

Competitive Parasitic Abilities of *Phytophthora parasitica* and *P. palmivora* on Fibrous Roots of Citrus

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ABSTRACT

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The competitive abilities of *Phytophthora parasitica* and *P. palmivora* to infect and multiply on fibrous roots of citrus were compared using the replacement series technique. Greenhouse-grown citrus seedlings were transplanted to infested soil containing inoculum ratios of *P. parasitica*:*P. palmivora* of 4:0, 3:1, 2:2, 1:3, and 0:4 propagules per cubic centimeter. At the end of the experiments, percentage of root infection and densities

of soil propagules were measured by plating on a selective medium. Yields of *P. palmivora* were usually near expected proportions for final propagule densities and for root infection, but yields of *P. parasitica* were nearly always depressed in mixed inoculations. Under screenhouse conditions, *P. palmivora* outcompeted *P. parasitica* whether measured in a short-term experiment, periodically in a multiple-harvest experiment, in a long-term experiment, or in sterilized or nonsterilized soil. *P. parasitica* is widespread in Florida citrus soils, whereas *P. palmivora* is very localized. The local distribution of *P. palmivora* could not be explained by its inability to compete with *P. parasitica*.

Replacement series have been used widely by ecologists to evaluate the competitive abilities of herbaceous plant species (8). This technique has been used rarely in plant pathology, although it offers a means to study competitive interactions for living or dead resources by plant pathogens (1). Adee et al (1) found that *Pyrenophora tritici-repentis* (Died.) Drechs. was a better competitor for the wheat leaf surface than was *Septoria nodorum* Berk. using the replacement series technique. We felt this method would also be useful for measuring competition of parasites for fibrous roots of citrus.

Phytophthora parasitica Dastur is the most widespread species of *Phytophthora* in Florida citrus groves and nurseries (2,13,14). It causes root rot, foot rot, and brown rot of fruit. Most of the commonly used rootstocks are resistant to bark infection but susceptible to fibrous root rot (3). Other species such as *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian and *P. palmivora* (E.J. Butler) E.J. Butler are present in Florida and are highly pathogenic to citrus but occur only locally (12,13). Whiteside (12) speculated that *P. citrophthora* remained localized because it was primarily a fruit-infecting species, and favorable conditions for fruit infection occurred infrequently. We found that *P. palmivora* was highly pathogenic to fibrous roots of citrus (14). However, this species was recovered originally from only two sites, and it has been found since at only one additional location. Since *P. palmivora* is unable to grow at 33 C (14), reproduction of this species may be inhibited in Florida groves during the summer. However, the lack of widespread distribution of this species is probably not explicable by temperature alone. In the absence of other obvious factors that might explain its restricted occurrence, we hypothesized that *P. palmivora* was unable to compete favorably for root tissue with the much more widespread *P. parasitica*. The objective of this study was to compare the competitive abilities of *P. parasitica* and *P. palmivora* to infect and reproduce on fibrous roots of citrus in potted soils under screenhouse conditions using the replacement series technique.

MATERIALS AND METHODS

Inoculum preparation and soil infestation. Cultures of *P. parasitica* isolated from citrus groves near Haines City, Ft. Pierce, and LaBelle, Florida, and a nursery near Bartow, as well as an isolate of *P. palmivora* from near Ft. Pierce investigated previously (14), were used in these studies. Cultures were maintained on V8 juice agar slants. Chlamydo spores of each isolate were produced by the method of Tsao (11) and were separated from the mycelium by repeated blending and low-speed centrifugation. The chlamydo spore preparation was added to several liters of autoclaved Candler fine sand (typic quartzsammets, pH 6.8, 1% organic matter) that had been moistened to about field capacity and mixed manually to produce the concentrated inoculum. This inoculum was incubated moist at room temperature (23–26 C) for 2 days, then assayed on the selective PARPH medium (4,5) using the procedures of Timmer et al (10) to determine the propagule density. In this study, pimarinin was used at 5.0 mg/L, hymexazol was used at 25 mg/L of medium, thiamine was added at 1.0 mg/L, and all other ingredients were as described previously (4,5).

