Association of Early-Season Vesicular-Arbuscular Mycorrhizae with Increased Growth and Development of Cotton

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ABSTRACT

Vesicular-arbuscular (VA) mycorrhizae are ubiquitously associated with cotton (*Gossypium hirsutum* L.) in Georgia. Increased early-season root and shoot growth in the presence of *Endogone calospora*, together with earlier flowering and boll maturation, suggests a beneficial relationship between this fungus and cotton. In field observations, VA mycorrhizae were present five days after seedling emergence and confined mainly to the radicle. Arbuscules were the predominant sign of infection. Seven days later, arbuscules and vesicles were equally predominant, and present only in the cortex of feeder roots. The logarithmic phase of growth for VA mycorrhizal infection of cotton was from 5 to 25 days after seedling emergence. Under field conditions, there was a significant positive correlation between early-season mycorrhizae and vegetative growth and development of cotton. The data are discussed in relation to control of plant parasitic nematodes and a possible joint role of VA mycorrhizae and nematodes in the cotton stunt disease complex.

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Vesicular-arbuscular (VA) mycorrhizae may be the most widespread root infections of plants (17). They have been reported associated with roots of cotton (*Gossypium hirsutum* L.) (4, 14), and have been found to increase the growth and development of many important agronomic plants (6, 12, 15, 20, 21). It has recently been found that VA mycorrhizae enable plants to remove phosphorus from soil beyond the rhizoplane (10). This was demonstrated to be the result of increased sorption surface provided by external hyphae. In unpublished scanning electron micrographs, the authors of the present publication observed an extensive biomass of VA mycorrhizal hyphae protruding from the rhizoplane of cotton. They have also recently shown that control of plant-parasitic nematodes results in significant increases in VA mycorrhizae of cotton (2).

Plant-parasitic nematodes are known to be primary pathogens and predisposition agents of a number of diseases of cotton (5, 9, 16, 18, 19). They have also been shown to be involved in the cotton stunt disease complex (CSDC), which has become a serious problem in the southeastern cotton belt (3). A complete understanding of the cause of this disease is still unknown.

Since phytopathogenic nematodes could have an indirect detrimental influence on root symbionts of diseased higher plants, there is need for additional information about VA mycorrhizae of cotton. The objectives of the present investigation were to determine the presence of VA mycorrhizae of cotton in Georgia, to make preliminary greenhouse observations on the influence of *Endogone calospora* Nicolson & Gerdemann on the growth and development of cotton, and to make field observations on the sequence of mycorrhizal infection of cotton roots in relation to nematode populations and symptom development of the CSDC.

MATERIALS AND METHODS.—*VA mycorrhizae survey.*—In late August, 1972, root samples were taken from seven widely scattered Georgia cotton fields. Six samples were taken from each field, with one-half of the samples from areas with symptoms of cotton stunt, and one-half from productive areas of each field. Samples were taken by removing a 10 × 20 × 15-cm-deep volume of soil from the base of selected cotton plants (cultivars Coker 201 or Coker 310). The samples were placed in plastic bags and stored at 10 C until processing. Roots were removed from the soil with a 0.67-mm (35-mesh) screen, rinsed in tap water, blotted dry, cleared, stained, and analyzed for VA mycorrhizae as previously described (2).

Growth and development study.—Six 150 × 30 × 15-cm glass-sided root observation boxes were filled with approximately 50 cm of beach sand, and then 100 cm of commercial sterile sand. Six to eight cotton seeds (cultivar Coker 310) were planted in each box. A tap water suspension of twenty spores of *E. calospora* was poured directly over the seeds planted in each of three boxes. Immediately after emergence, the seedlings were thinned to three per box. All plants received Hoagland's solution (11) twice a week, and the observation boxes were maintained under greenhouse conditions throughout the 150-day experiment.

Shoot and root growth were taken at various intervals throughout the experiment (Fig. 1). Eighty-three days after seeding, the upper two mature leaves were removed from the three plants of each replicate, combined and nutrient analysis made using emission spectroscopy. Shoot and boll wts were recorded at the end of the experiment, and the root systems were examined for VA mycorrhizae after clearing and staining with the technique previously cited.

Field experiment.—A field experiment was established in Burke County, Georgia, in 1972, on a sandy-loam soil site with a 12-year history of the CSDC. The experimental site (136.1 × 30.9 m) was subdivided into 48 four-row plots (15.2 × 4.5 m). The eight treatments listed in Table 1 were replicated six times in a completely randomized factorial block design. Four weeks before seeding, 1.26 kl of soil from a productive area of the same field was spread on the surface of each of 24 of the plots and the entire experimental area tilrovated. The textural, nutritional,

and pH values of the imported soil were the same as those of the experimental site. The imported soil contained fewer plant parasitic nematodes and approximately twice as many spores of *Endogone* spp. than that of the experimental site.

