Influence of Vesicular-Arbuscular Mycorrhizae on Phytophthora Root Rot of Three Crop Plants

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ABSTRACT

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Citrus, avocado, and alfalfa seedlings, with or without endomycorrhizae formed by *Glomus fasciculatus,* were inoculated with *Phytophthora parasitica, P. cinnamomi,* and *P. megasperma,* respectively. Little or no differences in response to the *Phytophthora* species were observed between mycorrhizal and nonmycorrhizal citrus or alfalfa seedlings, but mycorrhizal avocado seedlings were affected more severely by *P. cinnamomi* than were nonmycorrhizal avocado seedlings. In the citrus and avocado experiments, the growth stimulus caused by the mycorrhizal fungus was eliminated when *Phytophthora* was present. Sporulation by *G. fasciculatus* on citrus was affected adversely by *P. parasitica.* Phosphorus concentrations in leaves of citrus and avocado seedlings infected with *G. fasciculatus* alone were significantly greater than concentrations in leaves of nonmycorrhizal seedlings. Phosphorus concentrations in seedlings inoculated with both *G. fasciculatus* and *Phytophthora,* however, were similar to concentrations in nonmycorrhizal seedlings.

Additional key words: soilborne fungi.

The anatomy of mycorrhizae, the distribution of mycorrhizal fungi, and the beneficial influence of vesicular-arbuscular (VA) mycorrhizae on plant growth are well known (5, 9). Recently, several studies indicated that VA mycorrhizae may influence development of root diseases caused by soilborne fungi. Mycorrhizal soybean roots were damaged more severely by *Phytophthora megasperma* than were nonmycorrhizal roots (13). VA mycorrhizal fungi, however, caused a decrease in production and germination of chlamydospores of *Thielaviopsis basicola* (1), and increased resistance of tobacco to infection by *T. basicola* (2). Similarly, nonmycorrhizal roots of cotton were damaged more severely by *T. basicola* than were mycorrhizal roots (14). In contrast, the mycorrhizal fungus *Glomus mosseae* did not reduce the severity of disease caused by *Pythium ultimum* or *Phytophthora megasperma* on soybeans (3), no relationship was found between *P. palmivora* and VA mycorrhizal infection of papaya roots (12).

This study was initiated to (i) determine whether mycorrhizal plants differed from nonmycorrhizal plants in response to inoculation with *Phytophthora* spp. and (ii) study the influence of *Phytophthora* on the development of a mycorrhizal fungus.

MATERIALS AND METHODS

Citrus sweet orange (*Citrus sinensis* [L.] Osbeck cultivar 'Pineapple'), avocado (*Persea americana* Mill. 'Topa Topa'), and alfalfa (*Medicago sativa* L. 'Moapa 69') seedlings with or without mycorrhizae were inoculated with *Phytophthora parasitica* Dast., *P. cinnamomi* Rands, and *P. megasperma* Drechs., respectively. In all tests, the cycorrhizal fungus was *Glomus fasciculatus* (Thaxter) Gerd. and Trappe.

Citrus seeds were sown in two flats of sand that were autoclaved for two 1-hr periods with a 24-hr interval. One flat received a layer of inoculum of *G. fasciculatus* placed 6 cm beneath the seeds. Inoculum consisted of soil, roots, and spores from a pot containing sudan grass (*Sorghum vulgare* Pers.) infected with *G. fasciculatus.* The other flat received roots of sudan grass that had been grown free of mycorrhizal fungi. Eight weeks after planting, 47% of the citrus roots were infected with *G. fasciculatus.* Infection was determined by the presence of arbuscles, vesicles, spores, or hyphae of *G. fasciculatus,* or a combination of these, in 100 or more 1-mm^2 sections of root tissue. Roots were stained with 0.05% trypan blue in lactophenol by the method of Phillips and Hayman (11) prior to examination.

