Influence of *Glomus fasciculatus* and Soil Phosphorus on Phytophthora Root Rot of Citrus

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ABSTRACT

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In soil fertilized with less than 15 μg P/g soil, roots of sweet orange seedlings infected with *Phytophthora parasitica* and the mycorrhizal fungus *Glomus fasciculatus* were healthier and weighed more than roots infected with *P. parasitica* alone. However, the beneficial effect of *G. fasciculatus* was eliminated by *P. parasitica* in mycorrhizal seedlings fertilized with more than 56 μg P/g soil. In citrus seedlings with split root systems inoculated with *P. parasitica*, the percentage of healthy nonmycorrhizal roots was significantly greater when these roots were split from mycorrhizal roots than when they were split from nonmycorrhizal roots. Numbers of propagules of *P. parasitica* were greater in the rhizosphere of roots in which one or both sides of the root system were mycorrhizal than in the rhizosphere of roots in which both sides of the root system were nonmycorrhizal. All evidence suggests that tolerance to *P. parasitica* root rot in citrus infected with *G. fasciculatus* is caused by the ability of mycorrhizal roots to absorb more phosphorus and possibly other minerals than nonmycorrhizal roots.

Additional key words: vesicular-arbuscular mycorrhizae, endomycorrhizae, soilborne fungi.

Several studies indicate that root infection by vesicular-arbuscular (VA) mycorrhizal fungi influence diseases caused by soilborne fungi. Plants with mycorrhizae may be more severely affected by root pathogens than plants without mycorrhizae. Mycorrhizal roots of a cultivar of soybean susceptible to *Phytophthora* were more susceptible to *P. megasperma* than were nonmycorrhizal roots (14), and mycorrhizal roots of avocado were more susceptible to *P. cinnamomi* than were nonmycorrhizal roots (3). In contrast, VA mycorrhizal fungi increased resistance of tobacco to disease caused by *Thielaviopsis basicola* (1), and nonmycorrhizal cotton roots were more severely damaged by *T. basicola* than were mycorrhizal roots (16). Wilting of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* was reduced when plants were preinfected by the mycorrhizal fungus *Glomus mosseae* (4).

Since VA mycorrhizae explore greater amounts of soil and absorb more phosphorus and certain other minerals than do nonmycorrhizal roots (7,15), the influence of a mycorrhizal fungus on disease may be due to the associated increase in mineral absorption rather than to a direct influence of the mycorrhizal fungus itself. Earlier, it was found that the number of healthy roots in mycorrhizal citrus inoculated with *P. parasitica* was greater than those in nonmycorrhizal citrus inoculated with *P. parasitica* (2,3). However, responses to *P. parasitica* were influenced by phosphorus nutrition; in plants not inoculated with *P. parasitica*, concentrations of phosphorus in leaves of mycorrhizal plants were greater than those in leaves of nonmycorrhizal plants.

This study was initiated to determine the role of phosphorus in the interaction between VA mycorrhiza and *P. parasitica* on citrus. If similar concentrations of phophorus could be established in both nonmycorrhizal and mycorrhizal plants, the phosphorus effect in the mycorrhiza-pathogen interaction would be minimized. Citrus with split root systems also were utilized in an attempt to eliminate phosphorus nutrition as a factor in the interaction.

MATERIALS AND METHODS

Interaction between *G. fasciculatus* and *P. parasitica* on citrus with split root systems. After sweet orange (*Citrus sinensis* [L.] Osbeck 'Pineapple') seeds germinated in an autoclaved sandy loam soil and produced primary lateral roots, tap roots of the young seedlings were severed to encourage growth of lateral roots. When the seedlings were 7 wk old, each seedling was transplanted, with its root system divided, into two 10-cm-diameter clay pots containing autoclaved sandy loam (7 μg available phosphorus per gram of soil by Olsen's analysis). Seedlings with root systems of unequal lengths were discarded. Ten grams of inoculum of *Glomus fasciculatus* (Thaxter) Gerd. and Trappe was added to one pot, both pots, or neither of the pots at transplant (Table 1). Inoculum consisted of soil, roots, and spores (20–30 spores per gram of soil) from a pot containing sudan grass (*Sorghum vulgare* Pers.) infected with *G. fasciculatus*. Eleven weeks later, the presence of *G. fasciculatus* in roots was confirmed by observation of cleared and stained root samples and again plants with unequal root systems were discarded.