Three weeks before seeding, 1,3-dichloropropene and related C_3 hydrocarbons (1,3-D), was injected into the soil of 24 plots, using a broadcast application (209 liters/hectare) applied at a depth of 15-20 cm. The field was seeded with Coker 201 cotton. The seeds planted in the plots which were to receive foliar applications of oxamyl {methyl *N'*, *N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate} were also coated with oxamyl (27.2 g active/45.4 kg seed). The foliage of the cotton in these plots was sprayed to runoff 5, 12, 19, and 26 days after seedling emergence (oxamyl, 0.33 kg active/hectare, 2,400 μg/ml). A final application of oxamyl (0.66 kg/hectare, 4,800 μg/ml) was applied seven days later.

Soil and root samples and plant growth observations were taken at various intervals throughout the growing season. Soil nematode assays were made using a modified centrifugation-flotation technique (13), and root samples analyzed for nematodes using a gyratory shaker technique (1). Soil samples for nutrient analysis were taken before planting, and tissue samples for emission spectroscopy nutrient analysis were collected 118 days after seedling emergence. Root samples for VA mycorrhizae detection were placed in FAA in the field immediately after being removed from the soil. They were stored in FAA and then cleared and stained using the technique previously cited. Spores of *Endogone* spp. were obtained using the modified centrifugation-flotation technique (13) or the wet sieving and decanting technique of Gerdemann and Nicolson (8).

RESULTS.—*Survey.*—VA mycorrhizal fungi were present in the roots of all cotton plants sampled. Infections of cotton roots ranged from 30 to 95% of the individual root systems, with means of 65 and 71% from the stunted and productive cotton sites, respectively. Spores of *Endogone* spp. were found associated with 50% of the roots sampled, with no significant difference between the stunted and healthy cotton plants.

Growth and development study.—From 28 to 71 days after seeding, the lengths of the cotton taproots growing in the presence of *E. calospora* were significantly longer than those growing in the absence of the fungus (Fig. 1). From 36 to 71 days after seeding, the shoot ht of cotton growing in the presence of *E. calospora* was significantly greater than those growing in the absence of fungus. Both flowering and boll maturation occurred earlier in the presence of *E. calospora.* Boll and shoot wt of 150-day-old plants with *E. calospora* was greater than those from plants grown without the fungus; however, the differences were not statistically significant.

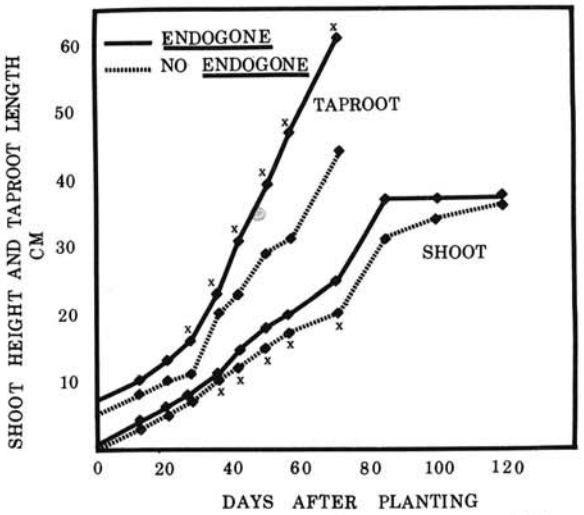

Fig. 1. Influence of *Endogone calospora* on root and shoot development of cotton grown in root observation boxes maintained under greenhouse conditions (Note: Comparable means on dates marked with an X are significantly different, $P = 0.05$).

Analysis of leaf tissue taken 83 days after seeding revealed that plants grown without *E. calospora* contained significantly higher amounts of K and Al than leaves from plants grown in the presence of the fungus. There were either no significant differences between treatments in concns of other elements, or both were within the range for optimum growth and development of cotton.

