Comparison of Rapid Methods for Evaluating Resistance to *Phytophthora cinnamomi* in Avocado Rootstocks

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ABSTRACT

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The clonally propagated avocado rootstock selections Thomas, Duke 7, Barr Duke, and Topa Topa (*Persea americana*); UCR 2007, UCR 2008, and UCR 2053 (*P. schiedeana*); Martin Grande (G755a, b, and c), UCR 2022, and UCR 2023 (*P. americana* × *P. schiedeana*); and UCR 2066 (*P. steyermarkii*) were evaluated for resistance to *Phytophthora cinnamomi* in excised etiolated shoots, excised roots, and intact root systems grown in vermiculite. Resistance reactions were similar in etiolated shoots and intact root systems with the *P. schiedeana, P. americana* × *P. schiedeana*, and *P. steyermarkii* selections. With the *P. americana* selections, in contrast, the etiolated shoot and intact root system reactions differed. Screening of excised avocado roots for differences in electrolyte leakage gave variable results and did not differentiate among some resistant and susceptible rootstocks. In general, screening of intact root systems grown in vermiculite gave the best results, with comparative resistance responses similar to those determined under field conditions.

Phytophthora cinnamomi Rands, the causal agent of Phytophthora root rot (PRR), is a serious worldwide problem on avocados (18). At present, about 60–75% of the acreage in California is infected (3). This is an especially serious problem because the bulk of the trees under production are grafted onto seedlings that are highly susceptible to PRR. Currently, the use of clonally propagated, moderately resistant rootstocks represents an important component of the integrated approach to control of PRR (3,18). Screening for resistance to PRR involves the collection and testing of seed populations from avocados in Central America and California. This is a lengthy procedure that initially involves growing seedlings in vermiculite for 6–8 wk. Their root systems are then flooded with zoospores of *P. cinnamomi*. After another 8 wk, roots are assessed for resistance to *P. cinnamomi*. Those seedlings showing the least PRR are potted in soil infested with *P. cinnamomi* and allowed to develop in the greenhouse until enough budwood can be obtained for initial increases. Then, clonally propagated cuttings grafted with commercial scions can be tested in the field over a period of 5–10 yr.

The search for resistant rootstocks initially focused on the Mexican horticultural race of *Persea americana* Miller because that race was best adapted to the soils and climate of California. Duke 7, Barr Duke, and Thomas are selections of this horticultural race that have shown moderate resistance to PRR (3,18). The identification of useful resistance in *P. americana* led to an expanded search for superior resistance in other closely related *Persea* species. This resulted in the discovery of such resistance in three naturally occurring *P. americana* × *P. schiedeana* Nees hybrids: G755a, G755b, and G755c (6), collectively referred to as Martin Grande (3,18). More recently, similar levels of resistance have been tentatively identified within *P. schiedeana* (3) in the UCR 2007, UCR 2008, and UCR 2053 selections. Some resistance also has been provisionally identified within *P. steyermarkii* Allen (19). With the large amount of material now being recovered from initial screening, it becomes essential that rapid techniques be developed that will provide additional information on the resistance potential of individual selections, prior to any decision regarding their field evaluation. Dolan and Coffey (5) reported on the evaluation of four rootstock selections, using a laboratory screening technique for detecting resistance that utilizes the etiolated shoots of clonally propagated budwood. More recently, Zilberstein and Pinkas (20) described a detached root technique in which the amount of electrolyte leakage from infected detached avocado roots was used for evaluating resistance to PRR. In both studies, only a few resistant rootstocks were utilized. To further evaluate the usefulness of such rapid screening techniques, a larger number and broader range of resistant germ lines need to be compared. In the present study, we used quantitative methods to compare resistance in etiolated shoot tissue, detached roots, and intact root systems of 12 rootstocks currently available in our research program.

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MATERIALS AND METHODS

Plant propagation and inoculum production. Twelve rootstock selections—*P. americana* Thomas, Duke 7, and Barr Duke; *P. schiedeana* UCR 2007, UCR 2008, and UCR 2053; *P. americana* × *P. schiedeana* hybrids G755a, G755b, and G755c (6) (collectively referred to as Martin Grande) and UCR 2022 and UCR 2023 (Martin Grande seedlings); and *P. steyermarkii* UCR 2066—were propagated vegetatively (5,7). Clonally propagated Topa Topa also was included as a *Phytophthora*-susceptible control. Isolate P2428 of *P. cinnamomi* was used in all the experiments. Zoospores were used as inoculum and were produced as described previously (5).