Roots of citrus seedlings with or without mycorrhizae were submerged for 1 hr in 600 ml of water containing 1,000 zoospores of *P. parasitica* (M114) per milliliter. Zoospores were obtained and suspended in water by the method of Menyonga and Tsao (8). Roots of seedlings with or without mycorrhizae that were not inoculated with *P. parasitica* were submerged in sterile distilled water. All seedlings were transplanted into 10-cm-diameter clay pots (one seedling per pot) of autoclaved sand. Treatments consisted of 10 replicate seedlings. Plants were grown in a glasshouse at temperatures ranging from 22 to 36 C and watered when necessary with a 14% Hoagland's solution lacking phosphorus (6). After 11 wk

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of growth, seedlings were lifted and plant weights and percentages of healthy roots were recorded. Roots that were discolored or necrotic were considered to be infected with *P. parasitica*. Heights were measured at the time of *Phytophthora* inoculation and at harvest.

Mycorrhizal and nonmycorrhizal avocado seedlings were obtained by the same procedure used in the citrus experiment. Seedlings 8 wk old, with or without established *G. fasciculatus*, were transplanted into 20-cm-diameter metal pots containing soil naturally infested with *P. cinnamomi*. The soil was obtained from an avocado orchard and contained approximately two propagules of *P. cinnamomi* per gram of soil. Numbers of propagules of *P. cinnamomi* were measured by a dilution-plate assay using a selective medium (10). Mycorrhizal and nonmycorrhizal seedlings not transplanted into infested soil were transplanted into autoclaved soil. Field soil was used in this experiment because *Phytophthora* infection was more consistent than with other methods of inoculation, and it did not cause an immediate decline of the avocados. Seedlings were grown in a glasshouse at temperatures ranging from 22 to 35 C and were harvested after 14 wk of growth. Avocados received the same nutrient solution as citrus but at weekly intervals. Treatments consisted of 10 replicate seedlings.

Alfalfa seeds were sown in autoclaved sand in 8-cm-diameter clay pots, half of which received a layer of inoculum of *G. fasciculatus* placed 5 cm beneath the seeds. The source of the inoculum of *G. fasciculatus* was identical to that used in the citrus and avocado experiments. Seedlings were later thinned to one plant per pot. When seedlings were 7 wk old, half of the pots containing mycorrhizal or nonmycorrhizal seedlings received 75 ml of a mycelial suspension of *P. megasperma* poured onto the soil of each pot. The mycelial suspension was prepared by grinding agar and mycelium in 750 ml of sterile water. The agar and mycelium were obtained from two 5-day-old cultures of *P. megasperma* growing in 90-mm petri dishes of cleared V-8 agar. Mycorrhizal and nonmycorrhizal seedlings not inoculated with *P. megasperma* received only ground, cleared V-8 agar. Treatments consisted of 10 replicate seedlings. Glasshouse temperatures and fertilization were identical to those of the citrus experiment. Seedlings were harvested after 13 wk of growth.

Chlamydospores of *G. fasciculatus* were collected from citrus soil by a procedure of decanting and wet sieving through a series of 350-, 250-, and 106-μm mesh screens (4). Citrus and avocado leaves were analyzed for phosphorus content by the method of Kitson and Mellon (7).

RESULTS AND DISCUSSION

Plant heights, top weights, and root weights of citrus seedlings inoculated with *G. fasciculatus* alone were significantly greater than those of noninfected seedlings, seedlings inoculated with *P. parasitica* alone, or seedlings inoculated with both *G. fasciculatus* and *P. parasitica* (Table 1, Fig. 1-A). In the presence of *P. parasitica*, growth response due to *G. fasciculatus* was negated. The percentage of healthy roots in mycorrhizal seedlings inoculated with *P. parasitica*, however, was significantly ($P = 0.05$) greater than in nonmycorrhizal seedlings inoculated with *P. parasitica* (Table 1). Sporulation by *G. fasciculatus* was reduced ($P = 0.05$) in the presence of *P. parasitica* (Table 2). Since *P. parasitica* severely reduced the amount of citrus root tissue, however, the numbers of chlamydospores of *G. fasciculatus* were not significantly different between treatments with or without *P. parasitica* when based on the number of chlamydospores per gram of root tissue (Table 2).