Roots of cotton in the presence of *E. calospora* were highly mycorrhizal; whereas, those grown without the

TABLE 1. Influence of imported soil and nematicides on seedling emergence, growth and development of cotton grown in a "cotton stunt" site

Treatment		Seedlings per 30.1-m row on 1 June 72	Seedling ht on 8 June 72 (cm)	Seed cotton per 30.1-m row (kg)
Soil[a]	Chemical			
Natural	...	113 A[c]	10.6 A	3.2 A
Imported	...	136 BC	14.1 B	4.4 B
Natural	1,3-D	113 A	15.1 BC	5.6 CD
Imported	1,3-D	129 AB	16.7 BC	5.6 CD
Natural	1,3-D + Oxamyl	126 AB	17.2 BC	5.4 BCD
Imported	1,3-D + Oxamyl	150 C	19.9 D	5.9 D
Natural	Oxamyl	123 AB	15.1 BC	4.4 B
Imported	Oxamyl	140 BC	15.0 BC	4.6 BC

[a]Soil imported from a productive site with the same nutrient and textural characteristics as the natural "cotton stunt" soil.
[b]Seeds planted on 27 April 1972.
[c]Column means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Influence of 1,3-dichloropropene related C_3 hydrocarbon (1,3-D) and methyl *N'*, *N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate (oxamyl) on the combined populations of *Meloidogyne incognita, Hoplolaimus columbus* and *Pratylenchus brachyurus*.

Treatment	Nematodes per 100 g soil		Nematodes per g root	
	12 July 72	23 Aug 72	12 July 72	23 Aug 72
1,3-D + Oxamyl	1.9 A[a]	21 A	1.5 A	13 A
1,3-D	0.2 A	64 A	0.2 A	10 A
Oxamyl	33.1 B	630 AB	28.8 A	51 A
Nontreated	43.1 B	1059 B	34.4 A	205 B

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

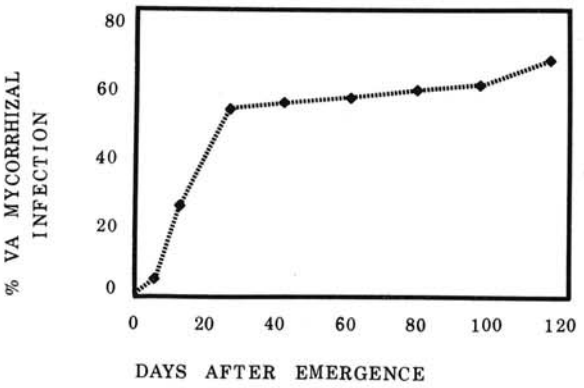

Fig. 2. Rate of vesicular-arbuscular mycorrhizae infection of cotton in a Georgia cotton field in 1972.

fungus contained no VA mycorrhizae of the type observed associated with the other treatment. An unidentified vesiculate mycorrhizal species, however, was found associated with both treatments; however, the extent of infection by this contaminant was very low.

Field experiment.—In general, seedling emergence was significantly enhanced when soil from the productive cotton site was added to the cotton stunt site (Table 1). The nematicide treatments, however, had no influence on seedling emergence. Thirty-two days after seeding, the growth and development of the cotton seedlings had been significantly enhanced by all treatments. Cotton yields were also significantly increased by all of the treatments. Plant growth was most rapid, and cotton yields the greatest, where the imported soil treatment was used in conjunction with the 1,3-D soil fumigation and foliar applications of oxamyl (Table 1).

Prior to soil fumigation, there were no significant differences in the plant parasitic nematode populations among the eight treatments. The importation of soil from the productive site had no significant influence on the population dynamics of plant parasitic nematodes. Soil and root assays made in July and August revealed that all nematicide treatments controlled the combined populations of *Meloidogyne incognita, Hoplolaimus columbus,* and *Pratylenchus brachyurus* to some extent (Table 2). The best nematode control, however, was obtained with the soil fumigant.

Five days after seedling emergence, VA mycorrhizal infections were detected in all seedlings examined except those germinated in natural "cotton stunt" soil without a nematicide treatment. Arbuscules of the symbiont were predominant signs of infection, and vesicles were few in number. At this early date, infection was confined mainly to the radicles. In later samples, however, only feeder roots were abundantly infected. Twelve days after emergence, arbuscules and vesicles were equally predominant in the root cortex. Combined VA mycorrhizal analyses for all treatments indicated that infection of cotton roots increased rapidly between 5 and 25 days after seedling emergence (Fig. 2). After 5 days, approximately 5.0% of each root system contained VA mycorrhizal infections, while infection increased to 54% 25 days after emergence. Subsequent VA mycorrhizal infection increased slowly until 68% of the roots sampled were infected 118 days after seedling emergence.