Plants with or without mycorrhizae (Table 1) were gently lifted from pots and were inoculated with zoospores of *P. parasitica* Dast. M114 (*P. nicotianae* var. *parasitica* [Dast.] Waterhouse) by submerging roots for 1 hr in 600 ml of water containing 500 zoospores per milliliter. Zoospores were obtained and suspended in water by the method of Menyonga and Tsao (12). Roots with or without mycorrhizae not inoculated with *P. parasitica* were submerged for 1 hr in sterile distilled water. Repotted plants were grown in a glasshouse at temperatures ranging from 22 to 36 C and were fertilized daily with a 14% Hoagland's solution (8) minus phosphorus. Plants were harvested after 12 wk. Percentages of healthy roots were recorded after roots were stained with tetrazolium for detection of rotting caused by *Phytophthora* spp. (10). The number of brightly stained healthy roots was estimated as a percentage of the total number of roots. Necrotic and inactive roots did not stain. Citrus leaves were analyzed for phosphorus content by the method of Kitson and Mellon (9). Samples of roots were randomly collected for estimation of mycorrhizal infection before each side of the root system was dried and weighed. The percentage of root tissue infected with *G. fasciculatus* was estimated by clearing, staining, and examining 100 or more 1-mm^2 sections of root tissue (3) under a microscope for the presence of arbuscules, vesicles, spores, and/or hyphae of *G. fasciculatus*.

Numbers of propagules of *P. parasitica* in the rhizosphere soil of each root system were determined at the time of harvest. The soil that remained on the roots after the bulk of the soil was shaken off

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TABLE 1. Top and root dry weights, (%) healthy roots, infection by *Glomus fasciculatus*, and associated populations of *Phytophthora parasitica* of sweet orange seedlings with split root systems inoculated with various combinations of *Glomus fasciculatus* and *Phytophthora parasitica*[a]

Treatment[b] (left-right root systems)	Number of replications	Top dry wt	Root dry wt (left-right root systems)	% healthy roots (left-right root systems)	Root tissue infected by *Glomus fasciculatus* (%) (left-right root systems)	No. of propagules of *P. parasitica*/g rhizosphere soil (left-right root systems)
0 -0	5	0.88 z	0.60 z -0.61 z	62 vw-60 vw	···	0-0
M - M	6	12.20 w	4.06 w-4.55 w	90 u -86 u	81 y-82 y	0-0
0 - M	5	10.42 w	3.03 x -4.15 w	86 u -86 u	-84 y	0-0
0 - 0P	5	1.13 z	0.84 z -0.63 z	46 wx-13 z	···	0-3.2 z
M - MP	6	8.69 x	3.15 wx-2.48 xy	89 u-70 uv	82 y-52 z	0-7.1 y
0P - M	4	8.14 x	1.35 yz -4.17 w	35 xy -73 uv	-86 y	8.3 y-0
0 - MP	6	5.57 y	2.03 xy -2.43 xy	54 vw-47 wx	-58 z	0-8.5 y
0P - MP	6	6.62 y	1.43 yz-3.29 wx	28 y -60 vw	-53 z	7.8 y-8.2 y

[a] Means in each column or means from both columns representing measurements from root systems split from the same plant followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] Index of abbreviations: 0 = nonmycorrhizal; M = roots infected by *G. fasciculatus;* P = roots infected by *P. parasitica.*

TABLE 2. Concentrations of phosphorus in leaves of sweet orange seedlings treated with various combinations of *Glomus fasciculatus, Phytophthora parasitica,* and three levels of soil phosphorus[a]

Treatment	Phosphorus in leaves (%)		
	Soil phosphorus (μg P/g soil)		
	6	56	600
Noninfected	0.08 z	0.17 wx	0.18 w
Phytophthora parasitica	0.07 z	0.15 xy	0.20 vw
Glomus fasciculatus	0.17 wx	0.17 wx	0.20 vw
P. parasitica + *G. fasciculatus*	0.14 y	0.15 xy	0.24 u

[a] Values represent mean of 10 seedlings. Means with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

the roots was considered to be rhizosphere soil. This soil was gently scraped off the roots and three 1-g samples from each root system were each divided into five subsamples. Each subsample was plated directly on a petri plate containing the *Phytophthora*-isolation selective medium (PVPH) of Tsao and Guy (17). Plates were incubated at 24 C for 5 days after which colonies of *P. parasitica* were easily recognized.

