration was adjusted to deliver 250 spores per pot in 25 ml of water which was poured over a germinated seed placed in the bottom of a 2.5-cm-deep hole in the soil mix. To standardize the microflora in all nonmycorrhizal treatments, a 25-ml aliquot of spore suspension filtrate gathered after passage through Whatman No. 1 filter paper was added to each pot. The seeds were covered and all pots were thoroughly watered immediately after planting.

Meloidogyne incognita was propagated in the greenhouse on tomato, *Lycopersicon esculentum* Mill. 'Rutgers', and eggs for use as inoculum were collected with 1.05% NaOCl according to the method of Hussey and Barker (7). One wk after planting, 9,000 eggs/seedling were poured into the soil around each plant.

Experimental design, plant maintenance, and collection of data.—Treatments consisted of individual inoculations with *G. margarita* and *M. incognita*, joint inoculations, and appropriate controls. The effects of cultivar and fertility level were evaluated for each of the above combinations (Table 1). Individual treatments were replicated 10 times in a randomized complete block design. The entire test was repeated once with data being reported from a single test. Routine spray programs were essential to control aphids, mites, and white flies. The study was terminated 89 days after planting.

Plant growth data collected were shoot height and fresh weight, root fresh weight, and number of squares. Roots were assayed for mycorrhiza development by clearing and staining (2). Azygospore populations in the soil were determined by a centrifugal-flotation method (8). Root-knot nematode reproduction was measured by separating second-stage larvae from 100 cm^3 of soil (10 replicates), also by centrifugal-flotation, and by determining the

TABLE 1. Influence of individual and joint inoculations with *Gigaspora margarita* and *Meloidogyne incognita* on growth and reproduction of root-knot nematode-resistant (McNair 511) and -susceptible (Stoneville 213) cotton cultivars at two fertility rates.

Treatment[x]			Shoot height[y] (cm)	Shoot fresh weight[y] (g)	Root fresh weight[y] (g)	Number of squares[y]
Inoculum	Cultivar	Fertility				
None	McNair 511	Low	26.9 f[z]	13.0 hi	5.6 gh	0.7 def
		High	34.5 d	29.1 fg	11.7 fg	4.1 c
	Stoneville 213	Low	20.8 g	10.4 i	3.6 h	0.1 ef
		High	32.8 de	34.4 f	8.7 gh	3.8 c
Gm	McNair 511	Low	47.4 bc	81.8 cde	17.1 def	3.5 c
		High	54.4 a	113.2 a	31.2 a	6.5 a
	Stoneville 213	Low	45.9 c	80.0 cde	16.8 ef	3.9 c
		High	52.3 ab	102.1 ab	23.9 bc	5.6 ab
Mi	McNair 511	Low	26.5 f	13.8 hi	5.2 h	0.7 def
		High	33.5 d	26.0 gh	7.5 gh	1.8 d
	Stoneville 213	Low	21.1 g	9.0 i	3.5 h	0.0 f
		High	27.0 e	19.4 ghi	9.2 gh	1.4 de
Gm+Mi	McNair 511	Low	50.2 abc	78.6 de	19.1 cde	4.7 bc
		High	52.1 ab	93.9 bc	26.9 ab	5.8 ab
	Stoneville 213	Low	47.7 bc	66.8 e	24.7 bc	3.5 c
		High	51.0 abc	89.9 bcd	23.2 bcd	4.5 bc

[x]Inoculum: Gm = *Gigaspora margarita*, Mi = *Meloidogyne incognita*; Fertilization: low (250 μg/g); high (500 μg/g) of 10-10-10 NPK.

[y]Based on an average of 10 replications per treatment.

[z]Column means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

number of eggs produced per root system by adult female nematodes (eight replicates) when the test was terminated. Eggs were collected from the root systems by cutting washed roots into 2.5-cm sections and mechanically stirring the sections at 1,600 rpm for 5 min in 400 ml of 1.05% NaOCl in a 1,000-ml beaker (7).

RESULTS

Growth and reproduction of cotton plants symbiotic with *G. margarita* were significantly ($P = 0.05$) greater than nonmycorrhizal plants, irrespective of either cultivar or fertility rate (Table 1). In all treatments, the degree of mycorrhizal stimulation of vegetative growth and square production was best at the low fertility rate. Shoot height and weight of plants inoculated with *G. margarita* ranged from 57-120% and 197-669%, respectively, greater than the controls (Fig. 1 and Table 1). Root-system weight increases of 167-366% occurred in the mycorrhizal plants with an accompanying 48-3,800% stimulation in square production. The degree of vegetative and reproductive growth was improved considerably more by mycorrhizal development than by doubling the fertility rate. At the low fertility level, total weight of both cultivars forming mycorrhizae ranged from 125-143% greater than the controls at 500 μg/g. Cultivar growth responses to mycorrhizal synthesis or formation did not differ markedly.

