The Influence of *Glomus mosseae* on *Tylenchulus semipenetrans*— Infected and Uninfected *Citrus limon* Seedlings

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Abstract: Greenhouse studies have shown that when rough lemon (*Citrus limon*) seedlings infected with *Tylenchulus semipenetrans* were transplanted into soil infested with *Glomus mosseae*, the mycorrhizal fungus infection increased seedling growth compared to nonmycorrhizal seedlings. *Tylenchulus semipenetrans* significantly suppressed seedling growth below that of mycorrhizal seedlings. Histological observations of nematode-free mycorrhizal roots showed that hyphae penetrated the epidermis and invaded the cortex, giving rise to arbuscules and vesicles. Nematode infection sites in *T. semipenetrans*-infected roots grown in soil infested with *G. mosseae* did not show evidence of vesicle development in the cortex but did show arbuscule development. *Key Words:* mycorrhizae, citrus nematode, rough lemon.

Several studies have discussed nematode-mycorrhiza interactions (2, 4, 11, 13, 14). Disease symptoms caused by pathogenic organisms may be less severe in the presence of vesicular-arbuscular mycorrhizae (VAM) (1, 11) or the effects may possibly be antagonistic (2, 12). The influence of VAM on seedling growth indicates that citrus is mycorrhiza-dependent and that nonmycorrhizal citrus seedlings may be severely stunted in soils low in phosphorus (6, 7, 15). Nothing is known of the combined effects of the citrus nematode, *Tylenchulus semipenetrans* Cobb, and the endomycorrhizal fungi that colonize citrus roots. Yet mycorrhizae are found in most citrus soils in Florida (8), and about half of the citrus groves are infested with the citrus nematode.

We report a study on the influence of a VAM fungus, *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, on rough lemon (*Citrus limon* (L.) Burm. f.) seedlings infected and uninfected with *T. semipenetrans*, a comparison of plant vigor between infected and uninfected seedlings, and histological observations of mycorrhiza-and-nematode-infected roots.

MATERIALS AND METHODS

To obtain uniform seedlings infected and uninfected with *T. semipenetrans*, rough lemon seed were planted in two flats containing a steam-pasteurized peat-vermiculite mix. Three months after germination, about 1 million *T. semipenetrans* juveniles surface-sterilized with 4 μg/g of ethoxyethyl mercury chloride and 3% dihydrostreptomycin sulfate for 12 h, were pipetted uniformly in the seedling rows in one flat by a method described previously (17). Six months after inoculation, uniform seedlings were selected from both flats and transplanted singly into 20-cm clay pots containing an Astatula fine sand (hyperthermic, uncoated typic quartzipsamments) subsoil (95% sand, 3% silt, 2% clay, 0.25% organic matter) which had been steam-sterilized. *Glomus mosseae* inoculum was added to half of the pots by amending the soil in each pot with 100 g of soil containing 160 chlamydospores from soil containing citrus seedlings infected with *G. mosseae*.

Treatments were: 1) *T. semipenetrans*-infected seedlings transplanted into *G. mosseae*-infested soil; 2) nematode-free seedlings in *G. mosseae*-infested soil; 3) *T. semipenetrans*-infected seedlings in *G. mosseae*-free soil; and 4) nematode-free seedlings in *G. mosseae*-free soil. Treatments were replicated 15 times and randomized on a greenhouse bench. One month after transplanting, all seedlings received a N-P-K dry commercial fertilizer (6% N, 2.6% P, 5% K) at 4 g/pot. Two additional fertilizations were applied after 5 and 7 months as a dilute liquid 12-0-6 N-P-K solution containing nitrogen in the urea form plus minor elements. The study was terminated 8 months after transplanting.

The stem diameter of each seedling was

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measured at harvest and the tops of the plants were cut at the ground line, oven-dried, and weighed. All roots were removed from the pots and carefully washed. Three-to-4-g portions of feeder roots were cut from each *T. semipenetrans*-infected seedling and placed in jars for extracting nematodes, which are reported as the total number of juveniles and males/g root (10). After nematode extraction, the roots were replaced with their respective root systems, which were oven-dried and weighed.

