Influence of *Aphelenchus avenae* on Vesicular-arbuscular Endomycorrhizal Growth Response in Cotton

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Abstract: The influence of *Aphelenchus avenae* on the relationship between cotton (*Gossypium hirsutum* 'Stoneville 213') and *Gigaspora margarita* or *Glomus etunicatus* was assessed by its effect on the mycorrhizal stimulation of plant growth and microorganism reproduction. The mycophagous nematode usually did not suppress stimulation of shoot growth resulting from mycorrhizae (*G. margarita*) at inoculum levels of 3,000 or 6,000 nematodes per pot, but retarded root growth at 6,000 per pot. When the nematode inoculum was increased to 10, 40, or 80 thousand, *G. margarita* stimulation of shoot or root growth was retarded at the two higher rates. Shoot growth enhancement by *G. etunicatus* was suppressed by 10 thousand *A. avenae* but not by 40 or 80 thousand. *A. avenae* reproduced better when the nematode was added 3 wk after *G. margarita* than with simultaneous inoculations. Sporulation of both fungi was affected little by the mycophagous nematode. The high numbers of *A. avenae* required for an antagonistic effect probably precludes the occurrence of any significant interaction between these two organisms under field conditions. *Key words:* mycophagous nematode, endomycorrhizal fungus.

Cotton growth is greatly stimulated by vesicular-arbuscular (VA) endomycorrhizal fungi, particularly in low phosphorus soils (8,13). VA mycorrhizal fungi colonize epidermal and cortical cells and form an extensive hyphal network bearing vesicles and spores outside the root (6). The extramatrical hyphae absorb nutrients, particularly phosphorus, and translocate these substances to the host's roots for utilization.

Although mycophagous nematodes are often present in the rhizosphere of agronomic crops, little is known about their effect on VA mycorrhizal formation and sub-

Received for publication 9 May 1980.

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This research was supported by the Science and Educational Administration of the U.S. Department of Agriculture under Grant No. 7800244 from the Competitive Research Grants Office and State and Hatch funds allocated to the Georgia Agricultural Experiment Stations.

sequent plant growth. Because of the production of extra-matrical hyphae in the rhizosphere, VA mycorrhizal fungi should be excellent hosts for mycophagous nematodes. Conceivably, VA mycorrhizal fungi would be less efficient at stimulating plant growth if parasitized by mycophagous nematodes. Such an effect has been reported in one study where *Aphelenchus avenae* retarded VA mycorrhizal stimulation of soybean and reduced sporulation (14). Similar results have also been reported with ectomycorrhizae (12,15,16). *A. avenae* reproduced on several species of ectomycorrhizal fungi and suppressed infection of red pine by *Suillus granulatus* (16). *Aphelenchoides* species also attack ectomycorrhizae (12). Mycophagous nematodes were also effective in reducing the pathogenicity of certain root-rotting fungi (1,10,11). *Aphelenchus avenae* reproduced on 54 out of 59 soil-inhabiting fungi (17) and retarded the development of several soil-borne fungal diseases under greenhouse conditions (1,10,11).

This paper reports the influence of *A. avenae* on endomycorrhizae growth responses in cotton. A preliminary report of this research has been published (7).

MATERIALS AND METHODS

Cotton seed, *Gossypium hirsutum* L. 'Stoneville 213,' were germinated in rolled germination paper at 30 C for 30 h and those with 4- to 6-mm long radicles were planted singly into 20-cm-diam plastic pots containing 4,000 cm^3 methyl bromide-fumigated soil (13). Soil (74% sand, 14% silt, 12% clay), with fertility adjusted by adding 10–10–10 N-P-K equivalent to 840 kg/ha and resulting in ca. 18 ppm available phosphorus, was prepared as previously described (13).

Aphelenchus avenae was propagated on *Rhizoctonia solani* Kuehn growing on autoclaved wheat (5). Nematodes were washed from the cultures with tap water and collected on a sieve.

Azygospores of *Gigaspora margarita* Becker and Hall and *Glomus etunicatus* Becker and Gerd. for inoculum were collected from greenhouse cultures as previously described (13). Seedlings were inoculated with 250 azygospores, and a 25-ml aliquant of spore suspension filtrate was added to the soil in each pot in all nonmycorrhizal treatments to standardize the contaminant microflora.