Influence of soil phosphorus and *G. fasciculatus* on Phytophthora root rot. Sweet orange seeds were germinated in two flats of the sandy loam soil used in the previous experiment. One flat received a layer of inoculum of *G. fasciculatus* as previously described placed 6 cm beneath the seeds. The other flat received a water filtrate from the inoculum of *G. fasciculatus* which included microorganisms associated with the mycorrhizal inoculum. The filtrate was passed through a 20-μm nylon sieve to remove spores of *G. fasciculatus.*

When the seedlings were 8 wk old, seedlings with or without mycorrhizae were inoculated with *P. parasitica* as described above. Roots of seedlings with or without mycorrhizae not inoculated with *P. parasitica* were submerged for 1 hr in sterile distilled water. Ten mycorrhizal or nonmycorrhizal seedlings with or without *P. parasitica* were planted in 10-cm-diameter clay pots (one seedling per pot) containing autoclaved sandy loam (7 μg available phosphorus per gram of soil) amended with single superphosphate ($Ca[H_2PO_4]_2 \cdot H_2O$) at 6, 56, or 600 μg of phophorus per gram of soil. The phosphorus fertilizer was mixed into the soil 48 hr before the seedlings were planted. The pH of the virgin soil was 7.2. The pH was reduced less than one pH unit by the addition of 600 μg P/g soil and at the end of the experiment, pHs of all treatments were similar. Seedlings were grown in a glasshouse at 22–36 C and watered when necessary with a 14% Hoagland's solution (8) lacking phosphorus. After 11 wk, seedlings were harvested and plant weights and percentages of healthy roots were recorded. Roots that were discolored or necrotic were considered to have been damaged by *P. parasitica.* Root samples were randomly selected and the percentage of root tissues infected by *G. fasciculatus* was determined as described above.

Chlamydospores of *G. fasciculatus* were collected from the soil in the pots by a procedure of decanting and wet-sieving through a series of 350-, 250-, and 106-μm wire sieves (6). Citrus leaves were analyzed for phosphorus content by the method of Kitson and Mellon (9), and the contents of potassium, sodium, iron, zinc, and copper were determined as described by Labanauskas et al (11).

Effect of phosphorus on *P. parasitica*. Ten milliliters of a liquid minimal synthetic growth medium (13) minus phosphorus and 10 ml of a phosphoric acid solution in the concentrations described in Table 2 were mixed in 60-mm-diameter petri plates. The pH of each solution of phosphoric acid was adjusted to 7.2 with 1N KOH and each solution was buffered (10% v/v) with a 0.01 M solution of MOPS buffer (3-[*N*-morpholino] propane sulfonic acid). Each test solution was inoculated with a 5-mm diameter agar disk of *P. parasitica* that was grown on solid minimal synthetic medium (1.5% agar) for 5 days. There were three replicate plates per treatment. After the plates were incubated at 24 C for 4 days, agar and colonies of *P. parasitica* were dried in an oven for 24 hr at 50 C and weighed.

The same solutions of phosphoric acid were used to test the release of zoospores of *P. parasitica* by a method developed by P. H. Tsao (*personal communication*). Eighteen-hour-old single spore colonies of *P. parasitica* produced in half-strength cleared V-8 broth were placed on 1-cm^2 sections of nylon mesh (100-μm mesh) and overlaid with 3 ml of half-strength cleared V-8 broth. After incubation for 24 hr in the V-8 broth, the colonies became entangled on the nylon sections and were washed three times in sterile distilled water and incubated in sterile distilled water for an additional 24 hr at 24 C. The colonies were then transferred into the test solutions of phosphoric acid and incubated for 30 min at 24 C, then 90 min at 18 C. The colonies of *P. parasitica* were immediately mounted in lactophenol on microscope slides, and the number of sporangia releasing zoospores in each treatment was counted under a microscope.

