# Phytophthora Root Rot Development on Mycorrhizal and Phosphorus-Fertilized Nonmycorrhizal Sweet Orange Seedlings

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**ABSTRACT**

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Comparable sweet orange seedlings were grown in soilless medium and either were inoculated with the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* (VAM) or were nonmycorrhizal and fertilized with soluble P (NM). Seedlings, 8 or 10 mo old, were transplanted into a low P (3.5 μg/g) citrus soil that was noninfested or infested with 1 or 10 chlamydospores of *Phytophthora parasitica* per cubic centimeter of soil. In one experiment, root dry weight and leaf P content of noninfested VAM seedlings were greater than those of NM plants, which were nearly deficient in P. *P. parasitica* reduced leaf P status of VAM and NM seedlings alike but reduced dry weight of only VAM plants. There were significantly fewer rotted root tips on VAM seedlings. *P. parasitica* reduced VAM colonization as a result of the loss of root tips. In a second experiment, at the higher inoculum density of *P. parasitica*, NM and VAM seedlings were similar in size and had sufficient levels of leaf P. *P. parasitica* infestation reduced leaf P content and root dry weight irrespective of mycorrhizal treatment. Root rot development occurred to about the same extent on NM and VAM root systems. *G. intraradices* did not increase the resistance or tolerance of sweet orange to Phytophthora root rot unless mycorrhizae conferred a P nutritional advantage over the NM plant.

Additional keywords: phosphorus nutrition

In Florida, greenhouse propagation of citrus in soilless media results in plants that lack vesicular-arbuscular mycorrhizal (VAM) fungi when transplanted into the orchard (6). Although additional P fertilization of containerized citrus may be substituted for mycorrhizal-mediated P uptake, other benefits of VAM colonization may be lost (7). If mycorrhizae reduce damage caused by root pathogens after outplanting into infested soils, the additional costs of inoculating containerized citrus with VAM fungi may be justified.

Attempts have been made to distinguish the effect of improved P nutrition by VAM fungi from other possible resistance or tolerance interactions with fungal root pathogens (1,3,8,11). In the earliest study of this kind, Davis and Menge (3) found that increased tolerance of mycorrhizal roots of sweet orange (*Citrus sinensis* (L.) Osb.) to root rot caused by *Phytophthora parasitica* Dastur (syn. *P. nicotianae* B. de Haan var. *parasitica* (Dastur) Waterh.) was associated with VAM improvement of host P nutrition. In a later study (4) of seven different mycorrhizal species, however, they concluded that sweet orange and Troyer citrange seedlings colonized by *Glomus mosseae* and *Gigaspora margarita* had some tolerance to *P. parasitica* when compared with nonmycorrhizal controls. In these studies (3,4), citrus roots were inoculated by submergence in zoospores, which resulted in severe root rot. This method of inoculation may have overcome or confounded observation of resistance or tolerance of mycorrhizal roots. Davis and Menge (4) did challenge sweet orange colonized by *Glomus fasciculatum* with chlamydospores of *P. parasitica* in soil and obtained moderate levels of root rot. In that experiment, mycorrhizae reduced the development of root rot, but this was in comparison to severely P-deficient nonmycorrhizal controls.

This paper describes two experiments designed to evaluate further the potential of VAM fungi to increase the resistance or tolerance of sweet orange to root rot caused by *P. parasitica*. Comparisons were made between seedlings colonized by *Glomus intraradices* Schenck & Smith and P-fertilized nonmycorrhizal seedlings of similar size and P sufficiency to evaluate the influence of VAM on root rot exclusive of P nutritional effects. We chose *G. intraradices* because it is an intraradical sporulator that has been used successfully as a root fragment inoculant for containerized citrus (7). Densities of *Phytophthora* chlamydospores, which approximate those encountered in Florida citrus soils, were utilized to obtain low to moderate levels of root rot.