Two separate studies were conducted. In the first test, which was repeated, treatments consisted of single inoculations with *A. avenae* (6,000 nematodes/pot) or *G. margarita*, or joint inoculations (3,000 or 6,000 *A. avenae* applied with *G. margarita* at planting or 3 wk after the fungus), and a noninoculated control. Ten replications of each treatment were arranged in a randomized complete-block design on a greenhouse bench. The study was terminated 82 d after planting.

In a second similar study the inoculum levels of *A. avenae* were increased (10,000, 40,000 and 80,000) and added simultaneously with the fungi, and both *G. margarita* and *G. etunicatus* were used.

At harvest fresh weight of shoots and roots were recorded. Azygospores and nematode populations in the soil were determined by the centrifugal-flotation method (9). Roots were assayed for mycorrhizal development by clearing and staining (3). The percentage of mycorrhizal roots in a 2–3-gm root sample from each plant in the second study was estimated visually under a stereoscopic microscope according to the following index: 1 = 0–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.

RESULTS

Mycorrhizae enhancement of plant vigor was reflected in shoot and root development. In the first experiment (Table 1), shoot and root weights were increased by *G. margarita* 40% and 77%, respectively, over those of nonmycorrhizal control plants. An even greater growth response occurred on mycorrhizal plants in the second experiment (Table 2). *G. margarita* and *G. etunicatus* stimulated shoot growth 156% and 224%, respectively, and root growth 325% and 216%, respectively.

The mycorrhizal stimulation of plant growth was affected little by *A. avenae*. The increase in shoot weight of mycorrhizal plants was not affected by the presence of mycophagus nematodes at either inoculum level or inoculation interval in the first experiment (Table 1). Similar results were obtained when the experiment was repeated

Table 1. Effect of inoculum densities and sequence of co-inoculations with *Aphelenchus avenae* and *Gigaspora margarita* on cotton growth and microorganism reproduction.

Treatment*		Shoot weight g	Root weight g	Nematodes/ 80 cm³ soil	Azygospores/ 80 cm³ soil
Mycorrhizal fungus	Nematode				
None	None	54.8 b†	20.2 c		
GM	None	76.5 a	25.7 a		1488 b
GM	AA-3	70.4 a	35.9 a	114 b	2384 a
GM	AA-6	73.7 a	23.2 bc	113 b	2072 ab
GM	AA-3 (3 wk)	73.7 a	30.7 ab	239 a	1472 b
GM	AA-6 (3 wk)	76.0 a	20.8 c	197 a	1464 b
None	AA-6	46.8 b	11.2 d	103 b	

*GM = *Gigaspora margarita;* AA = *Aphelenchus avenae;* nematodes were added at either 3,000 or 6,000 per pot simultaneously with the fungus at planting or 3 wk later.

†Column means followed by the same letter are not different according to Duncan's multiple range test ($P = 0.05$).

with the exception that shoot weight was significantly less ($P = 0.05$) when the low inoculum level of *A. avenae* was added simultaneously with *G. margarita*. Shoot growth of plants inoculated only with nematodes was not affected. Suppression of mycorrhizal stimulation of root growth, however, occurred at the high inoculum level of *A. avenae* at both inoculation intervals. *A. avenae* (6,000/pot) retarded root growth 35% and 42% when added simultaneously with, or 3 wk after, the mycorrhizal fungus, respectively. In the replicate experiment, though, root weights on mycorrhizal plants increased as a result of the simultaneous inoculation at both nematode inoculum levels. *A. avenae* alone suppressed root growth 45% (Table 1), but this was not observed in the replicate experiment.

Increasing inoculum levels of *A. avenae* had the greatest affect on the growth response from *G. margarita* inoculation. The two highest inoculum levels (40,000 and 80,000/pot) of *A. avenae* suppressed the *G. margarita* stimulation of cotton by 21–26%, whereas the beneficial effect of *G. etunicatus* was reduced 17% with only the low nematode inoculum level (Table 2). Similar results occurred with the mycorrhizae-enhanced root growth, although the re-

Table 2. Influence of *Aphelenchus avenae* on stimulation of cotton growth by *Gigaspora margarita* and *Glomus etunicatus* and reproduction of the microorganisms.