Large batches of soil were infested by adding the inoculum of previously determined propagule density singly or in various combinations of isolates and blending in a cement mixer. Amounts of concentrated inoculum were adjusted in all experiments so as to attain a final density of both species of four propagules per cubic centimeter of soil. Where mixtures of the two species were required, inoculum was added to achieve three, two, or one propagule per cubic centimeter of one species and one, two, or three propagules of the second species. Candler fine sand that had been sterilized by autoclaving was used in all experiments except one where the soil was not sterilized.

Seedlings were grown in Fafard Citrus Mix B (Conrad Fafard, Inc., Springfield, MA) in plastic tubes about 21 cm long and 4 cm in diameter. Three 4-mo-old seedlings about 15–20 cm tall with eight to 10 leaves were selected, rinsed free of potting mix, and transplanted to 2.3-L black plastic pots in infested or noninfested soil. Plants were maintained on benches in the screenhouse and arranged in a randomized complete block design. In most experiments, pots were flooded for the first 48 h after trans-

plant and for 48 h every 2 wk thereafter. Air temperatures usually ranged from 22 to 35 C during experiments. In the long-term experiment, plants were rearranged about every 2 mo to avoid locational effects. The long-term experiment was exposed to ambient weather conditions, with air temperatures as low as 5 C during winter and up to 35 C in summer. In this experiment, pots were flooded for 48 h after transplant and were watered to maintain plants in good condition thereafter.

Propagule density determination and root assays. At the end of the experiments, the final propagule densities and the percentages of root pieces infected by each species were determined. Seedlings were gently removed from the soil and the roots rinsed free of soil particles. The soil from each pot was mixed thoroughly, and then a 10-cm³ sample was removed from each pot. Soil samples from each pot were plated on the selective medium following the procedures of Timmer et al (10) using five plates per sample. In addition, 100 root pieces, each about 1 cm long, were selected at random from each plant and plated on the selective medium, 20 pieces per plate. Plates were incubated at 27 C for 3 days and the number of colonies from soil or infected root pieces were counted. The selective medium used was PARPH medium with a nutrient agar (NA) base rather than the standard cornmeal agar (CMA) base. On the CMA-based PARPH medium, *P. palmivora* formed small, dense colonies, whereas *P. parasitica* formed larger, more diffuse colonies. Use of the NA base enhanced the differences between colony types of the two species and thus facilitated colony counts. Three to five typical colonies of each species, as well as any questionable colonies, were selected in each experiment, grown out, and identified to confirm the correct recording of species.

In some experiments, the percentage of root rot was determined by visually examining up to 100 root tips, if available, on each seedling and determining the percentage of rotted tips. In the long-term experiment, root weight was determined by removing all of the fibrous roots, drying them at 65 C, and weighing them.

Experiments. In a preliminary short-term experiment, the competitive abilities of *P. parasitica* and *P. palmivora* were compared using inoculum ratios of 4:0, 2:2, and 0:4 plus a noninoculated control. In this experiment, 10 single-seedling replications of rough lemon (*Citrus jambhiri* Lush.) were used for each treatment. The *P. parasitica* isolate from Haines City was employed. This experiment was evaluated 6 wk after inoculation.

The competitive abilities of *P. palmivora* and the Ft. Pierce isolate of *P. parasitica* were compared in a multiple-harvest experiment where inoculum ratios of 4:0, 3:1, 2:2, 1:3, and 0:4 were established. Thirty-two rough lemon seedlings were used with each treatment. Eight seedlings from each treatment were evaluated at 4, 6, 8, and 10 wk after inoculation to determine the effect of time on the competitive interaction.

A long-term experiment was established to determine the competitive abilities of the Haines City isolate of *P. parasitica* and *P. palmivora* over a 16-mo period. Inoculum ratios used were 4:0, 3:1, 2:2, 1:3, and 0:4 plus a noninoculated control. Eight single-seedling replications of sweet orange (*C. sinensis* (L.) Osbeck) were used for each treatment. A 1-cm-diameter tube was used to collect soil core samples from pots to determine propagule densities of each species at 2, 6, 10, and 14 mo after

transplant to infested soil. At 16 mo, the percentages of root infection and propagule densities of each species were determined. The percentages of root rot and root weights were also measured at 16 mo.