For the histological study, feeder root sections 3-5 mm long were selected with the aid of a stereomicroscope from 30 seedlings infected with *G. mosseae* only or with the combination *T. semipenetrans* and *G. mosseae*. Only root pieces with mature citrus nematode females protruding from the root surface were selected from the nematode-infected seedlings. All root segments were fixed in FAA (formalin, acetic acid, alcohol) for 48 h, dehydrated in TBA (tertiary butyl alcohol), and embedded in paraffin. The cross sections 10 or 15 μm thick, were stained in safranin-fast green, mounted in Permount, and observed with a compound microscope (5).

The soil in each pot was passed through a 1-mm sieve to remove extraneous debris, a 25-cm^3 sample was withdrawn, and chlamydospore numbers were determined by the wet-sieve method, in which spores are trapped on 53- and 44-μm sieves and counted (9).

RESULTS AND DISCUSSION

Stem diameters and shoot and root weights were significantly greater (P = 0.05) for nematode-free seedlings transplanted into *G. mosseae*-infested soil than for nematode-infected seedlings in *G. mosseae*-infested soil (Table 1). Dry weights of both nematode-free and nematode-infected seedlings were greater (P = 0.01 and P = 0.05, respectively) in *G. mosseae*-infested soil than in soil not infested with *G. mosseae*. Mycorrhizal development increased plant growth over that of nonmycorrhizal seedlings although the nematode-infected mycorrhizal seedlings grew less than the seedlings infected only with *G. mosseae* (Fig. 1).

Glomus mosseae sporulation was not affected by *T. semipenetrans*. Because *T. semipenetrans* invades roots and feeds only in the cortex (16), the growth of *G. mosseae* in cortical tissues might be expected to affect establishment of a feeding site by this nematode, which could influence subsequent reproduction. In this study, however, there was no evidence of this effect. Numbers of *Tylenchulus semipenetrans*/g root from mycorrhizal seedlings growing in *G. mosseae*-infested soil were not significantly different from those growing on nonmycorrhizal seedlings (Table 1).

Significant growth differences between mycorrhizal and nonmycorrhizal seedlings showed that mycorrhizal fungi can provide citrus seedlings minimal nutrient requirements for growth stimulation. Because high phosphorus levels tend to mask mycorrhizal effects (3), minimal phosphorus was provided to maximize the influence of VAM and demonstrate its symbiotic influence.

All stained and sectioned feeder roots examined from nematode-free seedlings growing in *G. mosseae*-infested soil were

TABLE 1. The influence of *Glomus mosseae* on rough lemon seedlings infected and uninfected with *Tylenchulus semipenetrans*, and the combined effects on mycorrhizal sporulation and nematode reproduction.[z]

Treatment		Stem diam (mm)	Shoot weight (g)	Root weight (g)	Chlamydospores/25 cm^3	*T. semipenetrans* juveniles-males/g root
Glomus mosseae	*Tylenchulus semipenetrans*					
+	0	6.99a	11.29a	7.83a	177a	0
+	+	6.45b	9.48b	6.27b	186a	314a
0	+	6.25c	6.15c	5.13c	0	662a
0	0	5.87c	4.86c	3.58d	0	0

[z]Numbers followed by the same letter within a column do not differ significantly from each other (P = 0.05) according to Duncan's multiple-range test. Each number is the average of 15 replications.