RESULTS

Interaction between *G. fasciculatus* and *P. parasitica* on citrus with split root systems. Top dry weights of sweet orange with mycorrhizae on one or both sides of their split root systems were significantly greater ($P = 0.05$) than top dry weights of plants without mycorrhizae or plants inoculated with *P. parasitica* (Table 1, Fig. 1A). Top dry weights of plants with mycorrhizae on both of their split root systems and inoculated with *P. parasitica* were significantly greater than were top dry weights of nonmycorrhizal plants inoculated with *P. parasitica.* Top dry weights of plants with at least one side of their split root systems infected by *G. fasciculatus* alone were significantly greater than those of plants with one side infected by both *G. fasciculatus* and *P. parasitica.* Root dry weights of plants with mycorrhizae on one or both of their split root systems were significantly greater than were the root dry weights of plants without mycorrhizae (Table 1, Fig. 1B). Root dry weights of nonmycorrhizal roots inoculated with *P. parasitica* were not significantly different whether they were split from

nonmycorrhizal roots or mycorrhizal roots. Dry weights of mycorrhizal roots inoculated with *P. parasitica* also were similar whether they were split from mycorrhizal roots or nonmycorrhizal roots with or without *P. parasitica*. In plants not inoculated with *P. parasitica*, dry weights of roots infected by *G. fasciculatus* were significantly greater than were weights of nonmycorrhizal roots on the same plant. This difference in size between mycorrhizal and nonmycorrhizal roots on the same plant was visually evident and was responsible for many plants being discarded before they could be used in the experiment. Similarly, dry weights of roots infected by *G. fasciculatus* and *P. parasitica* together were significantly greater than were dry weights of nonmycorrhizal roots infected by *P. parasitica* on the same plant.

In plants not inoculated with *P. parasitica*, percentages of healthy roots of plants with mycorrhizae on one or both halves of split root systems were significantly greater ($P = 0.05$) than were percentages of healthy roots of plants with nonmycorrhizal roots on both halves of split root systems (Table 1). This difference probably was due to the inability of nonmycorrhizal plants to absorb adequate amounts of phosphorus. Generally, infection by *P. parasitica* reduced percentages of healthy roots with or without *G. fasciculatus*, but the percentage of healthy roots infected by both *P. parasitica* and *G. fasciculatus* split from roots infected by *G. fasciculatus* alone was not significantly reduced. The percentage of healthy nonmycorrhizal roots inoculated with *P. parasitica* was significantly greater when they were split from mycorrhizal roots than when they were split from nonmycorrhizal roots. The percentage of healthy mycorrhizal roots inoculated with *P. parasitica* was significantly greater than was the percentage of healthy nonmycorrhizal roots inoculated with *P. parasitica* on the same plant (Table 1, Fig. 1B).

The percentage of root tissue infected by *G. fasciculatus* was reduced only when roots were concomitantly infected by *P. parasitica* (Table 1). When *P. parasitica* and *G. fasciculatus* infected different halves of the root system of the same plant, root infection by *G. fasiculatus* was not reduced.

Numbers of colonies originating from propagules of *P. parasitica* recovered from rhizosphere soil of plants with mycorrhizal infection on one side of split root systems and numbers of propagules of *P. parasitica* in rhizosphere soil of plants with mycorrhizae on both sides of their split root systems were not significantly different (Table 1). Numbers of propagules of *P. parasitica* produced in the rhizosphere of plants with completely nonmycorrhizal root systems were significantly less ($P = 0.05$) than were those in the rhizosphere of plants with mycorrhizae on at least one side.

Concentrations of phosphorus in leaves of plants with mycorrhizae on one or both of their root systems, but not inoculated with *P. parasitica*, were significantly greater than were phosphorus concentrations in leaves of plants without mycorrhizae. Differences between the concentrations of phosphorus in plants with mycorrhizae on one side and plants with