Intact root system screening. Avocado rootstock selections were rooted in vermiculite in cylindrical plastic containers (30 cm long and 10 cm in diameter) until a well-developed root system was visible. Rootstocks were then inoculated with 250 ml of an aqueous suspension of *P. cinnamomi* zoospores containing approximately 6×10^5 zoospores. Fourteen days after inoculation, the shoots of the rootstocks were removed and the root system washed free of vermiculite. Root infection was assessed by determining the percentage of healthy roots, both visually and by means of a line intersect technique (14). The latter technique involved randomly selecting a sample of the diced root system, spreading it over a grid, and counting the number of line intersects for both the healthy and the necrotic roots. Percent healthy roots was calculated by dividing the number of line intersects for the healthy roots by the total present. Also, percent recovery of *P. cinnamomi* was determined by cutting the entire root system into 1-cm segments, of which 30 randomly selected segments were plated on the *Phytophthora*-selective medium

PARPH (12,13). The experiment was conducted twice, with seven replicates per selection.

Etiolated shoot screening. Lengths (7 cm) of etiolated shoots were placed in 10-cm petri dishes (5). Shoots were inoculated in the center of each segment with a 10-μl droplet containing 1×10^3 zoospores and incubated at 24 C in the dark for 72 hr before evaluation. Development of *P. cinnamomi* was determined by measurements of the lesion length. Lesion severity was rated on a scale of 1–5, where 1 = no lesion, 2 = lesion restricted to inoculation point, 3 = brown expanding lesion < 1 mm across, 4 = brown lesion girdling stem, and 5 = complete necrosis. Also, percent recovery of *P. cinnamomi* was determined by plating 20 etiolated shoot sections, 3 mm thick and cut from both above and below the inoculation point, on the *Phytophthora*-selective medium PARP (5,12). The plates were incubated at 24 C for 72 hr, then scored for the percentage of segments yielding colonies of *P. cinnamomi*. The experiment was conducted three times, with 10 replicates per selection.

Detached root screening. The relative electrolyte leakage of detached roots infected with *P. cinnamomi* compared with similar noninfected detached roots was determined by measuring the electrical conductivity of the bathing solutions. Detached juvenile root tips (7 cm long and approximately 3 mm in diameter) were selected and their cut surfaces sealed with silicon grease. The roots were placed in 10-cm-diameter petri dishes on a slant and with tips suspended in 5 ml of distilled water. These were then inoculated by adding a 1-ml suspension of 1% filtered soil extract containing 5×10^3 zoospores. Noninfected controls were inoculated with a 1-ml suspension without zoospores. After 4 hr, the suspensions were removed and the roots resuspended in 25 ml of distilled water. The electrical conductivity of the bathing solution was determined with a conductivity meter (Cole Parmer 1484-44, Chicago, IL) at 76, 100, and 124 hr after inoculation. The experiment was conducted twice with five replicates per selection.

RESULTS

An analysis of variance and Duncan's new multiple range test were used to determine significant differences among rootstock means ($P = 0.05$).

Intact root system screening. Values for percent healthy roots for UCR 2022 and UCR 2023, assessed both visually and by the line intersect method, were less than or equal to that of the susceptible Topa Topa at 14 days after inoculation. In contrast, percent healthy roots for UCR 2008, UCR 2007, UCR 2053, Thomas, Barr Duke, Duke 7, and UCR 2066 were equal to or greater than those for the moderately resistant selections G755a, G755b, and G755c (Table 1). Percent recovery of *P. cinnamomi* from roots of UCR 2022 and UCR 2023, although significantly less than that from roots of Topa Topa, was significantly greater than that of the other rootstocks (Table 1).

Etiolated shoot screening. Percent recovery of *P. cinnamomi* from shoot sections, lesion severity ratings, and lesion length measurements for Duke 7, Barr Duke, Thomas, UCR 2022, and UCR 2023 were equal to or greater than those for the susceptible Topa Topa (Table 2). The same measurements for UCR 2066, UCR 2007, UCR 2053, and UCR 2008 were equal to or less than those of the moderately resistant selections G755a, G755b, and G755c, with the exception of the lesion severity rating for UCR 2066, which fell between those of Topa Topa and Martin Grande (Table 2).

