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Influence of Mycorrhizal Fungus, Phosphorus, and Burrowing Nematode Interactions on Growth of Rough Lemon Citrus Seedlings[1]

G. S. SMITH AND D. T. KAPLAN[2]

Abstract: Rough lemon seedlings were grown in mycorrhizal-infested or phosphorus-amended soil (25 and 300 mg P/kg) in greenhouse experiments. Plants were inoculated with the citrus burrowing nematode, *Radopholus citrophilus* (0, 50, 100, or 200 nematodes per pot). Six months later, mycorrhizal plants and nonmycorrhizal, high-P plants had larger shoot and root weights than did nonmycorrhizal, low-P plants. Burrowing nematode population densities were lower in roots of mycorrhizal or nonmycorrhizal, high-P plants than in roots of nonmycorrhizal, low-P plants; however, differences in plant growth between mycorrhizal and nonmycorrhizal plants were not significant with respect to initial nematode inoculum densities. Phosphorus content in leaf tissue was significantly greater in mycorrhizal and nonmycorrhizal, high-P plants compared with nonmycorrhizal, low-P plants. Nutrient concentrations of K, Mg, and Zn were unaffected by nematode parasitism, whereas P, Ca, Fe, and Mn were less in nematode-infected plants. Enhanced growth associated with root colonization by the mycorrhizal fungus appeared to result from improved P nutrition and not antagonism between the fungus and the nematode.

Key words: symbiont, tolerance, *Glomus intraradices, Radopholus citrophilus, Citrus limon,* burrowing nematode, rough lemon.

Vesicular-arbuscular mycorrhizal (VAM) fungi and plant-parasitic nematodes are ubiquitous in agricultural soils. Both groups of organisms are obligately dependent on the host for nourishment and reproduction and are commonly found in association with host feeder roots. The potential of VAM fungi to alleviate nematode-induced plant stress has been widely investigated because of their ability to increase root growth and nutrient absorption (16). Numerous studies have reported that VAM fungi increase host tolerance or resistance in many plant–nematode systems. Most of these studies, however, did not address whether the improved host response was a result of improved host nutrition or antagonism or competition between the nematode and the mycorrhizal fungus.

The citrus burrowing nematode (CBN), *Radopholus citrophilus* Huettel, causes spreading decline, a destructive disease of citrus, in central Florida (5). CBN-infected trees in deep, well-drained sands may have 50% fewer functional feeder roots than uninfected trees. VAM fungi colonize citrus feeder roots and increase uptake of phosphorus and minor elements in Florida citrus soils (8,14).

Most mycorrhiza-nematode studies have investigated sedentary endoparasites, whereas few investigations have been conducted with migratory endoparasites (16). On cotton, Hussey and Roncadori (4) reported the absence of an interaction between *Pratylenchus brachyurus* (Godfrey) Filip. & Schuur.-Stehk. and the VAM fungus, *Gigaspora margarita* Becker & Hall; nematode reproduction and fungus sporulation were unaffected by the presence of either organism at two fertility levels. O'Bannon et al. (12,13) reported that the VAM fungus, *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, increased tolerance of rough lemon to both *R. citrophilus* (*R. similis*) and *Tylenchulus semipenetrans* Cobb. Because these studies were conducted in P-deficient soils, increased tolerance may have resulted from improved host nutrition of mycorrhizal plants.

The objectives of this study were to compare the effects of a VAM fungus and P fertilization on plant growth responses to

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[2] Research Plant Pathologists, USDA ARS, Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803. Present address of first author: IPM, 45 Agriculture Bldg., University of Missouri, Columbia, MO 65201.

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CBN infection, mineral nutrition, and CBN root population densities.

Materials and Methods

Treatments in two experiments included mycorrhizal (VAM) and nonmycorrhizal plants grown in soil amended with 25 mg P/kg soil (NMLP) and nonmycorrhizal plants grown in soil amended with 300 mg P/kg soil (NMHP). Seeds of *Citrus limon* (L.) Burm. f. cv. rough lemon were planted in two flats containing a 1:1 (v:v) mixture of steam-pasteurized Astatula fine sand (hyperthermic, uncoated typic quartzipsamments) and Lakeland fine sand (thermic, coated typic quartzipsamments, 97% sand, 2% silt, 1% clay) at pH 7.1 and P = 30 mg P/kg soil. Mycorrhizal seedlings were grown in one flat by infesting soil with 100 g soil and root inoculum of *Glomus intraradices* Schenck & Smith before planting. Nonmycorrhizal seedlings were grown in another flat in soil mix amended with 200 mg P/kg soil. Seedlings were grown in the greenhouse for 6 weeks before transplanting to 15-cm-d clay pots.