In the natural soil experiment, the competitive abilities of *P. parasitica*, using a mixture of all four isolates, and *P. palmivora* were compared using inoculum ratios of 4:0, 3:1, 2:2, 1:3, and 0:4. Ten single-seedling replications of sour orange (*C. aurantium* L.) were used in nonsterilized Candler fine sand. The experiment was evaluated 7 wk after inoculation.

Data presentation and statistical analysis. For replacement series experiments, the yield of each species in single culture, i.e., the percentage of root infection or the propagule density, is taken as unity and the yield of each species in mixed inoculations is expressed as a proportion of that recovered in single culture (1,7). If inter- and intraspecies competition is equal, then species recovery should be in the same proportion as in the inoculum. Data on percentages of root infection and on propagule densities in these experiments were expressed graphically in this manner and compared with the theoretical relative yields of each species. The pairwise *t* test was used to compare observed relative yields with theoretical yields. In addition, the lack-of-fit regression test (PROC RSREG procedure [7]) was used to determine whether observed responses were significantly different from the expected linear response (1). The relative yield total (RYT), the sum of the relative yield of *P. parasitica* plus the relative yield of *P. palmivora*, was compared with the theoretical relative yield of 1.0 using a nonparametric sign test. The observed RYT values for the 3:1, 2:2, and 1:3 inoculum ratios were categorized as greater or less than the theoretical (1.0), and the probability that the distribution was due to chance was determined (9).

Other factors such as root rot and root weights were subjected to analysis of variance, and means were separated using the Waller-Duncan *k*-ratio *t* test.

RESULTS

Preliminary experiment. Root rot in this experiment was much greater in plants inoculated with *P. palmivora* only than in those inoculated with *P. parasitica* only (Table 1). However, the percentage of root infection and the propagule densities were similar with both species in single-species inoculations. In plants inoculated with both species, the percentage of roots infected with *P. palmivora* and the number of propagules of *P. palmivora* recovered from soil was much greater than those of *P. parasitica*.

Multiple-harvest experiment. *P. palmivora* was recovered more often than *P. parasitica* from plants inoculated with different proportions of the two species and assayed at 4, 6, 8, and 10 wk after inoculation (Fig. 1). Isolation of *P. parasitica* from roots was significantly less than the reference value in almost all cases according to *t* tests. Propagule densities of *P. parasitica* in soil often differed from the reference line, and by 10 wk after inoculation, all points were significantly different. The hypothetical linear model was not appropriate for describing any of the observed relative yields for *P. parasitica* over time.

The response of *P. palmivora* seldom differed from the expected for root infection or propagule densities. However, the linear

TABLE 1. Effect of inoculation of rough lemon seedlings with *Phytophthora parasitica* or *P. palmivora* singly and in combination on root rot and the recovery of each species from roots and soil

| <i>P. parasitica</i> : <i>P. palmivora</i> ratio | Root rot (%) | Root infection (%) | | Propagules/cm ³ soil | |
|--|---------------------|----------------------|---------------------|---------------------------------|---------------------|
| | | <i>P. parasitica</i> | <i>P. palmivora</i> | <i>P. parasitica</i> | <i>P. palmivora</i> |
| 4:0 | 20.4 b ^x | 81.5 a | 0.0 ^y | 20.0 a | 0.0 |
| 2:2 | 53.4 a | 17.9 b | 69.9 | 4.5 b | 27.0 |
| 0:4 | 64.5 a | 0.0 | 74.5 | 0.0 | 20.3 |
| 0:0 | 5.5 c | 0.0 | 0.0 | 0.0 | 0.0 |
| | | | NS ^z | | NS |

^xMean separation within columns and experiments by the Waller-Duncan *k*-ratio *t* test, $P \leq 0.05$.

^yValues for which no recovery was expected were not included in the analysis of variance.

^zNS = analysis of variance of treatments where recovery of *P. palmivora* was expected indicated no significant difference between those treatments.

model accounted for a significant portion of the variation for the root infection data only at the 6-wk harvest and for the propagule density data only at the 4- and 8-wk harvests.

The RYT rarely differed significantly from the reference values in the early harvests. By the 10-wk harvest, however, the RYT was greatly depressed, indicating that the *P. parasitica* yield had been reduced but that the yield of *P. palmivora* had not been enhanced. The intersections of the observed lines for the two species were shifted strongly to the right for root infection and propagule densities for all harvests. Thus, interspecific competi-

tion was greater than intraspecific competition, and *P. palmivora* was infecting roots whereas *P. parasitica* was not competing successfully for the available susceptible tissue.

