# Increased Endomycorrhizae of Cotton Roots in Soil Treated With Nematicides

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ABSTRACT

Fumigation of nematode-infested field soil with nematicidally active rates of 1,2-dibromo-3-chloropropane (DBCP) or 1,3-dichloropropene and related $C_3$ hydrocarbons, resulted in significant increases in endomycorrhizal infection of cotton roots. While the mycorrhizal potentials of soils with different cotton productivity records were similar, the increases resulting from chemical treatments were expressed in different ways. Cotton roots grown in a DBCP-treated soil with a history of excellent cotton productivity, had significant increases in arbuscule, vesicle, and spore production by unidentified mycorrhizal fungi. In contrast, plants grown in DBCP-treated soil with a poor record of cotton productivity, had no significant increase in arbuscule production. The increase in vesicle production by the endomycorrhizal fungus, however, was significantly greater than in roots grown in the soil having the better productivity history. No endomycorrhizae were observed in cotton roots grown in methyl bromide-treated soils.

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Both endomycorrhizae and plant-parasitic nematodes are commonly associated with the roots of most cultivated plant species. Since both of these organisms alter the physiology of roots, it is highly probable that they influence the biology of each other. A number of studies have shown this to be true for ectomycorrhizae and plant-parasitic nematodes (1, 7, 8, 9, 10, 11, 13, 14), but little is known about the ecological relationships between endomycorrhizae and plant-parasitic nematodes. Fox & Spasoff (5) reported that *Heterodera solanacerarum* and *Endogone gigantea* mutually suppress the reproduction of each other on tobacco. Ruehle (12) found that the presence of endomycorrhizae in feeder roots of *Liriodendron tulipifera* failed to prevent *Pratylenchus brachyurus* from parasitizing cortical tissue. In a study of peach roots infected with *Meloidogyne* sp., Marx (D. H. Marx, *unpublished*) found that the cortex of feeder roots was colonized by endomycorrhizae, but that the fungus failed to invade tissue galled by nematodes. Deal et al. (4) found both plant-parasitic and saprophagous nematodes associated with endomycorrhizal grape roots. Using a soybean variety susceptible to Phytophthora-rot, Ross (10) reported a more severe and higher incidence of Phytophthora-rot in roots of plants grown in soil infected with a chlamydosporia species of *Endogone*, than in plots infested with *Phytophthora* alone. The phenomenon, however, was not observed with a more disease-tolerant cultivar.

During a preliminary survey, we found less endomycorrhizae in cotton roots infected with endoparasitic nematodes than in roots free of endoparasitic nematodes. This prompted us to investigate the influence of chemical nematode control on the growth and development of endomycorrhizae in cotton roots.

MATERIALS AND METHODS.—Two adjacent 4 X 15-m plots were randomly placed in a field in Burke County, Georgia. The experimental site had a 12-year history of cotton stunt (3). Nematode population densities were estimated in these plots prior to the nematicide treatment. Six individual soil samples from each plot were processed, using a modified centrifugation-flotation technique. One of the plots was fumigated at a depth of 15 - 20 cm with a broadcast application of 1,3-dichloropropene (1,3-D) and related chlorinated $C_3$ hydrocarbons (188 liters/hectare). Twenty days later, top soil from this plot was removed and used to fill four 150 X 30 X 15-cm glass-sided root observation boxes. Four observation boxes were also filled with top soil from the nontreated plot, and four with a methyl bromide-treated greenhouse soil mix. Two cotton

seeds ('Coker 201') were planted in each box and grown in the greenhouse for 37 days from the date of seeding.

The root systems were removed from the observation boxes and washed. Soil from each box was analyzed for nematodes using the modified centrifugation-flotation technique. The root systems were examined for the presence of endomycorrhizae, using a modified root clearing and staining technique similar to the one described by Phillips & Hayman (6). The roots were autoclaved for 10 min in 10% KOH at 121 C, washed with fresh KOH, immersed in 3% alkaline $H_2O_2$ for 10 min, rinsed thoroughly in $H_2O$, acidified in 1% HCl, stained for 30 min in acid fuchsin-chloryl hydrate (900 ml $H_2O$, 100 ml chloryl hydrate and 0.5 g acid fuchsin) at 121 C, and destained in lactophenol (500 ml phenol, 500 ml lactic acid, 1,000 ml glycerol, and 500 ml distilled $H_2O$). Most of the microscopic observations were made at ×80, with frequent confirmations at higher magnifications.