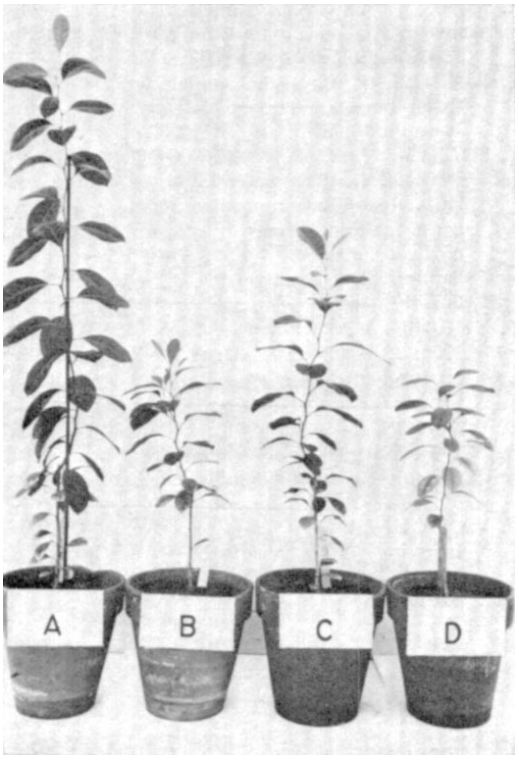

FIG. 1. Rough lemon (*Citrus limon*) seedlings infested by A) *Glomus mosseae,* B) Control, C) *Tylenchulus semipenetrans* plus *G. mosseae,* and D) *T. semipenetrans.*

colonized by the fungus. The hyphae penetrated the epidermis and invaded the cortex, giving rise to arbuscules and vesicles (Fig. 2). In some roots, vesicles and arbuscules developed in almost 50% of the cortical cells. In others, vesicle formation in the cortex along the endodermis caused a compression of endodermal and pericyclic cells. Vesicles were never observed in sections from seedlings infected with both organisms. However, arbuscules were observed in some cortical cells in proximity to nematode-induced nurse cells (Fig. 3). The presence of *T. semipenetrans* in feeder roots of rough lemon seedlings transplanted into *G. mosseae*-infested soil apparently prevented the fungus from producing vesicles, but did not prevent the formation of arbuscules in cortical tissue. Previous observations of new roots produced by nematode-infected seedlings transplanted into *G. mosseae*-infested soil have shown that they were rapidly invaded by the fungus, which produced vesicles as well as arbuscules before nematode invasion.

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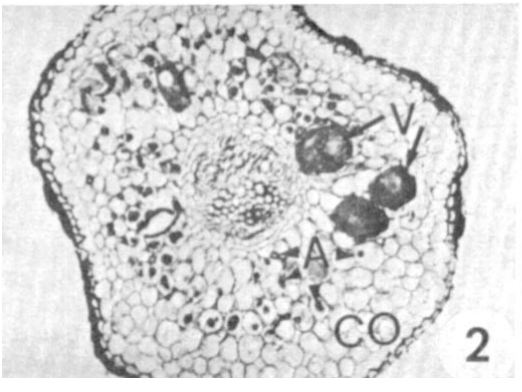

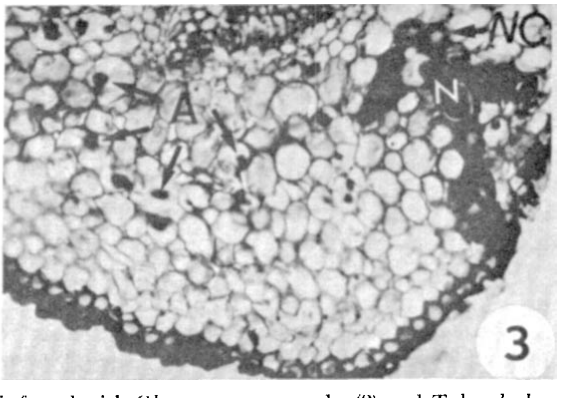

FIG. 2-3. Histology of rough lemon feeder roots infected with *Glomus mosseae* only (2) and *Tylenchulus semipenetrans* plus *G. mosseae* (3). 2) Vesicle (V) formation in cortex (CO); and arbuscules (A). 3) Cross section showing "nurse cells" (NC) induced by *T. semipenetrans* (N) and arbuscules (A) of *G. mosseae* in the cortex.

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