Long-term experiment. Propagule densities of *P. parasitica* were nearly always significantly less than expected reference values in samples collected 2–14 mo after inoculation (Fig. 2). In contrast, none of the observed propagule densities for *P. palmivora* differed significantly from expected reference values. Again, in this experiment observed RYT values were not significantly different from or were less than reference values in most cases, indicating that

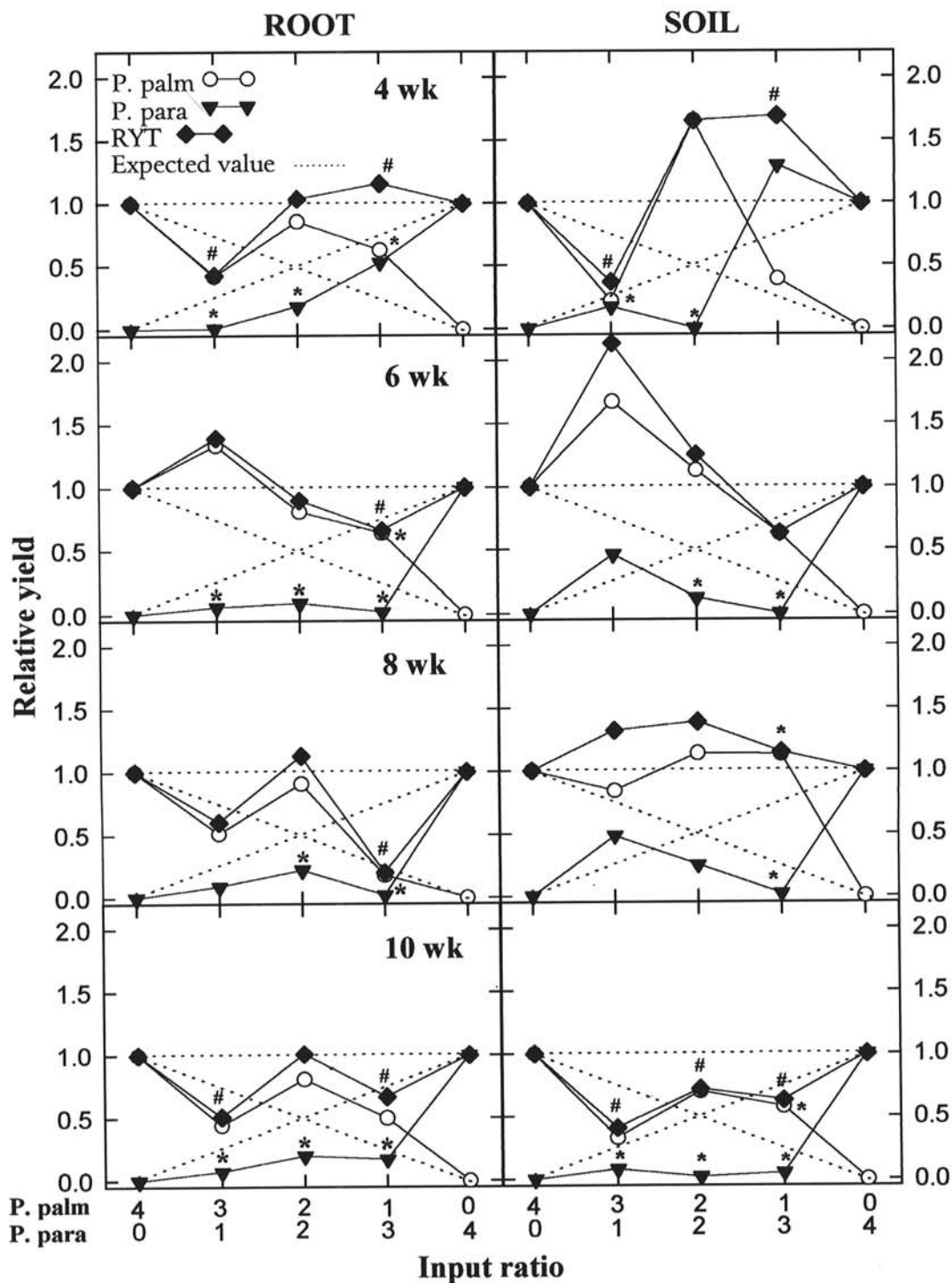


Fig. 1. Relative recoveries of *Phytophthora parasitica* (*P. para*) and *P. palmivora* (*P. palm*) from rough lemon roots and soil at 4, 6, 8, and 10 wk after inoculation with different inoculum density ratios of the two species in a multiple-harvest experiment. RYT = relative yield total of both species. Expected values are the reference values indicating equal inter- and intraspecific competition. # = Value of RYT significantly different from the reference value (1.0) by a nonparametric sign test. * = Significantly different from the reference value for each species according to the *t* test, $P \leq 0.05$.

interspecific competition was equal to or greater than intraspecific competition. As in the multiple-harvest experiment, intersection of the observed lines were shifted strongly to the right, indicating the ability of *P. palmivora* to outcompete *P. parasitica*.