In a second experiment, samples of top soil were collected from two sites in the same cotton field. Site No. 1 was selected because of its history of excellent cotton yields, whereas, Site No. 2 was selected because of its 12-year history of cotton stunt. The soils were of the same physical structure and nutritional composition. (Site No. 1. 74.4% sand; 10.8% silt; 16.8% clay; pH 6.6; P 102, K 143, Ca 940, and Mg 278 kg/hectare: Site No. 2. 76.2% sand; 9.0% silt; 14.8% clay; pH 6.5; P 108, K 134, Ca 806, and Mg 260 kg/hectare). Twenty-four 40-cm plastic pots were filled with soil from each site. Eight of the pots of soil from each site were treated with methyl bromide (454 g/16 pots) and eight with DBCP (7.5 liters/15-cm hectare). Twenty-four hr after treatment, the soil was aerated for 1 hr and returned to the pots, which were immediately seeded with Coker 201 cotton. Following germination, the stands were thinned to one seedling per pot. After 182 days in the greenhouse, the root systems were washed, and five grams of each root system were selected at random and processed for endoparasitic nematodes by incubating for 72 hr in flasks containing 100 ppm ethoxyethyl mercuric chloride and 50 ppm dihydrostreptomycin sulfate. During incubation, the flasks were rotated on a gyratory shaker at 100 rpm (2). Five grams of each root system were also examined for endomycorrhizae as previously described.

RESULTS AND DISCUSSION.—Four plant-parasitic nematode species were present in the experimental sites prior to fumigation with 1,3-D, and there were no significant differences among the initial population densities (Table 1). Fumigation with 1,3-D significantly reduced populations of

TABLE 1. Nematode populations in soil treated with 1,3-dichloropropene and related chlorinated $C_3$ hydrocarbons, and planted with cotton for 37 days

| Treatment | Nematodes/100 g soil | | | |
|---|---|---|---|---|
| | *Hoplolaimus columbus* | *Pratylenchus brachyurus* | *Meloidogyne incognita* | *Trichodorus christiei* |
| Initial density[y] | | | | |
| Field soil | 31 a[z] | 2 a | 0 a | 0 a |
| Field soil & 1,3-D | 12 a | 1 a | 4 a | 0 a |
| Greenhouse soil | 0 a | 0 a | 0 a | 0 a |
| Final density | | | | |
| Field soil | 13 b | 2 b | 0 b | 2 b |
| Field soil & 1,3-D | 0 c | 0 c | 0 b | 1 b |
| Greenhouse soil | 0 c | 0 c | 0 b | 0 b |

[y] Initial density = nematodes/100 g of soil at the beginning of the experiment. Final density = nematodes/100 g of soil at the conclusion of the experiment.

[z] Grouped 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 and related chlorinated $C_3$ hydrocarbons on the growth and development of endomycorrhizae in cotton roots

| Treatment | Percent of individual root system containing:[y] | | | |
|---|---|---|---|---|
| | Endomycorrhizae | Arbuscules | Vesicles | Chlamydospores |
| Field soil & 1,3-D | 68.0 a[z] | 64.0 a | 68.0 a | 17.6 a |
| Field soil | 24.0 b | 16.0 b | 18.0 b | 0.0 b |
| Greenhouse soil | 0.0 c | 0.0 c | 0.0 c | 0.0 b |

[y] Each category was derived by a separate microscopic analysis of each root system.

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

TABLE 3. Final population densities of nematodes from two soils treated with 1,2-dibromo-3-chloropropane or methyl bromide and planted with cotton for 182 days

| Treatment | Nematodes/g root tissue | |
|---|---|---|
| | *Hoplolaimus columbus* | *Pratylenchus brachyurus* |
| Soil from site No. 1 | | |
| DBCP | 0.0 a[z] | 0.0 a |
| Methyl bromide | 0.2 a | 0.1 a |
| Nontreated | 0.1 a | 108.4 b |
| Soil from site No. 2 | | |
| DBCP | 0.0 a | 0.1 a |
| Methyl bromide | 0.2 a | 0.0 a |
| Nontreated | 90.0 b | 12.2 a |

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

*Hoplolaimus columbus, Pratylenchus brachyurus*, and *Meloidogyne incognita*, and significantly increased endomycorrhizal infection of cotton roots (Table 2). The mycorrhizal increase was expressed through significant increases in arbuscules, vesicles, and chlamydospores produced by the endomycorrhizal fungus. The number of chlamydospores in the root tissue, however, was very low. Endomycorrhizae were not observed in roots grown in methyl bromide-treated greenhouse soil.