While initial VA mycorrhizal infections appeared to be more extensive in plots treated with nematicides, there were no statistically significant differences among the treatments. When 256 pairs of parameters studies in the experiment were evaluated in a correlation matrix, 30 of those pairs were found to be significantly correlated with each other. Four of the significant correlations involved

TABLE 3. Significant correlations involving vesicular-arbuscular mycorrhizal infection among 256 pairs of parameters studied in relation to a field experiment with cotton

Correlated characteristics (observation dates)	Correlation coefficients[a]
Mycorrhizae (5/9/72) – plant height (6/8/72)	+0.33
Mycorrhizae (5/15/72) – plant height (6/1/72)	+0.44
Mycorrhizae (5/9/72) – nematodes/g root (11/22/72)	−0.29
Mycorrhizae (5/29/72) – nematodes/100 g soil (11/22/72)	+0.33

[a]Correlation coefficients greater than 0.28 or less than −0.28 were significant ($P = 0.05$).

mycorrhizal infection (Table 3). High levels of early-season mycorrhizal infection were significantly correlated with increased growth and development of shoots of cotton plants. High levels of early-season mycorrhizal infection were negatively correlated with root populations of nematodes at the end of the growing season, but positively correlated with nematode population density in the soil.

There were no significant differences in soil populations of VA mycorrhizal fungus spores among the treatments in November, 1972, or March, 1973. The number of spores of *Endogone* spp. recovered from soil in March, 1973, however, was approximately one-half the number recovered in November, 1972.

Preplanting soil nutrient, pH, and textural analyses of the soil indicated no significant differences among the treatments. Analysis of leaf tissue taken 118 days after planting showed little or no difference in nutrient uptake by the plants of the eight treatments.

DISCUSSION.—VA mycorrhizae appear to be ubiquitously associated with cotton in Georgia. In addition to the limited survey reported in the present manuscript, the authors have sampled numerous other cotton fields, and have never found root systems of cotton completely devoid of VA mycorrhizae, except immediately after seed germination.

The increase in the rate of growth and development of root and shoot systems of cotton in the presence of *E. calospora,* in addition to the earlier flowering and boll maturation, suggests the existence of a physiologically beneficial relationship between this fungus and cotton. The lack of differences in shoot development at harvest can be explained by the extensive potential of cotton for vegetative growth which often takes place at the expense of reproductive growth and development.

The greenhouse and field data suggest a possible chronological order for the process of symbiosis. The fungus apparently required an initial period for extensive colonization of roots and possibly a significant physiological effect on the root system. VA mycorrhizae were present within five days after seedling emergence and confined mainly to the radicle. Arbuscules were the predominant sign of infection. Seven days later, arbuscules and vesicles were equally predominant and present only in the cortex of feeder roots. The logarithmic phase of the growth curve for VA mycorrhizal infection in the field appeared to occur between 5 and 25 days after seedling emergence. This was followed immediately by an increase in the rate of vegetative growth and development of cotton. Enhancement of root growth preceded increased shoot development. In both the greenhouse and field experiment, distinct modifications of vegetative growth and development were present approximately 30 days after seedling emergence. This was followed by earlier flowering and boll maturation. Mycorrhizal infection in the field experiment increased slowly until 68% of the roots were infested. This is nearly identical to the extent of infection observed in the survey. The soil population density of spores of *Endogone* spp. decreased during overwintering.

The cotton leaf tissue analyzed for nutrient differences was probably harvested too late. Earlier samples may be required to show any differences in nutrient uptake and accumulation. Increased water uptake by *E. calospora* should not be precluded as influencing the growth and development of cotton plants (21).

The imported soil treatment in the field experiment significantly increased seedling emergence and resulted in a denser stand of cotton. These results were confirmed in a similar test conducted in 1973. Apparently some unidentified factor protected the seedlings against early-season seedling disease.

Proportional differences in the yields among the treatments in the field test could have been predicted from vegetative growth measurements taken approximately 30 days after seedling emergence. Treatments that produced the greatest early-season vegetative growth yielded the greatest amount of cotton. As the growing season progressed, vigorous plants converted from vegetative to reproductive growth, while stunted plants continued a slow rate of vegetative growth. These observations and results are the same as those observed for the CSDC (1).

The significant negative correlation between mycorrhizae and root populations of nematodes is in agreement with the findings of Fox and Spasoff (7) and Bird et al. (2). At harvest, high populations of plant parasitic nematodes in the soil are frequently associated with vigorous plants, and it is not surprising that early-season mycorrhizal infection could be positively correlated with late-season soil populations of plant-parasitic nematodes.

The significant positive correlations between early-season VA mycorrhizal infection of cotton roots and early-season vegetative growth and development of cotton, together with negative correlations between VA mycorrhizal infection of cotton roots and the presence of plant parasitic nematodes, suggests that these two groups of soil-borne organisms may play a joint role in the CDSC. The data and observations from the present investigations support the hypothesis that phytopathogenic nematodes have indirect, detrimental influences on root symbionts of higher plants. If this hypothesis is proven to be correct, it is likely that the damage of higher plants caused by phytopathogenic nematodes is magnified many fold through detrimental influences on associated plant symbionts.

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