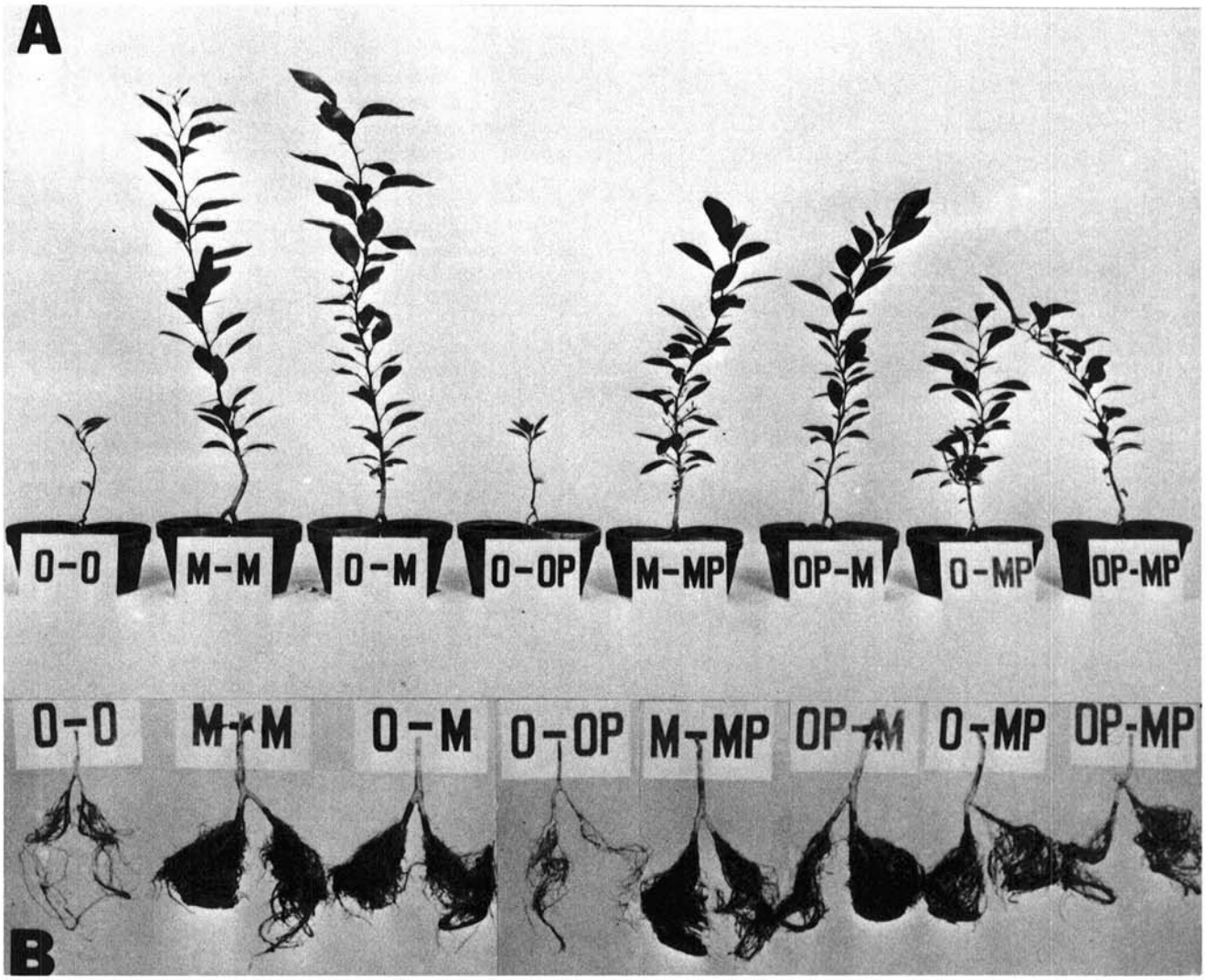

Fig. 1. A, Interactions between *Glomus fasciculatus* and *Phytophthora parasitica* on citrus with split root systems as indicated by growth responses of citrus, and **B,** split root systems stained with tetrazolium chloride, which stained healthy roots red (dark in photograph). Legend of abbreviations: 0 = nonmycorrhizal roots, M = mycorrhizal roots, and P = roots inoculated with *P. parasitica*.

mycorrhizae on both sides were not significant ($P = 0.05$). The effects of *P. parasitica* on concentrations of phosphorus in leaves were not consistent.

Influence of soil phosphorus and *G. fasciculatus* on Phytophthora root rot. Total dry weights of nonmycorrhizal sweet orange seedlings increased as the concentrations of phosphorus in the soil increased (Table 3). Plants infected by *G. fasciculatus*, however, were not as responsive to the increases of phosphorus in the soil and total dry weights of mycorrhizal plants at different concentrations of phosphorus in the soil were not significantly different ($P = 0.05$). Total dry weights of mycorrhizal plants were reduced by *P. parasitica* in soil fertilized with any concentration of phosphorus (Table 3). Total dry weights of nonmycorrhizal seedlings were significantly reduced by *P. parasitica* only in soil fertilized with 56 μg P/g soil. In soil fertilized with 6 μg P/g soil, total dry weights of mycorrhizal plants infected by *P. parasitica* were significantly greater than were those of nonmycorrhizal plants infected by *P. parasitica.* In soil fertilized with 56 μg P/g soil, total dry weights of mycorrhizal plants infected by *P. parasitica* and of nonmycorrhizal plants infected by *P. parasitica* were not significantly different. In soil fertilized with 600 μg P/g soil, total dry weights of plants infected by *P. parasitica* alone were significantly greater than those of plants infected by both *P. parasitica* and *G. fasciculatus.*