Upon completion of the experiment 16 mo after inoculation, root rot and root weight were evaluated as well as propagule density and percentage of root infection (Table 2). All inoculated plants had higher percentages of root rot than the noninoculated control, but there were few differences among treatments. Some inoculated treatments had lower root weights than the control, but there were no differences in root weights among the inoculated treatments. The percentage of roots infected by *P. parasitica* in mixed inoculum was significantly less than that in sole culture.

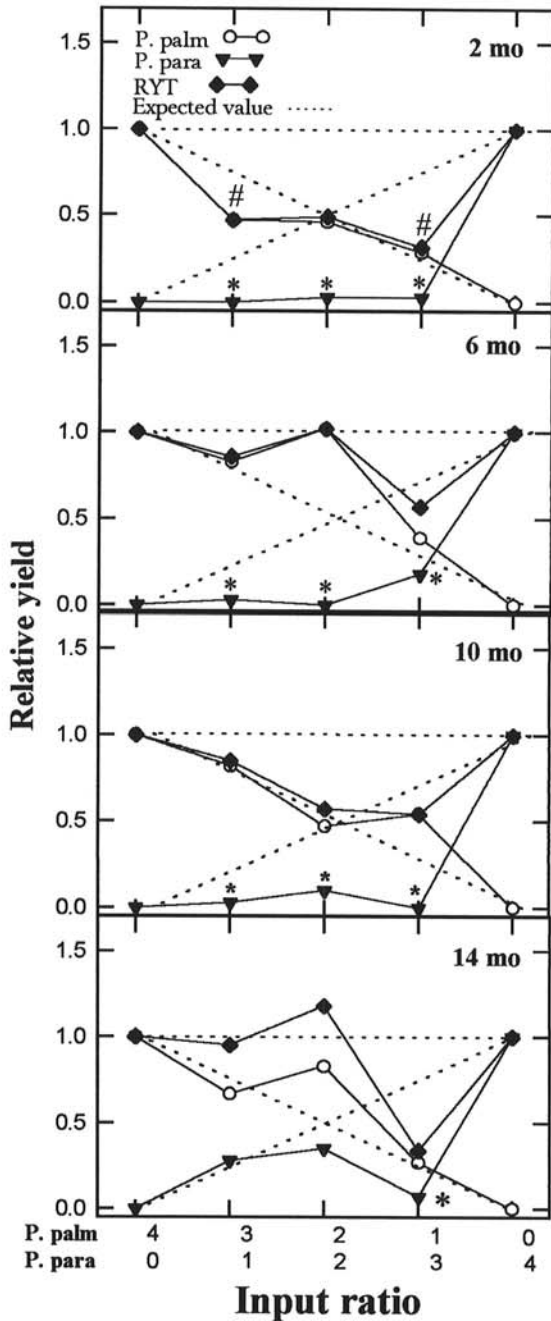


Fig. 2. Relative recoveries from soil of *Phytophthora parasitica* (*P. para*) and *P. palmivora* (*P. palm*) from soil at 2, 6, 10, and 14 mo after inoculation with different inoculum density ratios of the two species in a long-term experiment. RYT = relative yield total of both species. Expected values are the reference values indicating equal inter- and intraspecific competition. # = Value of RYT significantly different from the reference value (1.0) by a nonparametric sign test. * = Significantly different from the reference value for each species according to the *t* test, $P \leq 0.05$.

