A Rapid Method for Evaluating Citrus Seedlings for Resistance to Foot Rot Caused by *Phytophthora citrophthora*

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ABSTRACT

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A method was developed to evaluate the resistance of citrus rootstocks to foot rot disease caused by the fungus *Phytophthora citrophthora*. This method involved inoculating three-month-old branches of seedling rootstocks with an isolate of *P. citrophthora* and measuring the length of lesions that developed four days later. The degree of resistance was determined by comparing the lengths of lesions on seedlings of species of unknown resistance to lengths on seedlings of rootstock species with known resistance. The lengths on seedlings of resistant species were 2.8 mm for *Citrus macrophylla* and 3.2 mm for *Poncirus trifoliata;* lengths on moderately-resistant species were 5.0 mm for *C. aurantium* and 5.2 mm for *P. trifoliata* × *C. sinensis;* lengths on susceptible species were 11.0 mm for both *C. jambhiri* and *C. sinensis*. Thirty-two hybrids (*P. trifoliata* × 'poorman orange') were tested using this method. Of these, 14 hybrids were resistant, eight were moderately resistant, and 10 were susceptible.

Root and foot rot of citrus caused by *Phytophthora* spp. occur worldwide and are among the major causes of loss of production (13,15,16). Using rootstock resistant to Phytophthora foot rot when planting citrus orchards is one of the best ways to protect against this disease. A number of methods to evaluate resistance of citrus rootstocks to *Phytophthora* spp. have been reported. One method was to immerse root systems of citrus seedlings in a concentrated zoospore suspension of *Phytophthora* spp. and plant the seedlings in soil artificially infested with the same fungi. The percentage of seedlings that survive was then used as the criterion for estimating resistance (4,6,10). In a second method, the inoculated seedlings were planted in artificially-infested greenhouse soil and incubated for 4–6 wk at temperatures favorable to the pathogens. The seedlings were removed from the soil, the roots carefully washed, and the percent of decay estimated. This percentage was used in determining susceptibility of the rootstock to the pathogen (7,14). In a third method, intact root systems of two- to three-month-old citrus seedlings were inoculated with a suspension of zoospores and allowed to grow another 2–3 wk. The roots were treated with the vital stain 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) to determine the percentage of living roots. This percentage was then used to

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evaluate seedling resistance to the pathogen (3,12). A fourth method used seedlings at least one year old in which the basal stem tissues had become suberized. A small piece of stem bark was cut longitudinally with a scalpel just above the soil level and inoculum (hyphae, sporangia, and zoospores) was inserted under the bark and held in place with a strip of cheesecloth wrapped around the stem. After 6–8 wk, resistance to *Phytophthora* infection was evaluated according to the severity of symptoms on the stem (7,11,17). A rapid method for determing the pathogenicity and relative virulence of *Phytophthora* spp. in laboratory conditions was also developed in apple trees (2,8,9) and avocado trees (5).

The purpose of this study was to develop a screening method for resistance to Phytophthora foot rot that was faster than existing methods, and in which a large number of citrus seedlings could be screened without causing permanent damage.

MATERIALS AND METHODS

Plant material and fungi. The following three-year-old citrus seedlings from Kibbutz Netzer Syreni Nursery, Israel, were grown individually in 10-L pots: *Citrus sinensis* (L.) Osbeck cv. Shamouti; *C. aurantium* L. (sour orange); *Poncirus trifoliata* Raf. (trifoliate orange); *C. jambhiri* Lush. (rough lemon); *C. macrophylla* Wester (macrophylla); and *P. trifoliata* × *C. sinensis* cv. Troyer (Troyer citrange). These seedlings were used as standard plants in this study because of their known resistances to *P. citrophthora* infection. In an earlier study, trifoliate orange and macrophylla were ranked resistant; sour orange and Troyer citrange were ranked moderately resistant; and rough lemon and Shamouti were ranked susceptible (1).