Root dry weights of nonmycorrhizal plants increased as phophorus fertilization increased, but root dry weights of plants infected by *G. fasciculatus* alone in soil fertilized with 56 μg P/g soil were significantly greater ($P = 0.05$) than were those of plants infected by *G. fasciculatus* alone in soil fertilized with either 6 or 600 μg P/g soil (Table 4). In soil fertilized with 6 or 56 μg P/g soil, root dry weights of plants infected with *G. fasciculatus* alone were significantly greater than were those of noninfected plants. In soil fertilized with 6 μg P/g soil, root dry weights of mycorrhizal plants infected by *P. parasitica* were significantly greater than were those of nonmycorrhizal plants infected by *P. parasitica.* In soil fertilized with 56 or 600 μg P/g soil, however, differences between root dry weights of mycorrhizal plants infected by *P. parasitica* and those of nonmycorrhizal plants infected by *P. parasitica* were not significant.

Percentages of healthy roots of plants fertilized with any concentration of phosphorus were consistently decreased by *P. parasitica* (Table 5). However, percentages of healthy roots of plants infected by *P. parasitica* increased as the concentrations of phosphorus in the soil increased. Percentages of healthy roots of plants fertilized with 6 μg P/g soil and infected by *G. fasciculatus* and *P. parasitica* together were significantly greater ($P = 0.05$) than were percentages of healthy roots of plants infected by *P. parasitica* alone. Differences between the percentages of healthy roots of nonmycorrhizal and mycorrhizal plants infected by *P. parasitica* in soil fertilized with either 56 or 600 μg P/g soil were not significant.

Root infection by *G. fasciculatus* was significantly reduced as concentrations of phosphorus in the soil increased (Table 6). Furthermore, the percentage of root tissue infected by *G. fasiculatus* was reduced by *P. parasitica* in soil fertilized with 6 μg P/g soil. Numbers of spores produced by *G. fasciculatus* (calculated either as numbers of spores per gram of soil or root tissue) significantly decreased as concentrations of phosphorus in the soil increased. Sporulation by *G. fasciculatus* also was adversely affected by *P. parasitica* in plants fertilized with 6 or 56 μg P/g soil.

Concentrations of phosphorus in leaves of mycorrhizal plants were significantly greater ($P = 0.05$) than concentrations of phosphorus in leaves of nonmycorrhizal plants fertilized with 6 μg P/g soil (Table 2). In plants fertilized with 56 or 600 μg P/g soil, however, differences between the concentrations of phosphorus in mycorrhizal plants and those in nonmycorrhizal plants were not significant. In soil fertilized with 6 μg P/g soil, concentrations of phosphorus in leaves of mycorrhizal plants infected by *P. parasitica* were twice those of nonmycorrhizal plants infected by *P. parasitica.* In soil fertilized with 56 μg P/g soil, concentrations of phosphorus in leaves of mycorrhizal plants infected by *P. parasitica* were identical to the concentrations of phosphorus in nonmycorrhizal

TABLE 3. Total dry weight of sweet orange seedlings treated with various combinations of *Glomus fasciculatus, Phytophthora parasitica,* and three levels of soil phosphorus[a]

	Total dry wt (g)		
	Soil phosphorus (μg P/g soil)		
Treatment	6	56	600
Noninfected	0.74 yz	1.99 x	5.06 u
Phytophthora parasitica	0.36 z	1.14 y	4.27 uv
Glomus fasciculatus	3.50 vw	4.40 uv	4.33 uv
P. parasitica + *G. fasciculatus*	0.84 y	1.17 y	3.27 w

[a] Values represent mean of 10 seedlings. Means with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Dry root weight of sweet orange seedlings treated with various combinations of *Glomus fasciculatus, Phytophthora parasitica,* and three levels of soil phosphorus[a]

	Root dry wt (g)		
	Soil phosphorus (μg P/g soil)		
Treatment	6	56	600
Noninfected	0.37 yz	0.61 y	1.21 vw
Phytophthora parasitica	0.14 z	0.47 y	1.03 wx
Glomus fasciculatus	1.04 wx	1.37 v	1.03 wx
P. parasitica + *G. fasciculatus*	0.39 y	0.49 y	0.87 x