Before transplanting, nonamended soil mix sufficient to fill 80 15-cm-d pots was amended with 20 g finely ground triple super-phosphate (TSP at 25 mg P/kg soil) for the VAM and NMLP treatments and with 160 g TSP (300 mg P/kg soil) for the NMHP treatments and mixed for 3 minutes in a cement mixer. Soil P levels were selected to produce nonmycorrhizal plants that would not be severely deficient in P and would have similar-sized shoot and root systems as mycorrhizal plants. Soil, analyzed for P by Bray II double acid extraction 1 week after transplanting, contained 35 mg P/kg soil in the VAM and NMLP treatments and 200 mg P/kg soil in the NMHP treatment. Four weeks after transplanting, NMHP plants received an additional 1 g TSP (50 mg P/kg soil) per pot, and VAM plants were inoculated with 70 spores per pot of *G. intraradices* in a 10-ml aliquot poured around the base of each seedling.

Radopholus citrophilus biotype 1 was increased on monoxenic carrot disc cultures (10). Inoculum was extracted with 50% pectinol in water, retrieved by sugar centrifugation, and subsequently washed free of sucrose by centrifugation in sterile tap water. Soil was infested with 0, 50, 100, or 200 freshly extracted nematodes (mixed life cycle stages) per pot by pouring a 50-ml aliquot in a shallow furrow around the base of the seedling 9 and 11 weeks after transplanting in experiments 1 and 2, respectively. Each treatment-nematode inoculum level was replicated 10 times in experiment 1 and 9 times in experiment 2 and arranged in a randomized complete block design.

Seedlings were grown under shade cloth in a greenhouse. Soil temperatures ranged from 23 to 28 C. All plants were fertilized on a monthly basis with 20-2-20 (N = 20%, P = 0.8%, K = 16.6%) plus micronutrients to deliver approximately 90 mg N, 4 mg P, and 80 mg K per pot. Plants in experiment 1 received water from emittors every 24 hours, whereas plants in experiment 2 were manually watered at the onset of leaf wilt.

Plants were harvested 6 months after nematode inoculation. Plant height and shoot and root dry weights were recorded. Feeder roots were excised from the taproot, a 0.5-g subsample was removed from VAM root systems to assay for mycorrhizal colonization, and the remaining roots were incubated in glass jars at 25 C for 7 days (18) to determine the population densities (Pf) of *R. citrophilus*. Nematodes were collected by washing roots on a 500-μm-pore sieve over a 90-μm-pore sieve. Pf values were adjusted on mycorrhizal root systems to account for the 0.5-g subsample removed from the mycorrhizal assay. Nematode data were expressed as nematodes per gram dry root weight and Pf.

Roots from 0.5-g subsamples of all VAM plants and NMLP and NMHP control plants were cleared in hot 10% KOH for 30 minutes, rinsed three times in tap water, cleared again in 10% H_2O_2, rinsed overnight in tap water, and stained in 0.05% trypan blue in lactophenol. Percentage of

root colonization was determined by the gridline-intersect method (3). Shoots and roots were dried at 70 C for 48 hours before weighing. Three dried leaf samples were obtained by compositing leaves from three replicates from each treatment in each experiment and analyzed for Ca, Fe, K, Mg, Mn, P, and Zn. Phosphorus concentration was determined by the vanadomolybdo-phosphoric yellow color method (6) and the remaining elements determined by atomic absorption spectrophotometry.

The data were analyzed as a factorial design by analysis of variance. Main treatment effects consisted of NMLP, NMHP, and VAM treatments and nematode inoculum levels. Duncan's multiple-range test was used to determine differences between NMLP, NMHP, and VAM treatments. Nematode main effects were partitioned into linear, quadratic, and cubic components using orthogonal contrasts to determine host response to nematode inoculum levels. Root nematode population density data (CN) were log-transformed before analysis with the zero nematode inoculum level deleted from the analysis. Unbiased estimates of the means of log-transformed data were calculated (17). Data presented are from combined experiments 1 and 2.

Results

Only main effects are presented because interactions were not significant. NMHP and VAM plants had significantly greater shoot, root, and relative shoot dry weights than the NMLP plants (Table 1). Although shoot dry weights were similar between NMHP and VAM plants, root fresh weights were significantly greater in NMHP plants. The relationship between decreasing plant growth and increasing CBN inoculum densities was best described by the linear component ($P = 0.001$) for shoot dry weight and the linear and quadratic components ($P = 0.005$) for root fresh weight.