Meloidogyne incognita significantly suppressed plant development primarily in the susceptible cultivar, Stoneville 213 at high fertility (Table 1). Shoot weights of plants averaged 44% less than the controls. Square set was significantly retarded in both cultivars at the high fertility rate only, but root weight was not affected.

In concomitant culture, the mycorrhiza-induced plant growth response was affected little by the parasitism of *M. incognita*, regardless of cultivar or fertility rate (Fig. 2 and Table 1). Only shoot weight of the resistant cultivar was decreased at the high fertility level and root weight of the susceptible cultivar was increased at low fertility. The jointly inoculated plants generally developed as well as those inoculated only with *G. margarita*. Reduced shoot weight and poor square production in Stoneville 213

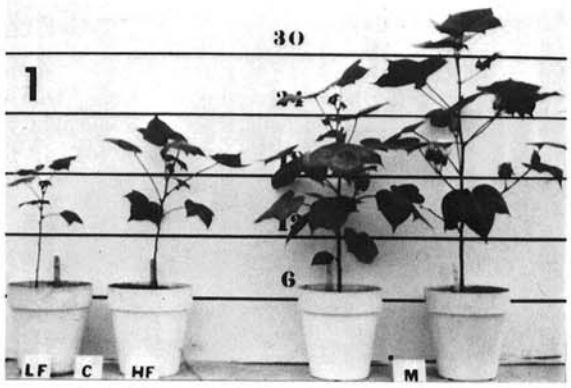

Fig. 1. Growth response of *Gossypium hirsutum* 'Stoneville 213' symbiotic with *Gigaspora margarita* at two soil fertility levels [250 and 500 μg/g (ppm) 10-10-10 NPK]. Left to right; controls (c), 250 (LF) and 500 (HF) μg/g and mycorrhizal plants (m), 250 and 500 μg/g.

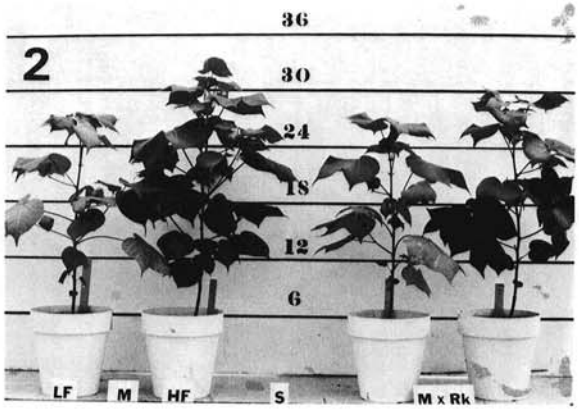

Fig. 2. Effect of individual and joint inoculations on height growth of *Gossypium hirsutum* 'Stoneville 213' at two fertility levels [250 and 500 μg/g (ppm) 10-10-10 NPK]. Left to right; plants mycorrhizal with *Gigaspora margarita* (M), 250 (LF) and 500 (HF) μg/g and plants mycorrhizal with *G. margarita* and challenged with *Meloidogyne incognita* (M+Rk), 250 and 500 μg/g.