[a] Values represent mean of 10 seedlings. Means with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Percentage of healthy roots of sweet orange seedlings treated with various combinations of *Glomus fasciculatus, Phytophthora parasitica,* and three levels of soil phosphorus[a]

	Healthy roots (%)		
	Soil phosphorus (μg P/g soil)		
Treatment	6	56	600
Noninfected	100 z	100 z	100 z
Phytophthora parasitica	33 y	74 vw	86 v
Glomus fasciculatus	100 z	100 z	100 z
P. parasitica + *G. fasciculatus*	50 x	72 w	79 vw

[a] Values represent mean of 10 seedlings. Means with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 6. Influence of *Phytophthora parasitica* and soil phosphorus on root infection and sporulation of *Glomus fasciculatus*[a]

	Root tissue infected with *G. fasciculatus* (%)			Spores per gram of soil			Spores per gram of dry root tissue		
	Soil P (μg P/g soil)			Soil P (μg P/g soil)			Soil P (μg P/g soil)		
Treatment	6	56	600	6	56	600	6	56	600
Glomus fasciculatus	54 z	38 y	12 x	8 y	5 y	1 z	6,304 w	3,138 x	575 yz
G. fasciculatus + *P. parasitica*	27 y	30 y	8 x	1 z	1 z	0 z	2,068 xy	2,166 yz	22 z

[a] Values represent mean of 10 seedlings. Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

plants infected by *P. parasitica*. In soil fertilized with 6 μg P/g soil, concentrations of phosphorus in leaves of plants infected by *P. parasitica* and *G. fasciculatus* were significantly less than were concentrations of phosphorus in plants infected by *G. fasciculatus* alone.

Root infection by *G. fasciculatus* also influenced uptake of other minerals. Although concentrations of potassium, sodium, iron, zinc, and copper in leaves of nonmycorrhizal plants decreased as concentrations of phosphorus in the soil increased, concentrations of these minerals in leaves of mycorrhizal plants were not influenced by phosphorus in the soil. Concentrations of sodium, iron, and zinc generally were greater in nonmycorrhizal and mycorrhizal plants infected by *P. parasitica* than in those infected with *G. fasciculatus* alone.

Effect of phosphorus on *P. parasitica*. Growth of colonies of *P. parasitica* was not affected by phosphorus in solution. Germination of sporangia, however, was more sensitive and was inhibited by phosphorus. A significant reduction in the release of zoospores was detected in solutions with 50 mg P/L water, and at 600 mg P/L water, no sporangia released zoospores (Table 7).

TABLE 7. Effect of phophorus on release of zoospores by sporangia of *Phytophthora parasitica*[a]

Phosphorus[b] (mg/L)	Equivalent conc. H_3PO_4 (mM)	Empty sporangia[c] (% of control)
0	0	100 w
10	0.3	89 w
50	1.6	43 x
100	3.3	13 y
300	9.9	3 yz
600	19.8	0 yz

[a] Values represent the mean of three trials. Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[b] Each solution was adjusted to pH 7.2 and buffered with MOPS buffer (10%, v/v).

[c] Colonies of *P. parasitica* incubated 90 min in test solutions at 18 C to induce zoospore release.

