Evaluation of Tolerance of Citrus Rootstocks to Phytophthora Root Rot in Chlamydospore-Infested Soil

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ABSTRACT

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Seven citrus rootstocks of commercial importance in Florida were evaluated for their tolerance to Phytophthora root rot. Six-month-old seedlings were planted in soil artificially infested with chlamydospores at 0, 0.5, 1, 5, and 10 propagules of *Phytophthora parasitica* per cm^3 of soil. For all rootstocks, at 6 wk after inoculation there was little increase in disease rating with an increase in inoculum density above 1 propagule/cm^3. Disease severity at the higher inoculum densities was significantly greater for the susceptible rootstocks (sour orange, Ridge Pineapple sweet orange, Cleopatra mandarin, and Carrizo citrange) than for the tolerant rootstocks (trifoliate orange and Swingle citrumelo). Volkamer lemon was intermediate between the two groups. Within the susceptible and tolerant groups, no significant differences among rootstocks were detected. Tolerance was related to the ability of trifoliate orange and Swingle citrumelo to regenerate roots in the presence of *P. parasitica*. The same grouping of rootstocks was observed when 3-mo-old seedlings were inoculated with high inoculum densities ($\geq$ 10 propagules/cm^3) and evaluated 3 wk later but not when older seedlings were used. Thus, a rapid method for primary screening of citrus rootstocks for tolerance to root rot was established through the use of chlamydospore-infested soil and very young seedlings.

Phytophthora spp. cause the most serious soilborne diseases of citrus worldwide (15). In Florida, *Phytophthora parasitica* Dastur causes losses from root and crown rot in nurseries and from foot rot and fibrous root rot (14,23) in orchards. Foot rot is recognized as an infection of bark near the ground level producing lesions on the trunk or crown roots that can girdle and kill the tree (14,15). *P. parasitica* also decays fibrous roots and can be especially severe on susceptible rootstocks in nurseries (15, 23). Fibrous root rot also occurs on susceptible rootstocks in bearing orchards where damage causes tree decline and yield losses (11,12,14). However, fibrous root loss on some tolerant rootstocks does not affect fruit yield (12). Here, tolerance is defined as the condition where plants are infected but show little or no net root loss either because infected roots do not rot or because root mass is maintained by root regeneration.

Although commonly planted scion varieties are susceptible to bark infection, most commercially used rootstocks are at least moderately resistant (4,15). Foot rot management, therefore, relies heavily on the use of resistant rootstocks as well as on cultural practices that minimize the risk of scion infection by the fungus (4,21).

In addition to foot rot resistance, other desirable characteristics of a Florida rootstock include tolerance of citrus blight and several viruses and nematode diseases, as well as acceptable horticultural performance (4). Most of the screening objectives for new rootstocks are long-term, so a procedure for preliminary screening for tolerance to root and foot rot has been practiced widely (3,6,8–10). It is necessary to evaluate both diseases because tolerance to root infection is generally less than resistance to bark infection (6,8,9). The initial test measures the severity of root rot on young seedlings compared to Carrizo citrange (*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osb.), which is judged to be a reference rootstock of moderate tolerance (8–10). For this "tank test," roots of seedlings are submerged in zoospore inoculum for 18–20 hr and then lined-out into outdoor planting frames where seedlings are flooded at the time of planting and periodically thereafter to promote root rot (3,8).

Tsao and Garber (19) indicated that the amount of citrus root rot resulting from zoospore inoculation depends on the interaction of the zoospores with the root surface and the rhizosphere. Nonuniformity of zoospore inocula on the root could lead to greater variability in the production of secondary infective propagules in the rhizosphere and the development of root rot. This may explain why various workers (3,6,8) have noted problems with erratic disease development after zoospore inoculation. Consequently, reinoculation or retesting of the same lots of seedlings was necessary (3).

Previous studies (5,7) indicated that inoculum prepared by adding *P. parasitica* chlamydospores to soil can provide conditions for uniform root rot development. The inoculum is assayed with a selective medium to adjust propagule density of the test soil (7,16). Thus, with chlamydospore-infested soil, I established a range of inoculum density and root rot severity for comparison of the relative tolerance of seven rootstocks of commercial importance in Florida. After ranking rootstocks, I sought to develop a rapid and reproducible screening method for evaluation of citrus rootstock germ plasm for tolerance to Phytophthora root rot.