Treatment*		Shoot weight g	Root weight g	Nematodes/ 80 cm³ soil	Agyzospores/ 80 cm³ soil	Mycorrhizae index†
Mycorrhizal fungus	Nematode					
None	None	28.8 f‡	8.9 d			
GM	None	73.7 cd	37.8 a		1079 b	4.7 a
GE	None	93.4 a	28.1 bc		2842 a	3.1 b
None	AA-80	27.4 f	12.1 d	34 bc		
GM	AA-10	63.9 de	32.3 ab	60 b	1789 ab	4.7 a
GM	AA-40	54.5 e	22.2 c	102 a	1549 ab	4.6 a
GM	AA-80	58.0 e	22.4 c	34 bc	2023 ab	4.7 a
GE	AA-10	77.8 bc	36.3 ab	48 bc	1549 ab	3.4 b
GE	AA-40	90.6 ab	34.3 ab	25 c	2408 ab	4.5 a
GE	AA-80	95.0 a	36.7 ab	41 bc	2786 a	3.3 b

*GM = *Gigaspora margarita;* GE = *Glomus etunicatus;* AA = *Aphelenchus avenae;* nematodes were added at either 10,000, 40,000, or 80,000 per pot simultaneously with the fungus at planting.

†Mycorrhizae index: 1 = 0-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100% mycorrhizal roots in a 2-3-g root sample.

‡Column means followed by the same letter are not different according to Duncan's multiple range test ($P = 0.05$).

sponse to *G. etunicatus* was not affected. *A. avenae* at the high inoculum level did not influence shoot or root development on nonmycorrhizal cotton in this experiment (Table 2).

Reproduction of *A. avenae* was influenced by the inoculation interval. Reproduction rates were highest when the nematode was added 3 wk after the mycorrhizal fungus (Table 1). This observation, however, was not confirmed in the duplicate experiment where nematode reproduction was considerably less overall. The population density of *A. avenae* on nonmycorrhizal cotton was comparable to that which occurred on mycorrhizal plants inoculated simultaneously (Table 1). Although the final population densities were erratic in the experiment with the higher inoculum levels of *A. avenae,* the nematode reproduced better on the plants inoculated with *G. margarita* (Table 2).

The influence of *A. avenae* on sporulation of the mycorrhizal fungi was inconsistent. In the first experiment the greatest spore production occurred when the nematodes and fungus were added simultaneously (Table 1), whereas in the replicate experiment the most azygospores were produced when the mycophagous nematode was added 3 wk after the fungus. No significant changes in sporulation occurred in the experiment with the higher nematode inoculum levels (Table 2). This was reflected in the mycorrhizal index which generally was not influenced by *A. avenae,* except where it increased on plants inoculated with *G. etunicatus* and 40,000 *A. avenae.*

DISCUSSION

In this study a mycophagous nematode at densities higher than normally found under natural conditions influenced plant growth responses to endomycorrhizal fungi. Our results with cotton showed a less drastic nematode effect than reported by Salawu and Estey who found that *A. avenae* suppressed the growth of mycorrhizal soybean (14). The development of the mycorrhizae possibly was so extensive on cotton that little overall damage resulted from the feeding activities of *A. avenae.* The suppression of root growth was the most consistent effect *A. avenae* had on mycorrhizal cotton plants. Root growth suppression on nonmycorrhizal cotton inoculated with nematodes in one experiment, suggests that *A. avenae* is capable of parasitizing higher plants, as reported previously (2,4). Perhaps the nematode retards root development by feeding on root hairs, since it usually is not found in healthy root tissue (16). *A. avenae,* however, has been observed in lesions on roots caused by pathogenic fungi (11). We did not examine cotton roots for nematodes.

The erratic reproduction of *A. avenae* on mycorrhizal cotton in our tests cannot be explained. As the cotton plants were extensively mycorrhizal, it is possible that different soil environmental conditions are needed for optimum reproduction of *A. avenae.* Although direct feeding by *A. avenae* on VA mycorrhizal fungi has not been observed, many fungi, including ectomycorrhizal fungi can serve as host for this nematode. Since VA mycorrhizal fungi cannot be cultured, the actual acts of mycophagous nematodes feeding on, and suppressing growth of, these fungi *in vitro* remain to be demonstrated.

Although *A. avenae* did not affect the sporulation of *G. margarita* or *G. etunicatus* on cotton, a *Glomus* species on soybean produced fewer spores in the presence of this nematode (14).

Even though *A. avenae* did not greatly limit the development or function of VA mycorrhizae on cotton in this investigation, studies under field conditions could have different results. Nonetheless, the occurrence of any antagonistic influence of mycophagous nematodes on the relationship between mycorrhizal fungi and agronomic plants under field conditions would probably be limited because of the high numbers of nematodes necessary for a deleterious effect.

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