DISCUSSION

Results from both experiments indicate that roots of sweet orange seedlings infected by *G. fasciculatus* were susceptible to root rot by *P. parasitica*. Mycorrhizal sweet orange seedlings appear, however, to exhibit greater tolerance to *P. parasitica* since size, root health, and phosphorus uptake were greater in mycorrhizal plants than in nonmycorrhizal plants when soil phosphorus was limiting. Tolerance in citrus to Phytophthora root rot caused by *G. fasciculatus* appears to be due to the ability of mycorrhizal roots to absorb more phosphorus and possibly other minerals than do nonmycorrhizal roots. Results of these experiments do not indicate that mycorrhizae formed by *G. fasciculatus* possess any resistance to *P. parasitica* due to a direct effect by the mycorrhizal fungus. In soil fertilized with 6 μg P/g soil, where phosphorus was limiting and uptake was greater in mycorrhizal plants, weights of mycorrhizal plants infected with *P. parasitica* were greater than those of nonmycorrhizal plants infected with *P. parasitica*. However, where fertilization with 56 μg P/g soil resulted in identical concentrations of phosphorus in nonmycorrhizal and mycorrhizal plants, there was no difference between the weights of nonmycorrhizal and mycorrhizal plants infected by *P. parasitica*. In fact, in soil fertilized with 56 μg P/g soil, mycorrhizal plants were relatively more severely affected by *P. parasitica* than were nonmycorrhizal plants since the relative reduction of growth (based on total dry weights) by *P. parasitica* was 57% in mycorrhizal plants and only 27% in nonmycorrhizal plants. In plants fertilized with 600 μg P/g soil, dry weights of mycorrhizal plants infected by *P. parasitica* were 23% less than dry weights of plants infected by *P. parasitica* alone. Therefore, *G. fasciculatus* provided tolerance to Phytophthora root rot only in soil with a total phosphorus content of less than 15 μg P/g soil. *G. fasciculatus* also offset effects by *P. parasitica* under conditions of low soil phosphorus in the split root experiment; mycorrhizal roots were less severely damaged by *P. parasitica* than were nonmycorrhizal roots and nonmycorrhizal roots infected by *P. parasitica* were healthier when split from mycorrhizal roots. Thus, tolerance to *P. parasitica* manifested by mycorrhizal roots may be at least partially transferred to nonmycorrhizal roots on the same plant. It is suggested again that this tolerance is simply a manifestation of improved nutrition.

Although nutrient concentrations in roots were not measured, it is postulated that mycorrhizal roots are nutritionally healthier than are nonmycorrhizal roots since root systems inoculated with *G. fasciculatus* were larger than nonmycorrhizal root systems on the same plant. This improved nutrition in mycorrhizal roots could explain the apparent resistance to *P. parasitica* exhibited by mycorrhizal roots when compared to nonmycorrhizal roots on the same plant. The mechanism that caused the increased growth of roots infected by *G. fasciculatus* is not known, but it has been reported that feeder roots proliferate in response to increases of phosphorus in the soil (5), and the better uptake of phosphorus due to *G. fasciculatus* might have caused the greater growth of mycorrhizal roots when mycorrhizal and nonmycorrhizal roots occurred on the same plant. Regardless of the mechanism involved, this phenomenon must be considered when using split root experiments in mycorrhizal studies.

In citrus with split root systems, growth of citrus was reduced when only one side of the split root system was infected by *P. parasitica*. This apparent change in the physiology of the plant caused by *P. parasitica*, however, did not affect the amount of root tissue infected by *G. fasciculatus*, which was only reduced when *G. fasciculatus* was in direct association with *P. parasitica*. This was not unexpected since *G. fasciculatus* and *P. parasitica* probably compete for the same food and space.

Growth of citrus seedlings without mycorrhizae on either side of split root systems was small because the soil contained less-than-optimum amounts of phosphorus for nonmycorrhizal plants. The population of *P. parasitica* recovered from the rhizosphere soil of the nonmycorrhizal plants was less than the population of *P. parasitica* recovered from soil of mycorrhizal plants. Apparently, the faster growing, more vigorous plants were able to support a larger population of *P. parasitica* than the plants stressed for phosphorus. Nonmycorrhizal roots of plants that also had mycorrhizal roots could support a population of *P. parasitica* as large as that found in the rhizosphere soil of mycorrhizal roots. It is therefore highly unlikely that *G. fasciculatus* produces antibiotics active against *P. parasitica* or induces phytoalexins in plant roots against *P. parasitica*. These data further support the contention that any difference in the responses of nonmycorrhizal citrus and citrus preinfected with *G. fasciculatus* to *P. parasitica* is due to nutritional superiority associated with infection by the mycorrhizal fungus.

Phytophthora root rot was not as severe in citrus fertilized with 600 μg P/g soil as it was in citrus fertilized with lesser amounts of phosphorus, which led to the investigation of the effects of phosphorus on release of zoospores by sporangia of *P. parasitica*. The reduction of disease severity might have been due to the levels of phosphorus in the soil which were toxic to *P. parasitica*. Although growth of *P. parasitica* was apparently not inhibited by phosphorus in solution, release of zoospores was reduced as the concentrations of phosphorus in solution were increased. Since zoospores are infectious units of *Phytophthora* spp., the reduction of severity of disease in soil fertilized with 600 μg P/g soil might have been due to the lower inoculum potential of *P. parasitica* as a result of the inability of the fungus to form viable zoospores in water with a high concentration of phosphorus.

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