MATERIALS AND METHODS

Chlamydospore inoculation. *P. parasitica* (isolate R-1) was isolated from the rhizosphere soil in an orchard of Hamlin orange (*C. sinensis*) trees on sour orange (*C. aurantium* L.) rootstock near Fort Pierce, Florida. Chlamydospores were

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produced by the method of Tsao (18) 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. The infested soil was incubated in a moist condition for 7 days, then the inoculum density was evaluated by dilution plating on pimaricin-ampicillin-rifampicin-pentachloronitrobenzene (PARP) selective medium using 125 mg/L instead of 250 mg/L of ampicillin and adding 25 mg of hymexazol per liter (7,16). Based on this assessment, the soil inoculum was thoroughly mixed with known volumes of autoclaved Candler fine sand soil to give the desired inoculum density. Each inoculum density level was confirmed by plating triplicate 50 cm^3 samples on PARP medium after soil mixing.

Inoculum density vs. disease severity. Seeds of the following rootstocks were obtained from registered seed source trees of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry: trifoliate orange (*P. trifoliata*), Ridge Pineapple sweet orange (*C. sinensis*), Carrizo citrange (*C. sinensis* × *P. trifoliata*), Swingle citrumelo (*C. paradisi* Macf. × *P. trifoliata*), sour orange (*C. aurantium* L.), Cleopatra mandarin (*C. reticulata* Blanco), and Volkamer lemon (*C. volkameriana* Pasq.). Seeds were sown in 150 cm^3 Leach tubes (Ray Leach Container Nursery, Canby, OR) containing Promix (Premier Brands, Inc.), and seedlings were fertilized weekly with Peter's 20-20-20 (N-P-K) Peat-lite Special (W. R. Grace, Fogelsville, PA). Six-month-old seedlings of each rootstock were selected for uniformity of root system size, within and among varieties, by inspection of the roots after they were washed free of peat moss. Root systems were transplanted into the center of 15-cm-diameter clay pots containing Candler fine sand soil with 0, 0.5, 1, 5, and 10 propagules of *P. parasitica* per cm^3 of soil. This range of propagule density is representative of that encountered in orchard soils in Florida (16,17). There were seven seedlings per rootstock-inoculum level treatment. After transplant, soil was flooded for the first 3 days by placing a dish under each pot and keeping the dishes full of water each day. On the third day, the water in each dish was poured out and watering during the subsequent 4 days occurred only when the soil surface was dry. The watering cycle was maintained for the remainder of the experiment. At 3 and 6 wk after inoculation of seedlings, the inoculum density was determined for the 1 and 10 propagules/cm^3 levels from Swingle citrumelo and Carrizo citrange. This was accomplished by taking a handful of approximately 50 cm^3 of soil from around the roots of each seedling (five replications per rootstock) and plating as described earlier. Two experiments were conducted during June and July (experiment 1) and October and November 1988 (experiment 2). Greenhouse conditions ranged from 25 to 35 C and 60 to 100% RH during the experiments.

After 6 wk, root systems were carefully washed free of soil to minimize loss of roots from treatment with root rot. The percentage of 200–300 root tips that were rotted was assessed by pinching each root tip 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 (7).

Before the root rot counts, a visual disease severity rating was also performed. Two people independently rated the seedlings of all rootstocks combined by placing them in 11 piles corresponding to ratings of 1 to 11, where 1 = no visible rot and 11 = no visible healthy roots. In experiment 1, the disease severity rating was compared with the percentage root rot determination by correlation analysis and was found to have an r = 0.90. For experiment 2, only visual disease ratings were performed.

The general response of rootstocks was that at the lower inoculum levels (0.5 and 1 propagules/cm^3 of soil) there was a relatively large increase in disease rating with increase in propagule density, whereas at the higher inoculum densities of 5 and 10 propagules, the corresponding increase in disease severity was smaller. An analysis of variance indicated that rootstock, inoculum density, and their interaction were highly significant ($P < 0.001$). Therefore, disease severity ratings (y) were fitted to a nonlinear asymptotic model, $y = x/A + Bx$, where x is the inoculum density (Fig. 1) and A and B are the slope and y-intercept, respectively. Thus, as x becomes larger, y approaches $1/B$. The parameter, $1/B$, and its standard deviation was used as an unbiased estimator of disease severity at the saturating inoculum levels.

