Laboratory Screening Technique for Assessing Resistance of Four Avocado Rootstocks to *Phytophthora cinnamomi*

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ABSTRACT

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Vegetatively propagated avocado rootstock selections G755c (G755), G1033, G6, and Duke 7 (D7) were screened in a laboratory procedure by point-inoculating 8-cm sections removed from the tips of young, etiolated shoots with about 1,000 zoospores of *Phytophthora cinnamomi*. After 3 days of incubation at 24 C in the light, each shoot section was evaluated for lesion length, lesion severity, and percent recovery of the pathogen. To determine if a correlation existed between shoot tissue and root tissue behavior, juvenile roots generated from etiolated shoot tissue of each rootstock selection were tested, both attached and detached, for resistance to *P. cinnamomi* by exposing them to zoospore suspensions. Selection G755 showed the highest level of resistance to *P. cinnamomi* in the etiolated shoot test, and this resistance was mirrored in root tissue behavior. Selection G1033, though more susceptible than G755, showed levels of shoot and root tissue resistance that were superior to those of either G6 or D7. The relative resistance of these rootstock selections to *P. cinnamomi* in the etiolated tissue test paralleled their performance in both greenhouse experiments and field trials. Use of etiolated avocado stem tissue shows promise for selecting resistant rootstocks.

Root rot of *Persea americana* Mill. caused by *Phytophthora cinnamomi* Rands was responsible in 1969 for crop losses of 20–25% in the major avocado production areas of southern California (8,13). Our research program is principally concerned with the development of an integrated approach to disease control (1) involving a combination of nursery practice, cultural practice, chemical control (3), biological control, and clonal rootstocks (2). Selection of clonal rootstocks possessing field resistance to *P. cinnamomi* is an integral part of an economically feasible control strategy for root rot. During 1984, 89 collections of *P. americana* and *P. schiedeana* from Central America were made as part of an ongoing program to gather new germ plasm sources with resistance to *P. cinnamomi*. No rapid and reliable method exists for screening large numbers of new selections for root rot resistance, however, and limited resources prevent us from evaluating all rootstocks in field trials. It is therefore necessary to develop a more economical means of selecting the most promising rootstocks from our extensive germ plasm collections.

Vegetative clonal propagation of avocado rootstocks uses a method involving grafted stem cuttings that are etiolated to promote root generation (4). The value of etiolated tissue as a tool for investigating host-pathogen relationships has been demonstrated with soybean hypocotyls (11,12) and maize mesocotyls (5), and we felt that the use of etiolated avocado tissue for examining resistance components should be explored. We initially chose to determine if resistance to *P. cinnamomi* would be expressed in etiolated stem tissue and if roots generated from such resistant stem tissue would likewise resist infection. If a positive correlation existed between stem and root tissue resistance, then promising rootstocks could be preselected for subsequent field trials.

A laboratory technique using *P. cinnamomi* zoospore inoculations was developed to determine the value of etiolated tissue for predicting rootstock resistance. Excised pieces of etiolated stems were point-inoculated with zoospore droplets, and the development of the pathogen in the tissue was measured by lesion length, lesion severity, and recovery of the pathogen from infected tissue. Comparisons were made between the performance of etiolated tissue and root tissue inoculated with zoospore suspensions.

MATERIALS AND METHODS

Plant material. Three selections of *Persea americana* (designated G6, Duke 7 [D7], and G1033) and one selection of *P. schiedeana* Nees (G755c [G755]) were vegetatively propagated using an etiolation technique (4). Budwood from each selection was tip-grafted to seedlings of *P. americana* cv. Topa Topa. The grafted shoot was covered with a small polyethylene bag to maintain humidity, and the plant was moved to the greenhouse until the union was well established, which usually took about 7 days. When the scion showed bud growth, the plant was placed in a dark chamber to promote shoot etiolation. The chamber was maintained under conditions of high humidity at 24 C. Within 3–5 wk, etiolated shoots grew to 20 cm and were excised for in vitro screening.

Root production was induced from the bases of etiolated stems. After etiolation, a stainless steel girdling ring was applied to the base of the stem just above the graft union. A solution of rooting hormone was sprayed on the base of the etiolated stem, a collar of opaque material was placed around the stem, and the collar was filled with vermiculite. The shoot tip remained exposed to light. The collar was composed of two concentric cylinders, an outer one of opaque plastic to exclude light and an inner one of clear acetate. This arrangement permitted periodic observations of root development by

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removing the opaque collar. Juvenile roots produced in this manner were ready for screening 2 mo after sleeving.

Pathogen isolate. *P. cinnamomi* culture Pc444, from the *Phytophthora* collection at UCR, was used in all experiments. Sporangia were produced in unsterile soil extract (SE) prepared by mixing 10 g (fresh weight) of soil with 1 L of demineralized water, which was allowed to stand overnight and then filtered through Whatman No. 1 paper. Disks taken from margins of cultures on cleared V8-$CaCO_3$ agar were placed in 10-cm-diameter petri dishes containing 1/10-strength cleared V8-$CaCO_3$ broth and incubated in the dark at 24 C for 24 hr. After incubation, mycelial mats were washed three times with sterile demineralized water and incubated in SE in the dark at 24 C for 6 days. Sporangium release was initiated by washing the mats in distilled water three times and chilling them at 4 C for 20 min. Zoospore viability was measured by germination on 1.5% water agar and varied from 40 to 60%.