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## MATERIALS AND METHODS

**VAM inoculation.** Sudangrass (*Sorghum bicolor* (L.) Moench var. *sudanense*) roots containing chlamydospores of *G. intraradices* were harvested from pot cultures. Roots were air-dried and cut into fragments 1 to 2 mm long, and VAM colonization potential was determined by the most-probable-number analysis (94 propagules/mg of root) as previously described (7). One milligram of root fragments was mixed per cubic centimeter of a medium consisting of peat and perlite amended with 7.5 mg P/cm$^3$ as rock phosphate (200 mesh, 15% P) and 5 mg/cm$^3$ dolomite dust contained in 150-cm$^3$ Leach tubes (Ray Leach Container Nursery, Canby, OR). Noninoculated medium consisted of the same ingredients without root fragments that received an extract of the inoculum passed through a 38-μm sieve to establish the same microflora associated with the inoculum. Two seeds of sweet orange were sown into each tube and thinned to one seedling after emergence. Inoculated seedlings were fertilized weekly with liquid 8-0-8 (N-P-K) plus micronutrients (VAM treatment) and noninoculated seedlings received 9-3-6 (NM treatment) to establish VAM and NM plants of comparable size and N, P, K sufficiency (7; J. H. Graham, *unpublished*).

***Phytophthora* inoculation.** After 8–10 mo, when *G. intraradices* was well established on the inoculated seedlings (approximately 50% root colonization), 10 VAM and 10 NM plants of comparable shoot size were selected for transplant into noninfested citrus soil or into soil infested with chlamydospores of *P. parasitica*. Chlamydospores were produced by the method of Tsao (13) and mixed with a small aliquot of moistened (5% w/w), unfertilized Candler fine sand soil (Typic quartzipsamments, 96.5% sand, 2% silt, 1.5% clay) that had a pH of 6.8, organic matter content of 1%, and extractable P of 3.5 μg/g of soil. Previously, the soil had been autoclaved for 6 hr to eliminate indigenous VAM fungi. The infested soil was incubated in moist condition for 7 days, then inoculum potential was evaluated by plating on pimaricin-ampicillin-rifampicin-pentachloronitrobenzene selective medium, using 125 mg/L instead of 250 mg/L of ampicillin and adding 25 mg/L of hymexazol (10). Based on this assessment, the soil inoculum was thoroughly mixed

with additional Candler fine sand soil to give an inoculum density of 1 or 10 chlamydospores/cm$^3$ of soil depending on the experiment. This range of inoculum density is representative of that encountered in citrus orchard soils in Florida (J. H. Graham and L. W. Timmer, *unpublished*). Seedlings with cylindrical root systems were transplanted into the center of 15-cm-diameter clay pots containing *Phytophthora*-infested soil or noninfested soil. Soil was flooded for the first 2 days after transplanting, then kept moist but drained for 5 days. This watering cycle was maintained for the remainder of the experiment. All treatments were fertilized weekly after the flooding period with Hoagland's solution (9) plus 5X phosphorus. Phosphorus fertilization and periodic flooding did not adversely affect colonization by *G. intraradices*, which increased from 50 to 83% during the subsequent 6-wk period. Two experiments were performed at 1 and 10 chlamydospores/cm$^3$ of soil inoculum density in a greenhouse where temperatures ranged from 25 to 35 C.

**Disease and VAM assessment.** After 6 wk, root systems were carefully washed free of soil to minimize loss of roots from treatments with root rot. The total number of root tips and the percentage of root tips with rot were assessed by pinching the root tips between the thumb and index finger to determine whether the cortex sloughed off the stele. Root tips that easily sloughed were scored positive for root rot, and those that did not slough were considered healthy. Randomly selected root tips were surface-sterilized and plated on pimaricin-vancomycin-pentachloronitrobenzene selective medium (14) containing 25 g/L of hymexazol to confirm the presence of *P. parasitica* in sloughing roots and the absence of *P. parasitica* in intact roots.

Roots and shoots were oven-dried for dry weight determinations. Roots were cleared and stained to assess colonization by *G. intraradices* as previously described (7). Leaf tissue was digested in perchloric-nitric acid and P content determined by the vanadate-molybdate method (15).