Rapid screen. To develop a simpler and more rapid process for evaluating root rot tolerance of rootstocks, seedlings younger than those used in the previous experiment and two high inoculum densities (10 and 100 propagules/cm^3 of soil) were utilized. Seedlings, either 3, 4, or 5 mo old (two- to three-, four- to six-, and seven- to 10-true leaf stages, respectively), of each rootstock were planted together in replicate 15-cm-diameter pots. There were five replicate pots of 3-mo-old seedlings and 10 replicate pots of 4- and 5-mo-old seedlings. Three weeks after inoculation, root systems were evaluated for root rot as before. Percentage of root rot and disease severity ratings were subjected to analysis of variance to test for inoculum density effects. The relationship between severity rating and seedling age was determined by linear regression. Comparison of disease ratings from the 3-wk tests with ratings from the 6-wk tests (experiments 1 and 2) for 5 and 10 propagules/cm^3 treatments was made by correlation analysis.

RESULTS

Inoculum density vs. disease severity. Three and six weeks after inoculation,

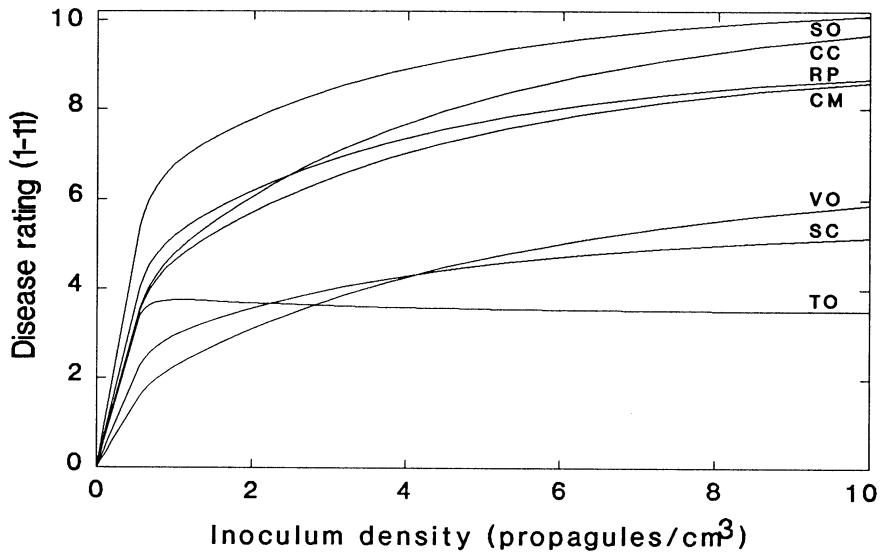

Fig. 1. Relationship between inoculum density of *Phytophthora parasitica* in chlamydospore-infested soil and disease severity 6 wk after inoculation of 6-mo-old seedlings of seven citrus rootstocks. SO = sour orange, CC = Carrizo citrange, RP = Ridge Pineapple sweet orange, CM = Cleopatra mandarin, VO = Volkamer lemon, SC = Swingle citrumelo, and TO = Trifoliate orange. Curves represent a least-squares fit of the data to the model $y = x/A + Bx$. Regression coefficients (r) for the different rootstocks varied from 0.86 to 0.99 and all were highly significant ($P < 0.001$). SO, CC, RP, and CM have a significantly ($P < 0.01$) greater disease rating than SC and TO according to Student-Newman-Keuls multiple comparisons test. VO is significantly different from SO and CC.

propagule counts from the 1 and 10 inoculum densities were approximately 40 propagules/cm^3 of soil, irrespective of the inoculum level or the rootstock sampled. Thus, an increase in inoculum density above 1 propagule/cm^3 of soil resulted in a relatively small additional increase in disease severity for all rootstocks (Fig. 1). Using $1/B$ as an estimator of disease severity at the saturating inoculum level, there were apparent differences in root rot tolerance among rootstocks. Two groups of rootstocks emerged that were significantly different from each other, but there were no significant differences among rootstocks within groups. Trifoliate orange and Swingle citrumelo were included within the tolerant group. Sour orange, Carrizo citrange, Ridge Pineapple orange, and Cleopatra mandarin formed a susceptible group. Volkamer lemon was intermediate in that it was significantly different from sour orange and Carrizo citrange but not from the other rootstocks in each group. This grouping of rootstocks was confirmed in the second experiment except that Volkamer lemon was not significant from any of the other rootstocks. Overall, the disease ratings from experiment 2 were significantly correlated with those in experiment 1 (Table 1).