Thirty-two one-year-old hybrid seedlings from a cross of trifoliate × poorman oranges were obtained from the Department of Fruit Tree Breeding and Genetics, Volcani Center, Israel. The seedlings were seed progeny, not clonal progeny. Their resistances to *P. citrophthora* were unknown, making them suitable for testing in this study. All plants, both standard and hybrid seedlings, were grown in a 25% shade house until one week prior to inoculation with *P. citrophthora.* At that time, they were moved to a greenhouse maintained at 24 ± 2 C.

P. citrophthora isolate C-5 was isolated from the grove at Kibbutz Givat Brener, Israel, in January 1981. This fungus was cultured on potato-dextrose agar (PDA) medium at 25 C to serve as inoculum.

Inoculation. Both branches and stems were inoculated with the fungus. Incisions (3 mm long, 0.2–0.5 mm deep) were made with a sterile scalpel into the bark of branch sections (25–30 cm long and 7–10 mm thick) from three-month-old citrus seedlings. Agar disks (3-mm diameter) were cut from an active PDA culture of *P. citrophthora* and placed over the incisions with the fungus side pressed against the wound. The inoculated branch sections were then incubated in growing chambers at 24 C and 90–95% relative humidity. The advance of the pathogen in the bark from the edge of the incision to the end of one side of the lesion's length were measured four days later. Stems were inoculated in a similar manner at soil level, except that the agar disks were held in place by wet strips of cheesecloth wrapped around each stem and sealed with parafilm to keep the inoculum moist. Seedlings with inoculated stems were incubated at 24 C and lesion lengths were measured 30 days later. Approximately 4–7 branches from each seedling were inoculated and only one inoculation was done on each stem. When we finished testing one group of seedlings, a new group was used for subsequent tests.

Evaluation. Resistance was evaluated by comparing the lengths of lesions on branches of seedlings with a known degree of resistance to *P. citrophthora* Fig. 1) to lesion lengths on hybrid seedlings with an unknown degree of resistance. Experiments were completely randomized in design and were repeated four times. Inoculations of the standard seedlings used five replicates, and inoculations of the hybrids used ten replicates. Similar results were obtained when experiments were repeated.

RESULTS AND DISCUSSION

On the standard seedlings, lesions developed on branches sooner than they did on stems. By the fourth day after inoculation, the lengths of lesions on branches were comparable to lengths of those found on stems 30 days after inoculation (Table 1). The average lengths of branch lesions were similar and were found to be correlated (r^2 = 0.910, $P < 0.01$).

No significant differences in virulence were found between isolate C-5 of *P. citrophthora* and 10 other isolates tested. This differs from the results of Jeffers and Aldwinckle (8) who found significant differences in virulence between isolates of *Phytophthora* spp. tested in apple rootstocks. In our study, we distinguished three groups differing in degree of susceptibility: rough lemon and Shamouti (susceptible); sour orange and Troyer citrange (moderately resistant); and macrophylla and trifoliate orange (resistant) (1).

In a screening program for superior rootstocks, the Department of Fruit Tree Breeding and Genetics, Agricultural Research Organization, Bet Dagan, developed 32 hybrids of trifoliate orange × poorman orange. We evaluated resistance of these hybrids to *P. citrophthora* by inoculating branches of year-old seedlings and assessing their degree of

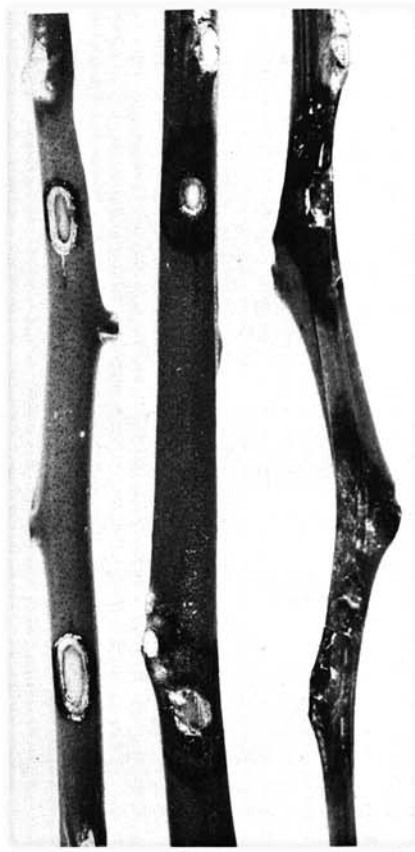

Fig. 1. Lesion lengths in three-month-old branches of three citrus species (from left to right: *Citrus macrophylla*, resistant; *C. aurantium*, moderately resistant; *C. sinensis*, susceptible) four days after inoculation with *Phytophthora citrophthora* C-5.