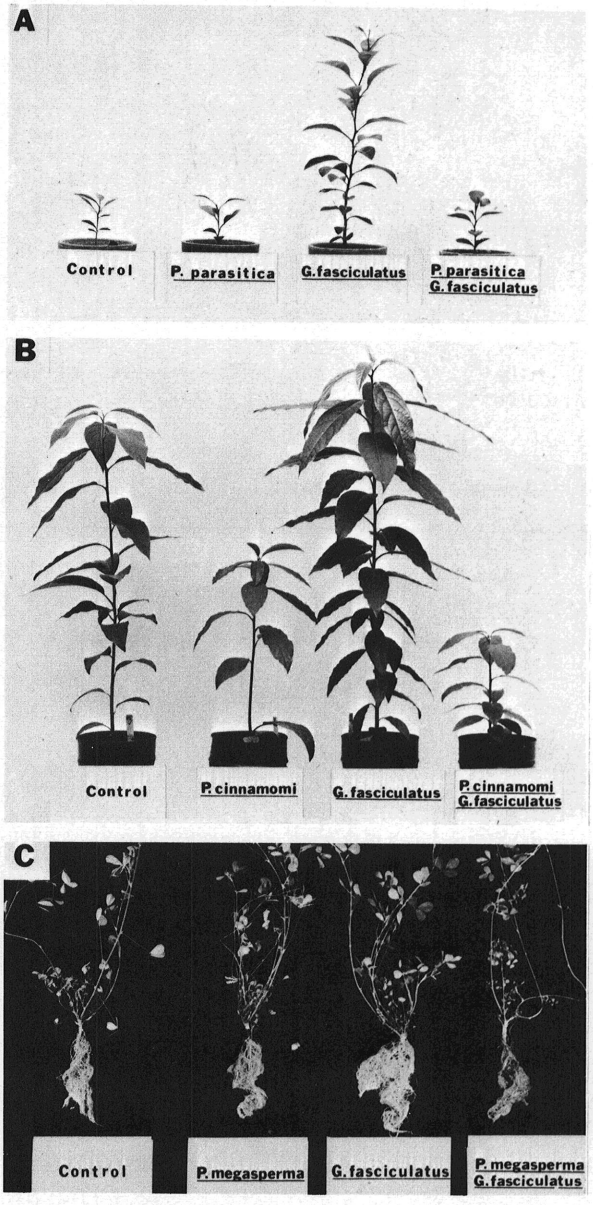

Fig. 1-(A to C). Interactions between three *Phytophthora* species and *Glomus fasciculatus* as indicated by growth responses of three crop plants. Mycorrhizal and nonmycorrhizal **A)** citrus, **B)** avocado, and **C)** alfalfa plants inoculated with or without *Phytophthora parasitica, P. cinnamomi,* or *P. megasperma,* respectively. Growth stimulus caused by *G. fasciculatus* is eliminated by *Phytophthora.*

TABLE 1. Effect of *Glomus fasciculatus* on growth responses and *Phytophthora parasitica* root rot of citrus[a]

Treatment	Height increase[b] (cm)	Top weight (g)	Root weight (g)	Healthy roots (%)
Noninfected	1.3 z	0.31 z	0.42 z	100 x
Phytophthora parasitica	0.6 z	0.29 z	0.10 z	9.2 y
Glomus fasciculatus	23.6 y	3.71 y	3.84 y	100 x
P. parasitica + *G. fasciculatus*	0.8 z	0.59 z	0.40 z	18.2 z

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

[b]Values represent increase in height between time seedlings were inoculated with *P. parasitica* to harvest—11 wk.

TABLE 2. Effect of *Phytophthora parasitica* on spore production by *Glomus fasciculatus* on citrus[a]

Treatment	Spores/g of soil (no.)	Spores/g of root tissue (no.)
G. fasciculatus	5.6 y	1,057.7 z
G. fasciculatus + *P. parasitica*	0.6 z	1,169.3 z

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

TABLE 3. Effect of *Glomus fasciculatus* on growth responses and *Phytophthora cinnamomi* root rot of avocado[a]

Treatment	Height increase[b] (cm)	Top weight (g)	Root weight (g)	Healthy roots (%)
Noninfected	23.3 x	19.5 x	17.2 y	80.1 x
Phytophthora cinnamomi	4.5 y	9.6 y	6.7 z	13.9 y
Glomus fasciculatus	37.4 w	28.8 w	30.2 x	89.0 x
P. cinnamomi + *G. fasciculatus*	1.7 z	6.3 z	4.5 z	3.6 z

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

[b]Values represent increase in height between time seedlings were inoculated with *P. cinnamomi* to harvest—14 wk.