The effect of CBN parasitism, P fertilization, and VAM fungal colonization on leaf nutrient concentrations was evaluated based on leaf tissue analysis of NMLP, NMHP, and VAM plants grown in soil free of CBN or soil infested with the highest inoculum level (Table 2). Concentrations of P, Ca, Fe, and Mn were lower in CBN-infected plants than in uninfected plants. Concentrations of P, K, Mg, and Mn, however, varied with P fertilization and mycorrhizal status. Leaf concentration of P was significantly greater in NMHP and VAM plants than in NMLP plants. Concentrations of Mn were significantly lower in NMLP and VAM than in NMHP leaves, K was higher in nonmycorrhizal than VAM leaves, and Mg was different among all treatments.

Final CBN root population densities (Pf) were not affected by initial nematode inoculum level. Because the high soil P level and mycorrhizal root colonization increased feeder root weight, CBN root population densities were calculated on whole root systems and on a per gram dry root weight basis (Table 3). Amending the soil with a high level of P or inoculation with a VAM fungus resulted in significantly fewer CBN per root system and per gram dry root weight.

Within VAM plants, mean percentage of root colonization of *G. intraradices* was 48% in CBN-infected and 55% in uninfected plants. NMLP and NMHP control plants were not colonized by mycorrhizal fungi.

Table 1. Shoot and root growth of rough lemon grown in soil infested with *Radopholus citrophilus* as affected by phosphorus fertilization or mycorrhizal inoculation.

Treatment†	Shoot dry weight (g)	Relative shoot dry weight	Root fresh weight (g)
NMLP	8.06 b	0.58 b	11.91 c
NMHP	9.74 a	0.71 a	15.94 a
VAM	9.84 a	0.71 a	13.80 b

Values are main effect means of plant response averaged over four inoculum densities (0, 50, 100, and 200 nematodes per pot) of *R. citrophilus*. Means within columns followed by the same letter are not significantly different according to Duncan's new multiple-range test.

† NMLP = nonmycorrhizal plants grown in soil amended with 25 mg P/kg soil at planting. NMHP = nonmycorrhizal plants grown in soil amended with 300 mg P/kg soil at planting. VAM = plants grown in soil amended with 25 mg P/kg soil and infested with soil and root inoculum of *Glomus intraradices* at planting.

TABLE 2. Influence of phosphorus fertilization, mycorrhizal inoculation, and citrus burrowing nematode (CBN) infection on the concentration of nutrients in rough lemon leaf tissue.

Treatment†	CBN level	Nutrient concentration in leaf tissue						
		Percentage				Dry weight tissue (μg/mg)		
		P	K	Ca	Mg	Fe	Mn	Zn
NMLP	0	0.162	2.83	2.57	0.202	82.9	32.0	25.0
	200	0.142	2.84	1.87	0.193	65.9	22.6	24.1
	Mean	0.153 b	2.83 a	2.25 a	0.198 c	75.2 a	27.8 b	24.6 a
NMHP	0	0.221	2.79	2.51	0.264	77.2	44.8	26.5
	200	0.201	2.90	2.22	0.271	70.7	39.8	23.9
	Mean	0.211 a	2.85 a	2.37 a	0.268 a	74.0 a	42.3 a	25.2 a
VAM	0	0.230	2.42	2.48	0.243	75.5	28.8	25.2
	200	0.207	2.47	2.03	0.219	65.6	21.7	24.8
	Mean	0.218 a	2.44 b	2.25 a	0.231 b	70.5 a	25.2 b	25.0 a
CBN main effect‡		***	NS	***	NS	***	***	NS

Values are means of six composite samples from experiments 1 and 2. Composite samples consisted of fully expanded leaves from three replicate plants in each treatment to give three samples per experiment. Treatment means within columns followed by the same letter are not significantly different according to Duncan's new multiple-range test.

† NMLP = nonmycorrhizal plants grown in soil amended with 25 mg P/kg soil at planting. NMHP = nonmycorrhizal plants grown in soil amended with 300 mg P/kg soil at planting. VAM = plants grown in soil amended with 25 mg P/kg soil and infested with soil and root inoculum of *Glomus intraradices* at planting. Initial nematode inoculum densities compared were 0 or 200 nematodes per pot applied at transplanting.

‡ For comparisons of 0 and 200 CBN inoculum levels averaged over VAM, NMLP, and NMHP treatments by Fisher's least significant difference. *** $P = 0.001$. NS = not significant.

DISCUSSION

The similarity in shoot and root growth and leaf nutrient concentrations between NMHP and VAM plants exemplifies the nutritional benefits that VAM fungi confer to citrus. Furthermore, the magnitude of decreasing plant growth to increasing CBN inoculum densities was not different between nonmycorrhizal and mycorrhizal plants. Although nonmycorrhizal and mycorrhizal plants had leaf P levels considered sufficient for nonbearing citrus (7), the significantly higher concentration of P in the NMHP and VAM plants was the single factor consistently associated with increased plant growth, and fewer CBN-infected plants is a direct result of improved P nutrition of the host. Under experimental conditions, however, tolerance of CBN in citrus is not conferred by mycorrhiza or P.