Detached root screening. The bathing solution electrical conductivity of infected UCR 2008, UCR 2066, Duke 7, and Barr Duke was significantly lower than that of susceptible Topa Topa at 76, 100, and 124 hr after inoculation (Table 3). In contrast, the electrolyte leakage from detached roots of Thomas, UCR 2023, and Martin Grande (G755c) was not significantly different from that of Topa Topa at 76 and 100 hr after inoculation (Table 3). Thomas differed from Topa Topa at 124 hr after inoculation but was not significantly different from the other resistant rootstocks studied. Lesions were formed at the inoculation point on all infected detached roots, and development was similar to the amount of electrolyte leakage. Generally, there was little or no electrolyte leakage from noninfected roots.

Table 1. Assessment of resistance to *Phytophthora cinnamomi* in 13 avocado rootstocks grown in vermiculite, 2 wk after inoculation with a 250-ml zoospore suspension (6×10^5 zoospores per rootstock)

Rootstock selection	Percent healthy roots[w]		Percent recovery[x]
	Visual rating	Line intersect method	
UCR 2008	90 a[y]	92 a	10 e
UCR 2007	90 a	79 bc	26 c
G755b	89 a	79 bc	18 cde
UCR 2053	88 a	87 ab	11 de
G755a	87 ab	82 abc	20 cde
Thomas	86 abc	79 bc	23 cd
G755c	79 bcd	78 bc	17 cde
Barr Duke	77 cd	78 bc	24 c
Duke 7	73 d	79 bc	25 c
UCR 2066	73 d	76 c	15 cde
Topa Topa[z]	56 e	51 d	58 a
UCR 2023	45 f	46 d	43 b
UCR 2022	43 f	48 d	37 b

[w] Visual rating was based on the entire root system. With the line intersect method, a random sample from the diced root system was placed on a grid and the number of line intersects for the healthy and necrotic roots counted. Percent healthy roots was determined as: (healthy root line intersects/total root line intersects) × 100.

[x] Percent recovery of *P. cinnamomi* from 30 root segments randomly selected from the entire root system and plated on the *Phytophthora*-selective medium PARPH.

[y] Means within a column followed by a different letter are significantly different according to Duncan's new multiple range test ($P = 0.05$).

[z] *Phytophthora*-susceptible control.

Table 2. Assessment of resistance to *Phytophthora cinnamomi* in detached etiolated shoots of 13 avocado rootstocks, 72 hr after point-inoculation with zoospores

Rootstock selection	Percent recovery[w]	Lesion severity[x]	Lesion length (mm)
UCR 2023	91 a[y]	4.5 a	54 a
Duke 7	81 a	4.1 b	53 a
Topa Topa[z]	67 b	3.5 c	37 b
Barr Duke	60 bc	3.2 cd	33 b
Thomas	58 bc	3.3 c	34 b
UCR 2022	53 bc	3.3 c	30 b
UCR 2066	46 cd	2.8 d	21 c
G755a	35 de	2.3 e	13 cd
G755b	33 de	2.2 e	8 de
UCR 2007	22 ef	1.6 f	2 e
G755c	12 f	1.4 f	2 e
UCR 2053	9 f	1.6 f	1 e
UCR 2008	8 f	1.7 f	2 e

[w] *P. cinnamomi* recovered from 20 etiolated shoot segments, 3 mm thick and cut from both above and below the inoculation point, plated on the *Phytophthora*-selective medium PARP.

[x] Rated on a scale where 1 = no lesion, 2 = lesion restricted to inoculation point, 3 = brown expanding lesion < 1 mm across, 4 = brown lesion girdling shoot, 5 = complete necrosis.

[y] Means within a column followed by a different letter are significantly different according to Duncan's new multiple range test ($P = 0.05$).

[z] *Phytophthora*-susceptible control.

