The Response of *Citrus limon* Seedlings to a Symbiont, *Glomus etunicatus*, and a Pathogen, *Radopholus similis*

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Abstract: The influences of a vesicular-arbuscular mycorrhiza (*Glomus etunicatus*) and burrowing nematode (*Radopholus similis*), alone and in combination, on the growth of rough lemon (*Citrus limon*) seedlings were studied in the greenhouse. Growth of mycorrhizal seedlings was significantly greater than that of nonmycorrhizal seedlings or seedlings inoculated with *R. similis*. Mycorrhizal stimulation of seedling growth was inhibited by nematode infection. When seedlings were inoculated with *G. etunicatus* and *R. similis*, suppression of seedling growth by *R. similis* was less on VAM seedlings than on nonmycorrhizal seedlings. Nonmycorrhizal seedlings infected with *R. similis* were significantly smaller than nonmycorrhizal seedlings free of *R. similis*. Vesicle formation and mycelia growth were less in nematode-infected roots. *Key Words:* Endomycorrhizae, burrowing nematode, rough lemon.

Nearly all flowering plants (angiosperms) are associated with symbiotic mycorrhizal fungi, which usually benefit plant development. Citrus is associated with a number of vesicular-arbuscular mycorrhizal (VAM) fungi (6, 8, 11). Under natural conditions citrus roots are readily colonized by mycorrhizal fungi, which improve the mineral nutrition of the host by improving the uptake of phosphorus (4, 6) and perhaps other minor elements (6, 9). If, however, these fungi are destroyed by heat or fumigation, plant growth is limited and vigor is reduced, particularly in soils low in phosphorus (6, 7, 18).

The combined influence on a host of obligate plant-parasitic nematodes and obligate endomycorrhizal fungi has been studied only recently (3, 5, 13, 15, 17, 19). There is variation in host response to the pathogen-mycorrhizal complex. Disease symptoms caused by pathogenic organisms may be less severe in the presence of mycorrhizae (2, 5, 15) or antagonism may occur whereby both organisms are adversely affected (3, 13) or the mycorrhiza may predispose its host to the effects of a pathogen (16).

Rough lemon [*Citrus limon* (L.) Burm. f.] is an important rootstock used in Florida and other citrus-growing areas. Previous studies (11, 13) have shown that rough lemon seedlings respond readily to mycorrhizal infection in an Astatula fine sand (hyperthermic, uncoated typic quartzipsamments; formerly Lakeland sand) common to the Central Ridge area of Florida. This soil contains the minimum phosphorus level of 40 μg/ml considered adequate for citrus culture in Florida (14). Only the addition of mycorrhizae or high rates of phosphate (20) to the soil will provide renewed growth of nonmycorrhizal seedlings.

It has often been demonstrated that *Radopholus similis* (Cobb) Thorne can suppress citrus development and that rough lemon rootstock is very susceptible to this nematode (12). The role of mycorrhizae in seedling development in the presence of this pathogen is unknown. This study was done to determine the effect of a VAM fungus, *Glomus etunicatus* Becker & Gerd., and of *R. similis* on the development of *Citrus limon*, and to define the interaction of the two.

MATERIALS AND METHODS

Seeds of rough lemon were planted in two 25 × 30 × 46-cm wooden flats containing steam-pasteurized Astatula fine sand (95% sand, 3% silt, 2% clay), 0.25% organic matter, pH 5.9, in a greenhouse. Two months later, when the seedlings were in the one- and two-leaf stages, 500 g of soil infested with *G. etunicatus* (250 chlamydospores/25 g of soil) was mixed in the seedling rows of one flat. The source of the *G. etunicatus* inoculum was soil containing VAM citrus seedlings free of *R. similis*. The other flat received similar soil free of mycorrhizal spores. Two months after the soil was infested with mycorrhizal inoculum,

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the seedlings were removed from each flat and washed in flowing tap water to remove adhering soil particles, and 20 mycorrhizal and 40 nonmycorrhizal seedlings of uniform height and size were selected.