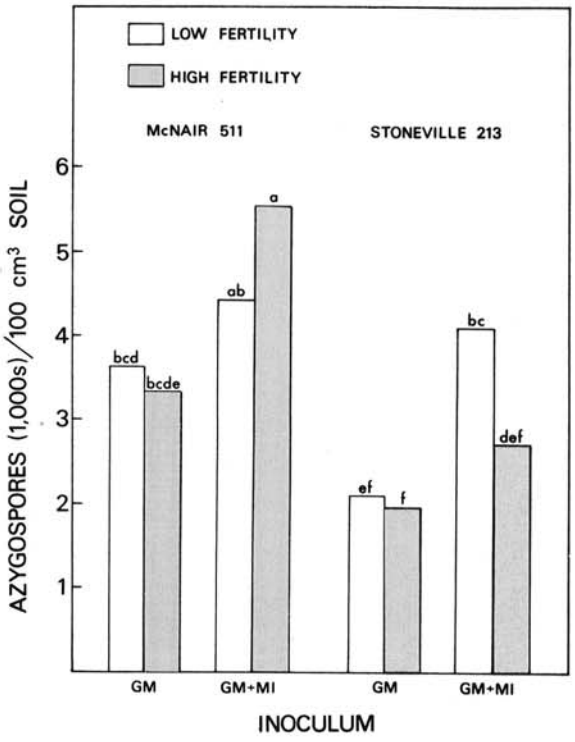

Fig. 3. Effect of cotton cultivar, fertility rate [250 and 500 μg/g (ppm) 10-10-10 NPK], and joint activities of *Gigaspora margarita* (GM) and *Meloidogyne incognita* (MI) on azygospore production by the mycorrhizal fungus. Column means labeled with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

caused by *M. incognita* were offset by mycorrhiza synthesis.

Azygospore production was influenced principally by cultivar and *M. incognita*, whereas fertility rate had a minimal influence (Fig. 3). Sporulation was significantly better on McNair 511 than on Stoneville 213. *Meloidogyne incognita* stimulated azygospore production on McNair 511 at the high fertility rate and Stoneville 213 at low fertility. Spore production in concomitant culture was variable, however, since there was no significant increase in the replicate test.

Nematode reproduction was affected by mycorrhiza synthesis and cultivar, but not by fertility level (Fig. 4). Individual mycorrhizal plants of Stoneville 213 supported significantly higher egg numbers than the smaller nonmycorrhizal controls. However, *G. margarita* was not associated with increased egg production on McNair 511. Number of eggs per gram of root was not related to either mycorrhizal development or soil fertility. Maximum nematode reproduction occurred on Stoneville 213, but the resistant McNair cultivar supported a moderate level of nematode egg production. A similar result was obtained when number of second-stage larva per 100 cm^3 of soil was determined. However, these data are not presented since we consider eggs produced per plant a more valid measure of nematode reproduction.

Stained and cleared root sections of mycorrhizal plants showed extensive development of intercellular hyphae, coiled intracellular hyphae, and arbuscules in the cortical parenchyma. The structures were not detected in nematode galls even though they were evident in abundance in the root tissue proximal and distal to the galls. Mycorrhizae formed on the roots of a few control plants; however, any plants with an estimated level of contamination higher than 1% of the total root system length were discarded.

DISCUSSION

Mycorrhiza formation and growth stimulation in cotton inoculated with *G. margarita* indicate that the crop is symbiotic with more than one VA mycorrhizal fungus species. Rich and Bird (9) reported growth increases of cotton in greenhouse tests with *G. calospora*; however, the beneficial effects were notably less than those associated with *G. margarita*. The varied responses may be due, at least in part, to the use of different plant cultivars and endophytes, growth media, fertility, and initial inoculum level.

Maximum cotton growth response to *G. margarita* at the low fertility level was expected since mycorrhizal efficiency generally is more pronounced in nutrient-poor soils (6). After addition of 250 μg/g 10-10-10 fertilizer, the rates of P (25 μg/g) and K (49 μg/g) were in the low to medium concentration range recommended for optimum