All data were tested for significance using analysis of variance and the *F* test to evaluate main treatments (*P. parasitica* and VAM) and their interaction. In some cases, individual Student's *t* tests were conducted to compare main treatments. Percentage root rot and VAM colonization were arcsin transformed to stabilize variance.

## RESULTS

In the first experiment, noninfested, NM sweet orange seedlings were similar to VAM seedlings in shoot dry weight ($P \leq 0.01$), but the total root and feeder root dry weights of NM plants were significantly ($P \leq 0.05$) less than those of VAM plants (Table 1). Leaf P content of NM seedlings was at the threshold of deficiency (0.1%), whereas mycorrhizal seedlings were well above this level.

*Phytophthora* inoculation significantly reduced feeder root, total root, and total shoot weight compared with noninfested plants (Table 1). There was an interaction between *P. parasitica* and VAM in that *Phytophthora* only reduced dry weight of VAM seedlings. Leaf P content of seedlings decreased as a result of infestation with *P. parasitica* but the effect was not significant. There was not a significant interaction between *Phytophthora* and mycorrhizae, indicating that the reduction in leaf P content was similar for VAM and NM treatments.

Total number of root tips on healthy VAM and NM plants did not differ significantly (Table 2). *P. parasitica* decreased the total number of root tips and healthy root tips. Although the reductions were greater for VAM plants than for NM plants, the interaction was only significant for total root tips. There were fewer rotted root tips on VAM root systems inoculated with *P. parasitica*, resulting in a significant decrease in the percentage of total tips with root rot compared with NM root systems ($P \leq 0.05$). *P. parasitica* reduced VAM colonization by over 50% as a result of the loss of root tips.

In the second experiment, NM plants were supplemented with additional P fertilization for 2 mo to increase leaf P content before *Phytophthora* inoculation. An inoculum density of 10 chlamydospores/cm$^3$ of soil was used to increase root rot development compared with the first experiment. In this case, noninfested NM and VAM seedlings had sufficient levels of leaf P, but P content of mycorrhizal plants was less than in the first experiment (Tables 1 and 3). Dry weights of feeder roots, total roots, and total shoots were not statistically different ($P \leq 0.05$) even though VAM plants were slightly smaller.

*Phytophthora* infestation significantly reduced feeder root weight by the same extent irrespective of mycorrhizae, as indicated by the lack of interaction between *P. parasitica* and VAM (Table 3). Reductions in total root and total shoot dry weights were insignificant and comparable between NM and VAM treatments. *P. parasitica* significantly reduced P content of leaves of NM and VAM seedlings alike. Although the interaction between VAM and *Phytophthora* was not significant, leaf P content of NM plants remained in the sufficiency range. In VAM plants, leaf P

**Table 1.** Dry weight and leaf phosphorus content of noninfested and *Phytophthora parasitica*-infested sweet orange seedlings either fertilized with phosphorus (NM) or inoculated with *Glomus intraradices* (VAM)

| Treatment | Dry weight (g) | | | Leaf P (%) |
|---|---|---|---|---|
| | Feeder roots | Total roots | Total shoots | |
| Noninfested | | | | |
| NM | 0.89 | 2.30 | 2.96 | 0.10 |
| VAM | 1.21 | 2.96 | 3.23 | 0.19 |
| *P. parasitica*[a] | | | | |
| NM | 0.75 | 2.18 | 2.95 | 0.07 |
| VAM | 0.64 | 1.82 | 2.09 | 0.13 |
| *P. parasitica* | **[b] | ** | * | NS |
| VAM | NS | NS | * | ** |
| *P. parasitica* × VAM | ** | ** | * | NS |

[a] Inoculum density of 1 propagule/cm$^3$ of soil.
[b] Main effect of interaction significant at $P \leq 0.05$ (*) or 0.01 (**) or nonsignificant (NS).