The same was true for propagule densities in soil. With *P. palmivora*, however, the percentage of roots infected and the propagule densities were not significantly different whether in sole culture or in mixtures with *P. parasitica*.

Natural soil experiment. Recoveries of *P. parasitica* from roots and soil were again significantly less than expected reference values (Fig. 3). In contrast, recoveries of *P. palmivora* were equal to or greater than expected. The hypothetical linear model accounted for a significant portion of the variability for root infection but not for propagule densities of both species. The RYT values, as in the previous experiments, were equal to or less than the expected values. The intersections of the observed lines were again shifted to the right, indicating the predominance of *P. palmivora* in nonsterilized soil as well as in the sterilized soil used in previous experiments.

DISCUSSION

In all of the experiments conducted in this study, *P. palmivora* clearly outcompeted *P. parasitica*. *P. palmivora* was more successful against all of the isolates of *P. parasitica* tested, on different citrus species, in long-term and short-term experiments, and in sterilized and nonsterilized soil. Little research has been conducted on interspecific competition between root-infecting fungi. Most studies have dealt with saprophytic exploitation of primary resources by parasitic and saprophytic fungi (6). Dandurand and Menge (2) found that prior inoculation of citrus roots with *Fusarium solani* (Mart.) Sacc. suppressed propagule densities of *P. parasitica* but not of *P. citrophthora* in the rhizosphere. *P. parasitica* is dependent on root tips for infection, and since *F. solani* reduced root tip production, it decreased the number of sites available for infection by *P. parasitica*. *P. citrophthora* is not dependent on the zone of elongation of the root tip for infection and thus was unaffected by prior inoculation with *F. solani*.

In our study, the two species competed directly for the fibrous roots of the citrus seedlings. Chlamyospore-infested soil maintains stable propagule densities for up to 8 days in soil moistened to field capacity (10). Upon flooding, sporangia are formed and zoospores are released. At that time, zoospores of the two species must compete for the available fibrous root tissue. Subsequent flooding cycles inducing sporangium production and release of more zoospores offer further opportunity for competition. The reasons for the dominance of *P. palmivora* are not immediately apparent. In culture, *P. parasitica* grows more rapidly and at a wider range of temperatures than *P. palmivora* (14), which should have been advantageous to the former species. However, *P. palmivora* produces sporangia more rapidly and in greater abundance on infected tissue than does *P. parasitica* (L. W. Timmer, unpublished). In our studies, a greater amount of root rot was noted with *P. palmivora* than with *P. parasitica*. Thus, *P. palmivora* may behave much like *P. citrophthora* (3) in that it invades the entire cortex and is not as dependent on the availability of root tips for reproduction.

The replacement series technique proved valuable for the study of interactions of these two *Phytophthora* species. Many types of interactions can be detected by comparing the slopes of the lines for each species with the theoretical values and by analyzing the values of the relative yield total (8). The technique may be useful for foliar or soilborne pathogens and may be used for living or dead substrates (1). The competitive abilities of species or strains on hosts with different types of resistance could be evaluated. We are currently utilizing this approach to evaluate the competitive ability of metalaxyl-resistant and -sensitive strains of *P. parasitica* (J. H. Graham, L. W. Timmer, and S. E. Zitko, unpublished).

Our hypothesis that *P. palmivora* remained localized because it could not compete favorably with *P. parasitica* is probably not valid, although it is not always possible to translate results of pot studies to the field. Whiteside (12) attributed the restricted occurrence of brown rot of fruit to the localized distribution of *P. citrophthora*. However, he found no definitive explanation for the limited distribution of *P. citrophthora*. Likewise, we find