Just before the seedlings were transplanted, 100 g of mycorrhiza-infested soil containing about 190 *G. etunicatus* chlamydospores was mixed in the top 10 cm of steam-pasteurized Astatula fine sand subsoil in each of twenty 20-cm clay pots; 100 g of steam-pasteurized nonmycorrhizal soil was mixed in each of forty similar pots. Astatula fine sand was used because it is a preferred soil for maximum activity of *R. similis* (12). Twenty mycorrhizal seedlings were transplanted into pots containing nonmycorrhiza-infested soil, and the 40 nonmycorrhizal seedlings were transplanted into 20 pots each of soil with and without *G. etunicatus* inoculum. One week later, 100 *R. similis* extracted by root incubation (21) from greenhouse-infected citrus seedlings were added to each of half of the pots in each group of the seedlings. The treatments were: 1) mycorrhizal seedlings in soil free of *G. etunicatus* and *R. similis;* 2) mycorrhizal seedlings in soil free of *G. etunicatus* but inoculated with *R. similis;* 3) nonmycorrhizal seedlings in soil infested with *G. etunicatus* but not inoculated with *R. similis;* 4) nonmycorrhizal seedlings in soil infested with *G. etunicatus* and inoculated with *R. similis;* 5) nonmycorrhizal seedlings in soil free of *G. etunicatus* and *R. similis;* and 6) nonmycorrhizal seedlings in soil free of *G. etunicatus* but inoculated with *R. similis.* Treatments were replicated 10 times, 1 plant/treatment, and randomized in a complete block design on a greenhouse bench. Fiberglass separators were placed between pots to prevent accidental cross-contamination.

Transplanted seedlings were maintained at ambient greenhouse temperatures ranging from 20–32 C and given regular cultural maintenance, except fertilization. Each seedling received 400 ml of an 8-8-8 (N-P-K) liquid nutrient solution at 1 and 5 months and a liquid 12-0-6 solution plus minor elements 3 and 7 months after transplanting. Stem diameter was measured bimonthly to determine growth rate.

Nine months after transplanting, plant height and stem internodes were measured and plants were harvested. Stems were cut at the soil line and tops of plants were oven-dried at 60 C for 48 h. Roots were removed and washed in flowing tap water. Two to 3 g of nematode-infected and nematode-free feeder roots from mycorrhizal plants were placed in hot acid-fuchsin lactophenol to stain vesicles, hyphae, and nematodes, and were cleared in lactophenol. Twenty-five 1-cm-long root sections from each replicate were examined with a dissecting microscope for vesicles and external mycelial development and were rated for each. The vesicle rating system was 0 = 0, 1 = 1–100, 2 = 101–200, and 3 = over 200 per 1-cm root section. Mycelial development was rated as follows: 0 = 0, 1 = slight, 2 = moderate, and 3 = extensive colonization. The *R. similis* was extracted from 2-to-3-g root portions from nematode-infected seedlings by incubation. Nematodes were counted at 3 and 5 days; moist roots were weighed and the numbers of *R. similis* per gram of root were recorded. After examination, all roots were oven-dried and weighed to obtain total root dry weights.

The soil in each pot was passed through a 1-mm sieve to remove extraneous debris. A 25-g sample was removed, and chlamydospores were recovered by the wet-sieve method, in which spores are trapped on 53- and 44-μm sieves, and counted (10). Data were subjected to analysis of variance, and differences between means were evaluated using Duncan's multiple-range test.

RESULTS

The influence of *G. etunicatus* and *R. similis* on the growth of rough lemon seedlings became evident 2 months after transplanting. Differences (P = 0.05) in stem diameter were first found 4 months after transplanting (Fig. 1). The stem diameter of mycorrhizal seedlings in uninfested soil (curve 1) was greater (P = 0.05) than that of seedlings in all other groups, whereas soil inoculation with *R. similis* suppressed the growth of mycorrhizal seedlings (curve 2). By 6 months after transplanting, the stimulative influence of soil infestation with mycorrhiza on nonmycorrhizal seedlings was apparent (curve 3), and by the end of the experiment these seedlings had achieved a growth rate similar to that of mycorrhizal seedlings. Seedling growth was less (P =