The tolerant rootstocks were distinguished from the susceptible group by the ability to grow roots in the presence of *P. parasitica* (Fig. 2). Fibrous root weight of trifoliate orange and Swingle citrumelo actually increased for the 0.5 and 1.0 propagule/cm^3 inoculum densities relative to the noninoculated controls (Fig. 2). Root loss of Volkamer lemon was more like that of the susceptible group, but Volkamer lemon had a greater weight of fibrous roots than the other rootstocks (*data not shown*) and hence had a somewhat lower visual disease rating.

Rapid screen. There was no significant interaction between inoculum densities $\geq$ 10 propagules/cm^3 of soil and disease severity of the young seedlings evaluated 3 wk after inoculation. However, percentage of root rot was negatively correlated with seedling age ($r = -0.70$, $P \geq 0.01$) when all inoculum levels and rootstocks were considered (Fig. 3). In both experiments, the mean disease rating of the 6-mo-old seedlings at the 5 and 10 inoculum levels was significantly correlated with that for the 3-mo-old seedlings but not for the 4- and 5-mo-old seedlings (Table 1). The groupings of rootstocks were the same for the 3-mo-old seedlings as for the 6-mo-old plants. The groups of tolerant and susceptible rootstocks were not differentiated on 4- and 5-mo-old seedlings because the extent of root rot development after 3 wk was less than that for the 3-mo-old seedlings or 6-mo-old plants after 6 wk in the first two experiments (Figs. 1 and 3). Thus, there was a significant interaction ($P \geq 0.01$) between seedling age and inoculum density.

DISCUSSION

Chlamydospore-infested soil adjusted to different propagule densities after assays with a selective medium yielded reproducible levels of Phytophthora root rot for quantitative ranking of rootstock susceptibility. Visual rating of disease severity was confirmed to be an effective evaluation of the extent of the root system affected by root rot. Examination of the tolerance of seven rootstocks revealed that disease severity was relatively constant for inoculum densities above 1 propagule/cm^3 of soil. This permitted the ranking of rootstocks at the higher inoculum densities.

Furthermore, 3-mo-old seedlings (two- to three-leaf stage) were less tolerant than older seedlings. Younger seedlings may have higher rates of root exudation than older plants, which renders them more susceptible to root infection (22), whereas phytoalexins may accumulate in roots of older seedlings (1,20). Even if the mechanisms of resistance of young and old seedlings were not the same, inoculation of 3-mo-old seedlings yielded ratings of rootstock tolerance after 3 wk similar to those obtained from tests conducted over a wide range of inoculum densities with 6-mo-old plants and a 6-wk period. Thus, a rapid

Table 1. Correlations (r) between the disease severity rating from the high inoculum density treatments (5 and 10 propagules/cm^3 of soil) of 6-mo-old seedlings evaluated after 6 wk (experiments 1 and 2) and of 3-, 4-, and 5-mo-old seedlings evaluated after 3 wk for seven citrus rootstocks

		Seedling age[a]		
	Experiment 2	3-mo-old	4-mo-old	5-mo-old
Experiment 1	0.84*[b]	0.95*	0.71	0.67
Experiment 2	...	0.88*	0.48	0.72

[a] 3-, 4-, and 5-mo-old seedlings inoculated with $\geq$ 10 propagules/cm^3 of soil (see text).
[b] Correlations significant at the $P \leq 0.05$ level are indicated by an asterisk.