TABLE 2. Effect of inoculation of sweet orange seedlings with *Phytophthora parasitica* or *P. palmivora* singly and in combination on the recovery of each species from roots and soil, root rot, and root weight 16 mo after inoculation

| <i>P. parasitica</i> : <i>P. palmivora</i> ratio | Root rot (%) | Root wt (g) | Root infection (%) | | Propagules/cm ³ soil | |
|--|---------------------|----------------|----------------------|---------------------|---------------------------------|---------------------|
| | | | <i>P. parasitica</i> | <i>P. palmivora</i> | <i>P. parasitica</i> | <i>P. palmivora</i> |
| 4:0 | 39.0 a ^x | 3.4 b | 29.0 a | 0.0 ^y | 53.1 a | 0.0 |
| 3:1 | 31.6 b | 4.5 ab | 6.3 b | 15.3 | 5.3 b | 23.3 |
| 2:2 | 50.8 a | 4.0 b | 12.8 ab | 20.6 | 10.0 b | 73.5 |
| 1:3 | 50.6 a | 4.8 ab | 8.8 b | 12.5 | 13.3 b | 75.8 |
| 0:4 | 35.5 ab | 4.8 ab | 0.0 | 26.0 | 0.0 | 54.3 |
| 0:0 | 4.5 c | 8.2 a | 0.0 | 0.0 | 0.0 | 0.0 |
| | | | | NS ^z | | NS |

^xMean separation within columns according to the Waller-Duncan *k*-ratio *t* test, $P \leq 0.05$.

^yValues for which no recovery was expected were not included in the analysis of variance.

^zNS = analysis of variance of treatments where recovery of *P. palmivora* was expected indicated no significant differences between those treatments.

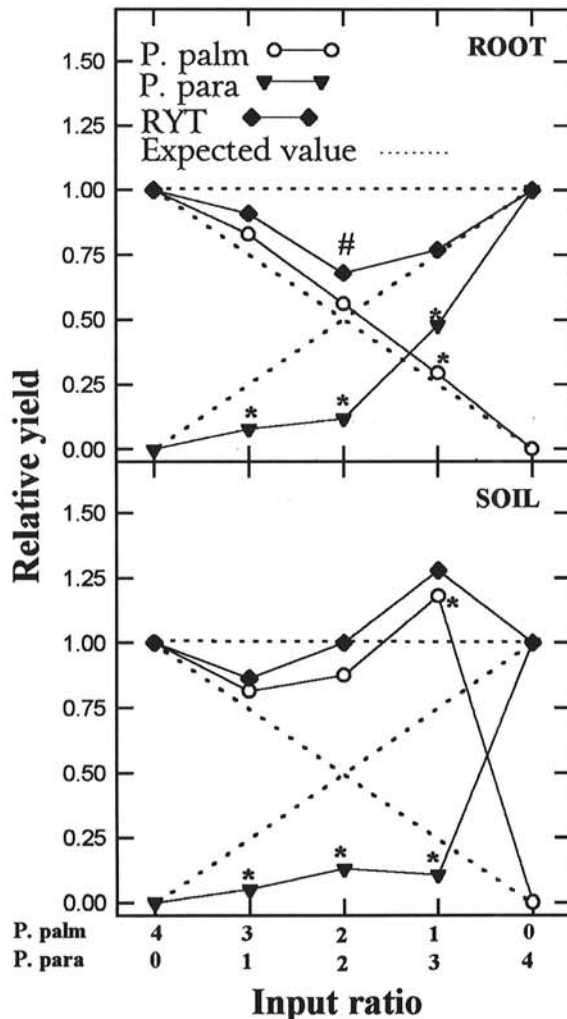


Fig. 3. Relative recoveries of *Phytophthora parasitica* (*P. para*) and *P. palmivora* (*P. palm*) from sour orange roots and soil 7 wk after inoculation with different inoculum density ratios in nonsterilized soil. RYT = relative yield total of both species. Expected values are the reference values indicating equal inter- and intraspecific competition. # = Value of RYT significantly different from the reference value (1.00) by a nonparametric sign test. * = Significantly different from the reference value for each species according to the *t* test, $P \leq 0.05$.

no obvious reasons why this species is not more widespread. Perhaps the citrus isolate of *P. palmivora* originated from palms or other native vegetation in the area and simply has not been further distributed. Dissemination of *Phytophthora* species in

Florida citrus groves is frequently on infested nursery stock. Of the nurseries tested, 50–80% have *P. parasitica* present (13; J. H. Graham and L. W. Timmer, unpublished). Although other methods of spread, such as with runoff water or equipment, are possible, *P. palmivora* and *P. citrophthora* may be locally distributed, because they have never found their way into Florida nurseries. Precautions should be taken to avoid introduction of this species into nurseries, since it is highly pathogenic to roots, shoots, and fruit of citrus (14).

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