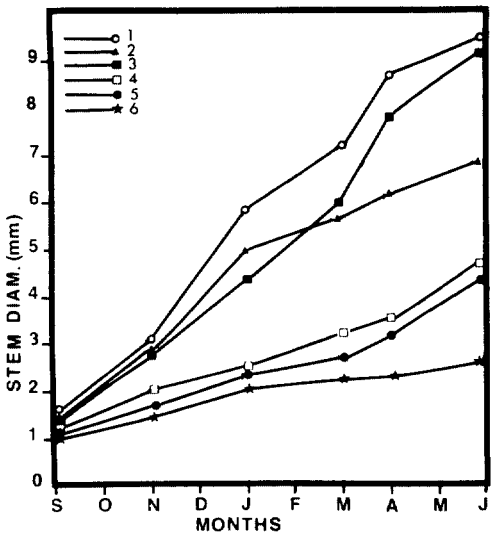

FIG. 1. Influence of single and combined inoculations with *Glomus etunicatus* and *Radopholus similis* on development of rough lemon seedlings during 9 months as demonstrated by stem diameter of: (1) mycorrhizal seedlings in soil not infested with *G. etunicatus* or inoculated with *R. similis;* (2) mycorrhizal seedlings in soil inoculated with *R. similis alone;* (3) nonmycorrhizal seedlings in soil infested with *G. etunicatus* alone; (4) nonmycorrhizal seedlings in soil infested with *G. etunicatus* and inoculated with *R. similis;* (5) nonmycorrhizal seedlings in soil free of *G. etunicatus* and *R. similis;* and (6) nonmycorrhizal seedlings in soil inoculated with *R. similis* alone.

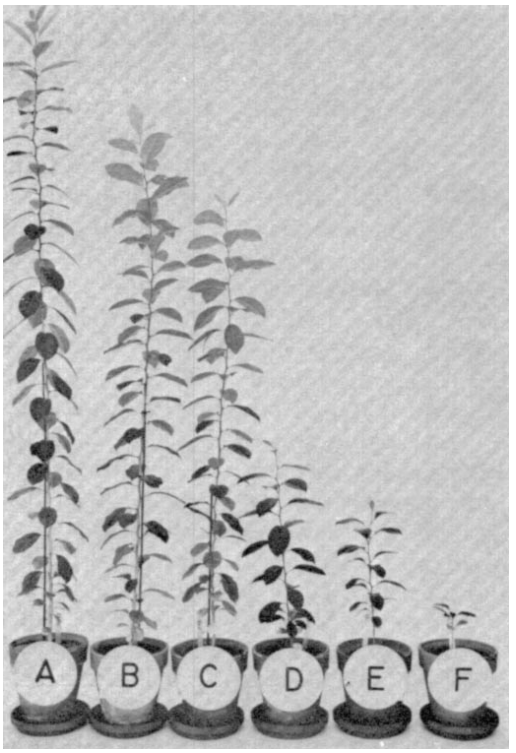

FIG. 2. Influence of *Glomus etunicatus* and *Radopholus similis* on growth of rough lemon seedlings after 9 months. A) mycorrhizal seedlings alone; B) nonmycorrhizal seedlings transplanted in soil infested with *G. etunicatus;* C) mycorrhizal seedlings inoculated with *R. similis;* D) nonmycorrhizal seedlings in soil infested with *G. etunicatus* and inoculated with *R. similis;* E) nonmycorrhizal seedlings not infested with *G. etunicatus* or *R. similis;* and F) nonmycorrhizal seedlings inoculated with *R. similis* alone.

0.01) with the other treatments. The stem diameter of nonmycorrhizal seedlings in nematode-infested soil (curve 6) was significantly different (P = 0.01) from that of all other seedlings after 7 months (Fig. 1) and at harvest (Fig. 2). The stimulative effect of mycorrhizae on seedlings transplanted into soil infested with *G. etunicatus* and inoculated with *R. similis* was negated by the pathogenic effect of the nematodes (curve 4). Nonmycorrhizal seedling growth was dependent upon fertilization, particularly with P.

When the experiment was terminated, mycorrhizal seedlings in soil free of *G. etunicatus* and *R. similis* (treatment 1) were respectively about 4 and 11 times as tall as nonmycorrhizal seedlings in uninfested soil (treatment 5) and nonmycorrhizal seedlings in soil free of *G. etunicatus* but inoculated with *R. similis* (treatment 6) (Table 1, Fig. 2). Stem internode increment growth was shorter (P = 0.05) in nematode-infected seedlings than in nematode-free seedlings (Table 1).

Shoot and root oven-dry weights paralleled the other growth data, verifying the stimulative influence of *G. etunicatus* and the suppressive influence of *R. similis* on plant growth (Table 1).