**Table 2.** Phytophthora root rot and mycorrhizal development on sweet orange seedlings either fertilized with phosphorus (NM) or inoculated with *Glomus intraradices* (VAM)

| Treatment | Root tips | | | Rotted (%) | VAM (%) |
|---|---|---|---|---|---|
| | Total | Healthy | Rotted | | |
| Noninfested | | | | | |
| NM | 646 | 639 | 7 | 1 | 0 |
| VAM | 767 | 748 | 19 | 2 | 83 |
| *P. parasitica*[a] | | | | | |
| NM | 374 | 305 | 69 | 19 | 0 |
| VAM | 302 | 265 | 37 | 13 | 40 |
| *P. parasitica* | **[b] | ** | ** | ** | ** |
| VAM | NS | NS | * | NS | ** |
| *P. parasitica* × VAM | * | NS | ** | * | ** |

[a] Inoculum density of 1 propagule/cm$^3$ of soil.
[b] Main effect or interaction significant at $P \leq 0.05$ (*) or 0.01 (**) or nonsignificant (NS).

dropped below the sufficiency range as a result of *Phytophthora* infestation.

In the second experiment, mycorrhizal seedlings had fewer healthy root tips than NM seedlings whether or not they were infested with *P. parasitica* (Table 4). There was an interaction between *P. parasitica* and VAM because *Phytophthora* reduced the total number of root tips to a greater extent on NM than on VAM root systems. The number of rotted roots observed on NM and VAM plants was comparable, however, and the difference in percentage of rotted roots was not significant.

## DISCUSSION

*G. intraradices* did not appear to increase the resistance of sweet orange roots to infection by *P. parasitica*, nor did mycorrhizae increase the tolerance of the plant to the loss of roots responsible for P uptake. Several earlier reports for citrus and other hosts indicated that VAM fungi did not reduce disease and sometimes even predisposed plants to Phytophthora root rot (2,4,5,12). In the most recent investigation of citrus root rot, Davis and Menge (4) screened several VAM fungi for their ability to increase resistance or tolerance of citrus seedlings to *P. parasitica*. Most often there was no reduction in root rot development even though the VAM plant was P sufficient and the NM control deficient. VAM fungi that conferred little or no P nutritional benefit were purported to be the most effective in increasing resistance or tolerance. Overall, we cannot conclude from their study whether the VAM fungi tested increased tolerance or resistance in the absence of P nutritional effects. Roots were dipped in high concentrations of zoospore inoculum (500/ml), which resulted in root rot development that exceeded 50% and was as high as 70% after 12 wk. The method of disease assessment was not quantitative but visual.

We used a more natural system for inoculation with *P. parasitica* by mixing chlamydospores uniformly in soil at densities that approximate those found in Florida citrus soils. We also attempted to quantify root loss by evaluating the condition of each root tip. When Davis and Menge (4) challenged sweet orange colonized by *G. fasciculatum* with 20 and 50 chlamydospores/g of soil, they obtained moderate levels of root rot and found that mycorrhizae reduced the development of root rot. Unfortunately, this was in comparison to severely P-deficient nonmycorrhizal controls. In the first experiment, we confirmed earlier reports (3,4) in that *G. intraradices* reduced root rot development when it conferred a nutritional advantage to sweet orange before challenge with *P. parasitica*. Also, as previously reported (3,4), root rot severely reduced VAM colonization, resulting in a decrease in leaf P status equivalent to that for the NM plant. Consequently, growth reduction of infested VAM plants compared with noninfested plants was relatively greater than that for NM plants, which were P-deficient and grew less in the absence of *P. parasitica*.

In the second experiment, noninfested VAM and NM plants were sufficient in P, yet *Phytophthora* reduced root dry weight and leaf P content irrespective of mycorrhizae. The development of root rot in terms of reduction of total roots, number of rotted roots, and percentage of root rot was comparable for VAM and NM plants. *G. intraradices* not only did not increase resistance of roots to *Phytophthora* infection, but it did not counteract the lack of P uptake by roots. This might be expected, since VAM fungi and *P. parasitica* primarily colonize the same portion of the root system, the zone of elongation behind the root tip. *Phytophthora* destroys root tips, which drastically reduces VAM colonization. Consequently, mycorrhizae are apparently no longer as effective in uptake of P.