Table 1. Lesion lengths (mm) on three-month-old branches and three-year-old stems of citrus species inoculated with *Phytophthora citrophthora* 4 days and 30 days after inoculation, respectively

Species	Branches	Stems
Citrus jambhiri	11.0[y] a[z]	9.0[y]a[z]
C. sinensis	11.0 a	13.6 a
C. aurantium	5.0 b	4.0 b
Poncirus trifoliata × *C. sinensis*	5.2 b	3.4 b
C. macrophylla	2.8 c	1.0 c
P. trifoliata	3.2 c	1.2 c

[y] Each value is the mean of five replicates.
[z] Values followed by the same letter are significantly different according to Duncan's multiple range test ($P = 0.05$).

Table 2. Degree of resistance of 32 citrus hybrids (*Poncirus trifoliata* × poorman orange) determined by four-day-old lesion lengths on three-month-old branches inoculated with *Phytophthora citrophthora*

Hybrid number	Lesion length[y] (mm)	Range[z] (mm)	Degree of resistance
1	11.3	9–12	Susceptible
2	1.7	1–3	Resistant
3	1.4	1–2	Resistant
4	1.4	1–2	Resistant
5	10.0	8–15	Susceptible
6	8.3	7–10	Susceptible
7	4.4	3–6	Moderately resistant
8	2.4	2–4	Resistant
9	5.4	3–6	Moderately resistant
10	8.0	6–12	Susceptible
11	4.7	4–6	Moderately resistant
12	9.2	6–13	Susceptible
13	7.9	6–10	Susceptible
14	1.9	1–3	Resistant
15	4.2	3–6	Moderately resistant
16	4.0	2–6	Moderately resistant
17	1.6	1–3	Resistant
18	1.5	1–3	Resistant
19	2.2	1–3	Resistant
20	5.0	3–7	Moderately resistant
21	1.5	1–2	Resistant
22	1.6	1–2	Resistant
23	1.9	1–3	Resistant
24	4.2	3–6	Moderately resistant
25	8.5	7–10	Susceptible
26	2.1	1–3	Resistant
27	8.2	5–10	Susceptible
28	4.4	2–7	Moderately resistant
29	7.1	6–9	Susceptible
30	2.0	1–3	Resistant
31	7.2	5–9	Susceptible
32	2.0	1–3	Resistant

[y] Each value is an average of 10 replicates.
[z] Range between the maximum and minimum lesion lengths of the replicates of each hybrid.

resistance by comparing the lengths of their lesions to the lengths of those found on known resistant seedlings (Table 1). From this evaluation, we ranked 14 hybrids as resistant, eight hybrids as moderately resistant, and 10 hybrids as susceptible (Table 2).

Similar screening tests for evaluating resistance to other *Phytophthora* spp. in woody host plant species have been developed by other researchers. Dolan and Coffey (5) developed a laboratory screening technique for assessing the resistance of avocado rootstocks to *P. cinnamomi.* Borecki and Millikan (2), Jeffers and Aldwinckle (8), and Jeffers et al (9) developed laboratory screening techniques for assessing the resistance of apple rootstocks to *Phytophthora* spp. Their techniques, and the technique described in this study, make it possible to screen a large number of rootstocks under laboratory conditions within a relatively short time period.

Our screening method is inexpensive, fast, and easy to use. Although its scale for ranking susceptibility is arbitrary, the method can indicate resistant candidates in a screening program with a high degree of confidence. Our method is appropriate only for testing resistance of rootstocks against foot rot caused by *P. citrophthora,* however. We recommend developing a different standard numeric scale for each different fungus or pathogen isolate of *P. citrophthora* to be tested.

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