TABLE 3. Citrus burrowing nematode (CBN) population densities in roots of rough lemon seedlings as affected by phosphorus fertilization or mycorrhizal inoculation.

Treatment†	CBN/g dry root weight	CBN/plant (Pf)
NMLP	754 a	1,479 a
NMHP	313 b	289 b
VAM	225 b	238 b

Values are main effect means of log transformed data with 0 nematode inoculum level deleted from analysis. Means presented are antilogs of unbiased estimates of log transformed data. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple-range test.

† NMLP = nonmycorrhizal plants grown in soil amended with 25 mg P/kg soil at planting. NMHP = nonmycorrhizal plants grown in soil amended with 300 mg P/kg soil at planting. VAM = plants grown in soil amended with 25 mg P/kg soil and infested with soil and root inoculum of *Glomus intraradices* at planting.

O'Bannon and Nemec (12) reported that preinoculation with a mycorrhizal fungus increased tolerance of rough lemon seedlings to CBN, compared with nonmycorrhizal seedlings grown in a P-deficient soil. There were dramatic increases, however, in growth of mycorrhizal, compared with nonmycorrhizal, seedlings in the absence of CBN. The lack of P-fertilized, nonmycorrhizal plants that were similar in size and nutrient status to the mycorrhizal plants precluded an assessment that the increased tolerance in mycorrhizal citrus to CBN, compared with nonmycorrhizal citrus, primarily results from improved P nutrition.

Root population densities of CBN were less on NMHP and VAM root systems than on NMLP root systems in our experiments. O'Bannon and Nemec (12) reported no dif-

ferences in CBN root population densities between mycorrhizal and nonmycorrhizal plants. Although initial inoculum densities were similar, differences in harvest dates, sampling procedures, and the number of replications could sufficiently account for the disparate results between these two studies.

The mechanism through which improved P nutrition reduces CBN root population densities in a CBN-susceptible citrus cultivar warrants further research. Nematode tolerance and reproduction in citrus have been related previously to root P levels and soil type (9,11). Soil population densities of *T. semipenetrans* were lower when the root P concentration of sweet orange was greater than 0.3% (9). Shoot and root growth of citrus were less affected and CBN root population densities were lower on plants grown in a Florida Leon loamy sand (sandy, siliceous, thermic aeric haploquods, 7.5% clay, 2.3% organic matter) than on plants grown in a Florida Lakeland sand (1.7% clay, 0.2 organic matter) (11). Phosphorus nutrition may have been a factor because mineral soils with higher clay and OM content and near neutral pH generally have higher available soil P levels (2).

The effect of CBN infection on leaf K, P, and Ca concentrations of greenhouse-grown plants varied from that reported in 8–40-year-old trees on rough lemon rootstock in various stages of CBN decline (1). Nutrient absorption may be influenced by many factors, such as soil temperature, water availability, and light intensity. Differences in nutrient uptake between field and greenhouse CBN-infected plants were probably caused by factors related to feeder root densities and mobility of these mineral elements in the soil profile. Phosphorus and Ca are not readily leached through the soil profile, and absorption by roots is an active process that is proportional to root absorptive area (2). Nitrate and K are highly mobile ions, however, with root absorption more a function of mass flow and diffusion.

In field trees grown in CBN-infested soil, leaf concentrations of N and K are lower, whereas P and Ca are unaffected (1). Destruction of feeder roots by CBN is five times greater at 75 cm deep than in the top 39 cm of the soil profile (5). Concentrations of P and Ca in Florida ridge soils are usually greatest in the top 30 cm soil, whereas the highly mobile NO_3 and K are easily leached below the soil profile with the highest density of feeder roots in CBN-infected trees. Failure of K fertilizations to increase leaf K of CBN-infected trees was previously attributed to autumn tree dormancy and leaching of K below the root zone (1). Conversely, plant nutrient availability may be limited in containers because of the limited soil volume. The destruction of feeder roots by CBN should affect P and Ca uptake more than N and K uptake.

The lack of an effective nematicide for CBN management in Florida has eliminated the "push-and-treat" and chemical barrier control programs (5). This has placed added emphasis on cultural practices designed to minimize stress-related factors that predispose CBN-infected citrus trees to decline. Although Smith et al. (15) determined that P application to healthy citrus groves did not increase yield, our findings suggest that increased P fertilization in CBN-infested groves is worth considering as a stress-reducing component in the management strategy for spreading decline sites.

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