Recent studies with other fungal root pathogen-host plant systems indicate that VAM fungi may decrease root disease or reproduction of the fungus even though the NM plant is sufficient in P and comparable in size. Rosendahl (11) concluded that induced resistance of pea roots to *Aphanomyces euteiches* was systemic. *G. fasciculatum* reduced oospore production on both the mycorrhizal and the nonmycorrhizal side of a split-root system compared with less P-sufficient nonmycorrhizal controls. This systemic effect appears to be analogous to that demonstrated for split-root systems of citrus wherein mycorrhizae increased P content of roots on the NM side of root system and thereby decreased development of Phytophthora root rot (3).

More recently, Caron et al (1) demonstrated that a wide range of P levels in soil and in tomato plants had no effect on the development of soil populations of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and root and crown rot. Only the presence of *G. intraradices* in the roots resulted in significant reductions in populations of the pathogen and root necrosis. This effect was observed irrespective of the extent of VAM colonization and growth effects that were reduced under high P fertility conditions. They concluded that improved P nutrition was not responsible for the increased resistance of tomato. This apparent VAM induction of

**Table 3.** Dry weight and leaf phosphorus content of noninfested and *Phytophthora parasitica*-infested sweet orange seedlings either fertilized with phosphorus (NM) or inoculated with *Glomus intraradices* (VAM)

| Treatment | Dry weight (g) | | | Leaf P (%) |
|---|---|---|---|---|
| | Feeder roots | Total roots | Total shoots | |
| Noninfested | | | | |
| NM | 0.99 | 3.32 | 4.22 | 0.20 |
| VAM | 0.78 | 2.81 | 3.33 | 0.12 |
| *P. parasitica*[a] | | | | |
| NM | 0.38 | 2.97 | 4.11 | 0.12 |
| VAM | 0.43 | 2.40 | 3.25 | 0.08 |
| *P. parasitica* | **[b] | NS | NS | ** |
| VAM | NS | NS | NS | ** |
| *P. parasitica* × VAM | NS | NS | NS | NS |

[a] Inoculum density of 10 propagules/cm$^3$ of soil.
[b] Main effect or interaction significant at $P \leq 0.05$ (*) or 0.01 (**) or nonsignificant (NS).

**Table 4.** Phytophthora root rot development on sweet orange seedlings either fertilized with phosphorus (NM) or inoculated with *Glomus intraradices* (VAM)

| Treatment | Root tips | | | Rotted (%) |
|---|---|---|---|---|
| | Total | Healthy | Rotted | |
| Noninfested | | | | |
| NM | 506 | 506 | 0 | 0 |
| VAM | 311 | 311 | 0 | 0 |
| *P. parasitica*[a] | | | | |
| NM | 151 | 80 | 71 | 48 |
| VAM | 133 | 72 | 61 | 43 |
| *P. parasitica* | **[b] | ** | ** | ** |
| VAM | ** | ** | NS | NS |
| *P. parasitica* × VAM | ** | ** | NS | NS |

[a] Inoculum density of 10 propagules/cm$^3$ of soil.
[b] Main effect or interaction significant at $P \leq 0.05$ (*) or 0.01 (**) or nonsignificant (NS).

resistance to a fungal pathogen is unique and deserves further investigation.

The mechanisms of induced resistance or increased tolerance of mycorrhizal sweet orange to Phytophthora root rot do not appear to be operable unless there is a P nutritional advantage conferred to the VAM plant. Even then, the reduction in root rot by mycorrhizae is not significant from the standpoint of biological control. Phosphorus does not limit growth of nonmycorrhizal citrus when transplanted into fertilized Florida orchards soils (J. H. Graham and L. W. Timmer, *unpublished*). Thus, the additional cost of inoculating containerized citrus to reduce *Phytophthora* damage does not appear to be justified.

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