Table 3. Comparison of electrolyte leakage in detached roots of nine avocado rootstocks inoculated with zoospores of *Phytophthora cinnamomi*

Rootstock selection	Postinoculation electrical conductivity[x] (μS/g fresh weight of root)		
	76 hr	100 hr	124 hr
Topa Topa[y]	168 a[z]	234 a	305 a
G755c	148 ab	218 ab	260 ab
UCR 2023	116 abc	175 abc	213 abc
Thomas	107 abc	136 abc	157 bc
UCR 2022	82 bc	151 abc	193 abc
UCR 2008	73 bc	107 c	140 c
UCR 2066	58 c	93 c	132 c
Duke 7	57 c	105 c	159 bc
Barr Duke	56 c	113 bc	139 c

[x] Roots were resuspended in 25 ml of distilled water 4 hr after inoculation, and electrical conductivity of this solution was measured.

[y] *Phytophthora*-susceptible control.

[z] Means within a column followed by a different letter are significantly different according to Duncan's new multiple range test ($P = 0.05$).

DISCUSSION

The reactions expressed in the intact root systems of Topa Topa, Thomas, Duke 7, Barr Duke, Martin Grande, and UCR 2023 were similar to results obtained in previous field and greenhouse experiments (3,8,9,18). On the basis of results obtained with intact root systems, the avocado rootstocks Thomas, Martin Grande, Barr Duke, and Duke 7 possess moderate resistance to PRR, as previously described (3,8,9,18). In addition, the *P. schiedeana* rootstocks UCR 2007, UCR 2008, and UCR 2053 and the *P. steyermarkii* rootstock UCR 2066 also expressed moderate resistance to PRR. In contrast, rootstocks propagated from two seedlings of Martin Grande, UCR 2022, and UCR 2023 were found to be almost as susceptible as Topa Topa.

The reaction expressed in the etiolated shoots was similar to that expressed in the intact root systems of the *P. schiedeana* rootstocks UCR 2007, UCR 2008, and UCR 2053 and the *P. americana* × *P. schiedeana* hybrid rootstocks Martin Grande, UCR 2022, and UCR 2023. Thus, the inoculation of etiolated shoots may provide a convenient and rapid screening method for detecting resistance to *P. cinnamomi* in *P. schiedeana* and *P. americana* × *P. schiedeana* germ plasm. This is supported by previous work (5) in which similar reactions were expressed in the etiolated shoots and the intact root systems of the *P. americana* × *P. schiedeana* selection G755c. Screening of etiolated shoots for resistance to *P. cinnamomi* may also prove to be useful for *P. steyermarkii* selections, since UCR 2066 showed a similar host reaction in both etiolated shoots and the intact root system. More selections of *P. steyermarkii* will have to be tested to confirm this, however.

Dixon et al (4) and McCredie et al (15) also found stem inoculations with *P. cinnamomi* to be useful in screening *Banksia* species for resistance to PRR. Similarly, Smith and Marks (16) were able to effectively use stem inoculations with *P. cinnamomi* to distinguish between water-stressed and unstressed *Eucalyptus sieberi* L. A. S. Johnson. Also, Afek et al (1) have used the inoculation of branches and shoots for assessing resistance to *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian in citrus rootstocks. Also, Jeffers et al (11) found an excised twig assay to be useful in determining pathogenicity of different species of pythiaceous fungi to different apple scion and rootstock selections.

In contrast, the reaction expressed in the etiolated shoots of the *P. americana* selections Thomas, Barr Duke, and Duke 7 was different from that of their intact root systems. Thomas, Barr Duke, and Duke 7 are known to have a moderate level of field resistance to PRR (3,8,9,18), but the results of etiolated shoot screening actually suggested that they were susceptible. Consequently, etiolated shoot tissue would appear unlikely to provide a useful screening tissue for *P. americana* selections.

Previous reports (2,10,20) have shown that measuring electrolyte leakage of infected host tissue may provide an estimation of the level of host resistance. We found that the detached root screening technique separated the moderately resistant rootstock Duke 7 from the susceptible Topa Topa as previously described (20). However, we were not always able to separate the moderately resistant rootstocks Martin Grande and Thomas from susceptible Topa Topa. In line with this apparent discrepancy, it has been reported that resistant *Acacia melanoxylon* R. Br. had a higher electrolyte leakage than susceptible *Xanthorrhoea australis* R. Br. after inoculation with *P. cinnamomi* (17).

We found screening the intact root systems gave results similar to those found under field conditions, which suggests this might provide the most useful short-term method of assessing resistance to *P. cinnamomi.*

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