Plant heights, top weights, and percentages of healthy roots of avocado seedlings inoculated with both *P. cinnamomi* and *G. fasciculatus* were significantly less than those of avocado seedlings inoculated with *P. cinnamomi* alone (Table 3, Fig. 1-B). Plant heights, top weights, and root weights of mycorrhizal avocado seedlings were significantly ($P = 0.05$) greater than those of noninfected seedlings, seedlings inoculated with *P. cinnamomi,* or seedlings inoculated with both *P. cinnamomi* and *G. fasciculatus.*

In the alfalfa experiment, mycorrhizal seedlings were not significantly larger than nonmycorrhizal seedlings, and there was no significant difference between the mycorrhizal and nonmycorrhizal seedlings inoculated with *P. megasperma* (Table 4, Fig. 1-C). *Phytophthora megasperma,* however, caused a significant decrease in growth of both nonmycorrhizal and mycorrhizal seedlings.

Phosphorus levels were markedly higher in avocado and citrus mycorrhizal seedlings than in nonmycorrhizal seedlings, seedlings inoculated with *Phytophthora* alone, or seedlings inoculated with both *Phytophthora* and *G.*

TABLE 4. Effect of *Glomus fasciculatus* on growth responses and *Phytophthora megasperma* root rot of alfalfa[a]

Treatment	Mean total plant weight (g)
Noninfected	7.94 y
P. megasperma	2.79 z
G. fasciculatus	10.36 y
P. megasperma + *G. fasciculatus*	3.00 z

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

TABLE 5. Percent phosphorus in leaf tissue of citrus and avocado seedlings[a]

Treatment	Percent phosphorus[b]	
	Citrus	Avocado
Noninfected	0.067 z	0.062 z
Phytophthora	0.047 z	0.055 z
Glomus fasciculatus	0.102 y	0.095 y
Phytophthora + *G. fasciculatus*	0.055 z	0.059 z

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

[b]Values based on dry weight of leaf tissue.

fasciculatus (Table 5). The concentration of phosphorus in mycorrhizal seedlings inoculated with *Phytophthora* was not significantly different from the phosphorus concentration in nonmycorrhizal seedlings with or without *Phytophthora.*

In these experiments, little or no resistance to *Phytophthora* was conferred on plants previously infected with *G. fasciculatus.* Mycorrhizal avocado seedlings were damaged even more severely by *P. cinnamomi* than were nonmycorrhizal seedlings. These results were not unexpected, since the majority of all cultivated plants in the field are mycorrhizal and root rots commonly occur.

The low phosphorus levels measured in mycorrhizal citrus and avocado plants infected with *Phytophthora* indicate that mycorrhizal symbiosis may be damaged severely if not eliminated by heavy *Phytophthora* infections. Because *Phytophthora* may reduce the inoculum potential of *G. fasciculatus* in the soil, subsequent infection by these beneficial fungi also may be reduced.

Poor growth of nonmycorrhizal seedlings can be attributed to the inability of nonmycorrhizal roots to absorb as much phosphorus as mycorrhizal roots. Adequate phosphorus levels and lush growth coincided in the mycorrhizal seedlings, but both the phosphorus level and growth of mycorrhizal seedlings were reduced to that of the nonmycorrhizal seedlings where *Phytophthora* was present. Since phosphorus status in the plants was indicative of functioning mycorrhizae, phosphorus may be an important aspect of plant response in mycorrhiza-soilborne plant pathogen interactions. From these results and those of others (2, 3, 12–14), the influence of VA mycorrhiza on fungal root pathogens appears to vary with the disease complex.

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