Small sporocarps of an *Endogone* sp. were present in the rhizosphere and were attached to cotton roots by hyphae. Since it seems unlikely that exogenous sporocarps would be produced during a 37-day experimental period, it was assumed that they were introduced with the field soil and served as inoculum for the endomycorrhizal infection.

In the second experiment, although both soils contained the same nematodes, *P. brachyurus* was the predominant species in Site No. 1, and *H. columbus* the predominant species in the cotton stunt site. Root assays after the 182-day experimental period, showed that DBCP and methyl bromide significantly controlled both nematode species (Table 3), and significantly increased endomycorrhizal infection of cotton roots (Table 4). Whereas treatment of soil from Site No. 1 significantly increased the amount of each root system containing arbuscules, fumigation of soil from the cotton stunt site did not result in a significant increase in arbuscule production, but did increase the percent of each root system containing vesicles. The increase, however, was significantly greater in roots grown in the cotton stunt soil than in those grown in soil from Site No. 1. When the extent of arbuscule and vesicle production was considered simultaneously, there were no significant differences in the endomycorrhizal potentials between the two soils. DBCP had no significant influence on spore production, although spores were quite numerous in many of the older lateral roots. Endomycorrhizae were not observed in roots grown in methyl bromide-treated soil.

The negative correlation between endomycorrhizae and endoparasitic nematodes suggests that nematodes might eventually limit the mycorrhizal potentials of nematode-infested soils. In the present investigation, however, both soils appeared to have the same endomycorrhizal potential, in spite of vastly different histories of annual crop yields. The mycorrhizal potentials of the two soils, however, were expressed in different manners, with roots grown in one DBCP-treated soil being predominantly arbuscular, while roots grown in the fumigated cotton stunt soil were predominantly vesicular.

In unpublished studies with cotton, we have noted that arbuscules are frequently associated with juvenile feeder roots; whereas, vesicles are more frequently associated with older feeder roots. In some cases arbuscule formation appears to precede vesicle formation. The difference in endomycorrhizal

TABLE 4. Influence of 1,2-dibromo-3-chloropropane and methyl bromide on the growth and development of endomycorrhizae in cotton roots grown in soil from two sites

| Treatment | Percent of each root system containing:[y] | | | | |
|---|---|---|---|---|---|
| | Endomycorrhizae | Arbuscules | Vesicles | Arbuscules and vesicles | Mycorrhizal spores |
| Soil from site No. 1 | | | | | |
| DBCP | 47.0 a[z] | 30.5 a | 27.5 b | 58.0 a | 10.0 a |
| Nontreated | 29.5 b | 8.5 b | 16.0 c | 24.5 b | 10.0 a |
| Methyl bromide | 0.0 c | 0.0 d | 0.0 d | 0.0 c | 0.0 a |
| Soil from site No. 2 | | | | | |
| DBCP | 58.5 a | 8.0 bc | 47.0 a | 55.0 a | 6.5 a |
| Nontreated | 29.5 b | 3.5 cd | 20.0 bc | 23.5 b | 9.0 a |
| Methyl bromide | 0.0 c | 0.0 d | 0.0 d | 0.0 c | 0.0 a |

[y] Each category except the one labeled arbuscules and vesicles was derived by a separate microscopic analysis of each root system. Statistics in the column labeled arbuscules and vesicles were derived from the sums of the arbuscule and vesicle estimate for each root system.

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

expression of roots grown in the two soils, therefore, may be due to different rates of physiological aging of the roots. The probable presence of more than one species of *Endogone* in the soils used in these experiments could also explain the differences in the endomycorrhizal responses in the roots grown in the two soils. It is also possible that over a period of years, nematodes or other factors prevented arbuscuate species from colonizing the cotton stunt soil. Research with defined isolates and known species will be necessary to determine which of the above hypotheses are correct.

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