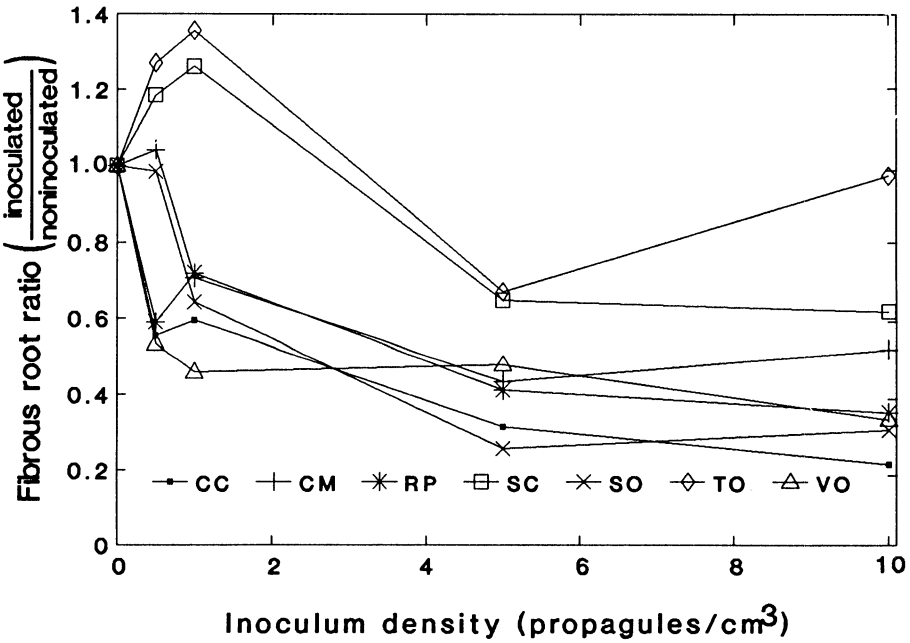

Fig. 2. Ratios of the fibrous root weight of seedlings infected with *Phytophthora parasitica* and noninfected seedlings over a range of inoculum densities in chlamydospore-infested soil for seven citrus rootstocks in experiment 1. See Fig. 1 for identification of rootstocks.

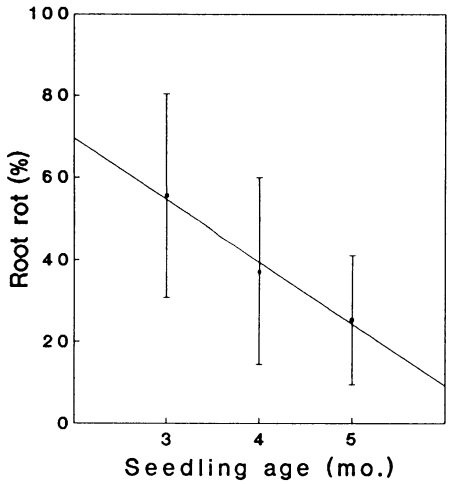

Fig. 3. Relationship between percentage of root rot caused by *Phytophthora parasitica* and seedling age for seven citrus rootstocks. The correlation ($r = -0.70$) is significant at $P < 0.01$. Rootstocks are as in Figs. 1 and 2.

and reproducible method for primary screening of citrus rootstocks for tolerance to root rot was indicated by using young seedlings.

The quantitative ranking of root rot susceptibility separated rootstocks into two general groups. In the tolerant group were trifoliate orange and Swingle citrumelo, and in the susceptible group were sour orange, Carrizo citrange, Cleopatra mandarin, and Ridge Pineapple sweet orange. Volkamer lemon was considered intermediate because it did not differ from rootstocks in both the susceptible and tolerant groups. This classification of these rootstocks is generally in agreement with the results of zoospore inoculation and outplanting tests with notable exceptions (3,9). Carpenter and Furr (3) reported that several trifoliate orange varieties survived poorly and that results with Carrizo citrange were highly variable. Elsewhere, Carrizo citrange was considered moderately tolerant (8–10), yet here it was ranked with sweet orange and Cleopatra mandarin rootstocks with a well-known susceptibility to *Phytophthora* spp. (4,15). Also somewhat surprisingly, sour orange was included within the susceptible group. The field tolerance of Carrizo citrange and sour orange to foot rot (4,15) confirms that there is not always a good correspondence between tolerance to root rot and foot rot resistance (3,6,8,9). However, neither of the two rootstocks that were rated tolerant in this study are susceptible to stem infections. Thus, root rot tolerance may serve as an acceptable indicator of bark resistance in a preliminary screen. This is significant because foot rot tests require larger seedlings for stem inoculations which increases the time and cost of evaluation. Furthermore, foot rot is subject to variability in disease development and rating (13,21).

Previously, Carrizo citrange was used as a standard rootstock of moderate tolerance in comparisons (8–10). Swingle citrumelo, shown to be consistently tolerant of root rot in these tests, should be used as a more rigorous standard for identifying *Phytophthora*-tolerant germ plasm.

The regeneration of fibrous roots by Swingle citrumelo and trifoliate orange at low inoculum densities is perhaps indicative of their mode of tolerance to *Phytophthora* spp. Swingle citrumelo and trifoliate orange are not immune to infection because their roots do support populations of *P. parasitica* in greenhouse and field soils (2; *unpublished data*). These rootstocks apparently have the ability to grow new roots subtending infected root tips. The resistance factors that limit infection to the root tip are not known but may be related to the phytoalexins found in woody tissue of citrus infected with *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian (1,20).

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