Examination of roots in lactophenol for frequency of vesicle and mycelial development showed that 100% of the roots from each mycorrhizal seedling (treatment 1) and each nonmycorrhizal seedling grown in soil infested with *G. etunicatus* (treatment 3) contained both vesicles and hyphae. Roots of only 50% of the mycorrhizal seedlings from soil infested with *R. similis* (treatment 2) contained vesicles, but all showed mycelial development, whereas roots of all but one nonmycorrhizal seedling grown in soil infested with *G. etunicatus* and *R. similis* (treatment 6) contained vesicles and hyphae. Vesicle and mycelial

TABLE 1. Effects of *Glomus etunicatus* and *Radopholus similis*, alone and in combination, on growth of *Citrus limon*, nematode and chlamydospore populations, and mycorrhizal development.

Treatment no.	Treatment: *G. etunicatus*	Treatment: *R. similis*	Growth of *C. limon*: Plant height (cm)	Growth of *C. limon*: Internode growth (mm)	Growth of *C. limon*: Oven-dry weight: Shoot (g)	Growth of *C. limon*: Oven-dry weight: Root (g)	Population: *R. similis*/ g of root	Population: Chlamydospores/ 25g of root	Mycorrhizal development rating of roots[a]: Vesicles	Mycorrhizal development rating of roots[a]: Mycelia
Mycorrhizal seedling										
1	+	0	182.1a[b]	2.53a	42.5a	14.6a		135.0a	1.05a	1.02a
2	+	+	100.5c	2.19b	16.1c	7.0c	81.9a	64.8b	0.22b	0.50b
Nonmycorrhizal seedlings										
3	+	0	161.5b	2.49a	33.5b	10.6b		48.8b	1.07a	1.11a
4	+	+	50.9d	1.95c	6.1d	2.8d	85.9a	12.8b	0.52b	0.84b
5	0	0	37.7d	2.44a	3.3d	2.6d				
6	0	+	15.0e	1.01d	0.5e	0.3e	157.6a			

[a]Root ratings: vesicles (no./25 1-cm root sections: 0 = 0, 1 = 1–100, 2 = 101-200, 3 = over 200); mycelial colonization (in 25 1-cm root sections: 0 = none, 1 = slight, 2 = moderate, 3 = extensive).

[b]Each value is the mean for 10 plants. Within a column, means followed by the same letter do not differ significantly (P = 0.05) according to Duncan's multiple-range test.

development were significantly (P = 0.05) less in mycorrhizal nematode-infected seedlings than in the comparable nematode-free seedlings (Table 1).

DISCUSSION

In this study and a similar study not reported here, *R. similis* numbers from mycorrhizal seedlings were not different from numbers from nonmycorrhizal seedlings; however, the reduction in vesicle formation was significant (Table 1). Since vesicles of *G. etunicatus* can occupy much of the root cortex, as can *R. similis,* it suggests that disruption of corticle tissue by *R. similis* leaves little space for colonization by the symbiont. The severity of root damage also adversely effects mycelial growth; therefore *R. similis* severely limits the development of *G. etunicatus.* Similarly, Atilano *et al.* (1) found that *Meloidogyne arenaria* on mycorrhizal grape roots negated the beneficial effects of *Glomus fasciculatus.* On the other hand, *Pratylenchus brachyurus* or *Meloidogyne incognita* on cotton apparently do not adversely influence *Gigaspora margarita* development (5, 15), and *Heterodera solanacearum* and *Endogone gigantea* are mutually antagonistic on tobacco (3). Because *G. etunicatus* root infection was reduced by *R. similis,* its role in increasing the uptake of nutrients, particularly phosphorus (4, 20), was probably severely inhibited in nematode-infected seedlings. This inhibition, combined with the virulence of the pathogen, suppressed plant growth. While mycorrhizal development may slow infection and disease production by *R. similis,* disease symptoms caused by the pathogen suppressed the beneficial influence of the symbiont.

Fungus sporulation appeared variable, and chlamydospore numbers were lower from soils containing *R. similis*—infected seedlings. There is no indication that *R. similis* influenced sporulation, but rather spore production was less because of an undesirable substrate for mycelia growth.

The deleterious effect of *R. similis* on plant development was particularly noticeable in the length of internodal growth. Shortened internodes resulted from infection with *R. similis,* regardless of mycorrhizal involvement.

Particularly important in greenhouse studies is the general practice of sterilizing the soil or potting media before planting and inoculating with the pest to be studied. This experiment graphically illustrates the need to account for the effects of other organisms in interpretation of results.

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