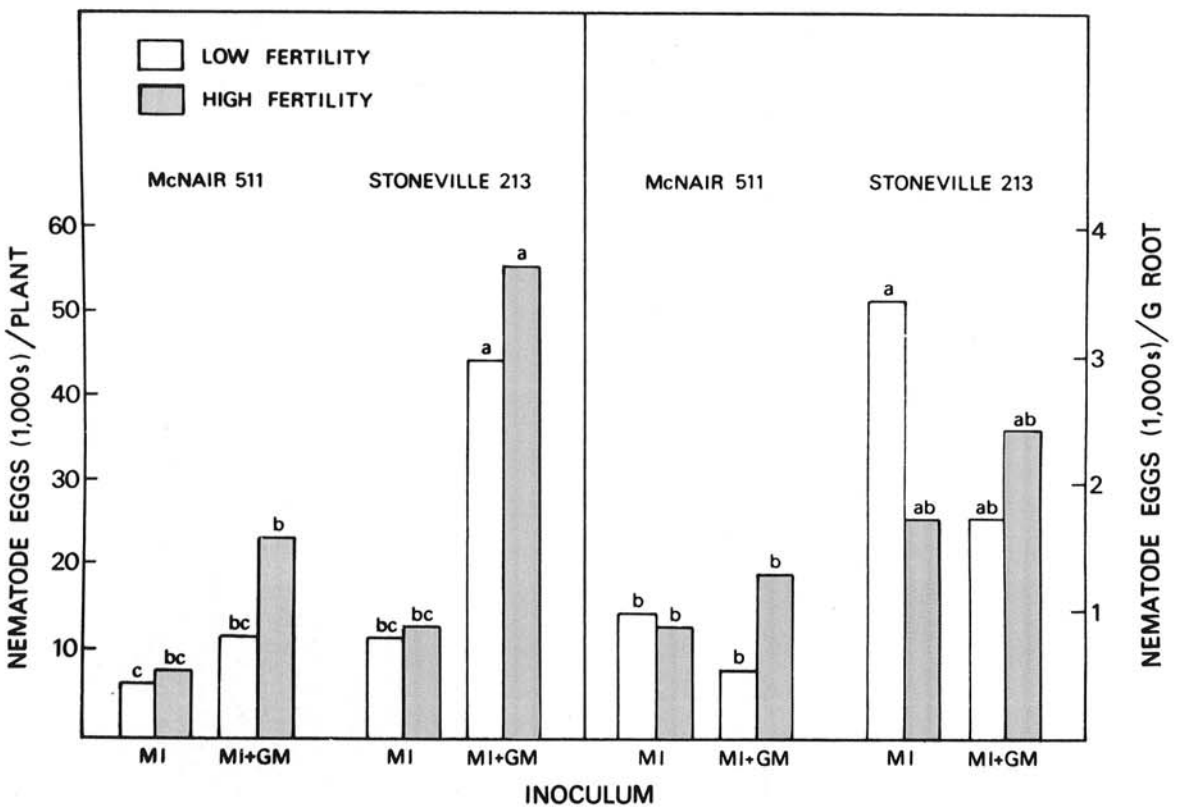

Fig. 4. Influence of cultivar, fertility rate [250 and 500 μg/g (ppm) 10-10-10 NPK), and mycorrhiza formation by *Gigaspora margarita* (GM) on egg production by *Meloidogyne incognita* (MI). Column means labeled with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

cotton growth by the Soil and Plant Testing Laboratory. The magnitude of mycorrhiza-induced growth increases of 192-253% at the high fertility level were somewhat surprising since the P (36 μg/g) and K (70 μg/g) concentrations were within the limits favorable for good plant growth. Consequently, this reaction may be related to a reduction or alteration in the competitive soil microflora induced by fumigation which could provide an ecological advantage in favor of *G. margarita*.

Mycorrhiza development in Stoneville 213 offset the shoot stunting and poor square set caused by *M. incognita*. The effect apparently was due to increased host vigor rather than any antagonistic physiological change induced by symbiosis. This view is supported by the quantitative similarity in egg number per gram of root in both mycorrhizal and control plants. However, mycorrhizal plants, because of the larger root systems, supported a greater root-knot nematode population than did their nonmycorrhizal counterparts; this would be important in maintaining a higher soil population of nematodes.

Meloidogyne incognita did not markedly retard plant development of either cultivar when mycorrhizae formed. Likewise, there was no apparent nematode antagonism toward the degree of mycorrhiza synthesis or fungus sporulation. Stimulation of azygospore production in concomitant culture suggests that *M. incognita* may have induced physiological changes favoring sporulation, but since the trend was not significant in the replicate study, additional information is needed to verify the relationship. Schenck et al. (14) found that spore production by *G. calospora* on soybean was variable; it was stimulated by a low inoculum root-knot nematode level on a resistant cultivar, but was suppressed on a susceptible cultivar. Experiments or surveys in field soils have shown a negative correlation between VA mycorrhiza development in cotton (9) and soybean (13) and the activities of a mixed population of plant-parasitic nematodes. This general trend in soybean was verified in greenhouse tests using *M. incognita* (14). However, variable interactions between certain VA mycorrhizae and *M. incognita*, along with the effects of nematode inoculum levels and cultivar resistance, make it difficult to relate specific results of the study to field observations.

The absence of endomycorrhizal structures in nematode-galled tissue, even though the adjacent proximal and distal portions of the root contained the endophyte, corresponds with other findings (2, 11). Consequently, galls have been suggested as a physiologically undesirable substrate for VA mycorrhizae synthesis (11). Examination of galls 82 days after inoculation revealed that the cortex and epidermis had been sloughed off in that region, but not in the adjoining unaffected root areas. Therefore, we were unable to determine whether the cortex surrounding the young gall was mycorrhizal at any time during its development.

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