In vitro screening of etiolated shoots. Laboratory screening was conducted on excised pieces of etiolated shoot tissue. Plants were removed from etiolation, the shoot was excised from the nurse seed, and the cut end of the shoot was dipped in silicon vacuum grease. Groups of shoots were sealed in plastic bags and returned to the laboratory, where the tip 8-cm section was excised from each shoot, the cut ends of the sections were sealed with vacuum grease, and the shoot sections were placed in a 15-cm-diameter glass petri dish containing a 13-cm-diameter disk of No. 1 Whatman paper saturated with distilled water. Zoospore inoculum, as a 10-μl drop containing about 1,000 zoospores, was applied to the center of each shoot section. The sections were incubated at about 24 C on a bench top in the light for 3 days before evaluation.

Pathogen development in the tissues was evaluated by direct lesion measurement and by plating stem sections on selective media to determine percent recovery of pathogen. Lesion severity was also determined 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. After lesion length and severity were recorded, alternate 2-mm-thick pieces were excised 28 mm above and below the inoculation point and plated onto PARP medium (6), modified by substituting 125 μg/ml of ampicillin trihydrate for 250 μg/ml of sodium ampicillin. Plates were incubated for 3 days at room temperature and were scored for the percentage of segments yielding colonies. Values for lesion length, lesion severity, and percent recovery represent the mean of 10 replicates per selection. The experiment was repeated twice and the data were combined.

Root tissue challenge. Juvenile roots generated directly from etiolated shoots were screened in situ by dipping the root system of a plant in a zoospore suspension. A rooted shoot was removed from the collar, excised from the nurse seed, and the root system washed free of vermiculite. The bare-rooted shoot was placed in a 1-L plastic cup containing 800 ml of zoospore suspension containing about 100 zoospores per milliliter of distilled water. After 24 hr of incubation at room temperature, the zoospore suspension was replaced with distilled water after rinsing the cup with a stream of demineralized water. Plants were incubated at about 24 C for 2 days. Five-centimeter root-tip sections were removed from the plants and 15 were chosen at random for evaluation. Root-tip lesions, if present, were measured and all root tips were plated on PARP to determine percent infection. Root pieces with lesions were also evaluated for lesion severity on a scale of 1–4, where 1 = no lesion, 2 = brown lesion restricted to root tip, 3 = brown expanding lesion, and 4 = complete root necrosis. The data represent the mean of five plants per selection, repeated once.

Resistance of roots of five plants of each selection was assessed in vivo by drenching vermiculite-filled collars of rooted shoots with zoospore suspensions. The vermiculite was watered to saturation and 25 ml of a zoospore suspension was washed into it with 100 ml of distilled water. Three zoospore concentrations were used: 1×10^5, 5×10^5, and 1.5×10^6 per milliliter. The plants were incubated in the greenhouse for 7 days, all root tips were excised, and 40 root tips were chosen at random for plating on PARP medium to determine percent infection.

The performance of excised roots was also evaluated. Roots about 1 and 3 mm in diameter were excised, trimmed to 5 cm long, and the cut ends inserted in 0.8% water agar that had been poured into covers of 100-mm plastic petri dishes. The covers were inverted over crystalizing dishes containing 250 ml of zoospore suspension containing 100 zoospores per milliliter of distilled water. About 1 cm of the root tip was immersed in the suspensions. After 2 days of incubation at room temperature, the length of the lesions was measured and the roots were plated on PARP medium for percent recovery of *P. cinnamomi*. Ten roots of each diameter for each selection were tested. The experiment was repeated once.

Table 1. Resistance to *Phytophthora cinnamomi* of detached etiolated tissue segments produced during vegetative clonal propagation of avocado rootstock selections[x]

Selection	Mean lesion length	Lesion severity index[y]	Percent recovery of pathogen
G755	2.3 a[z]	1.6 a	10.8 a
G1033	5.9 a	2.4 b	28.3 b
G6	26.2 b	4.0 c	60.0 c
D7	27.8 b	4.1 c	89.7 d

[x] Ten 8-cm-long tip segments were excised from etiolated shoots, point-inoculated with a 10-μl drop of zoospores, and incubated for 3 days before assessment.
[y] Lesion severity index of 1–5: 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.
[z] Different letters in each column indicate a significant difference ($P = 0.05$) according to Duncan's new multiple range test.

Table 2. Resistance in situ to *Phytophthora cinnamomi* of juvenile roots generated from etiolated tissue during vegetative clonal propagation of avocado rootstock selections[x]

Selection	Mean lesion length	Percent roots infected	Lesion severity index[y]
G755	1.2 a[z]	53.8 a	1.7 a
G1033	5.8 b	65.4 a	2.2 b
G6	6.5 bc	96.2 b	3.2 c
D7	8.7 c	92.3 b	2.8 c

[x] Bare-rooted shoots were dipped in zoospore suspensions and incubated for 2 days at about 24 C.
[y] Lesion severity index of 1–4: 1 = no lesion, 2 = brown lesion restricted to root tip, 3 = brown expanding lesion, and 4 = complete root necrosis.
[z] Different letters in each column indicate a significant difference ($P = 0.05$) according to Duncan's new multiple range test.

RESULTS

Etiolated tissue inoculation. Mean lesion lengths in etiolated tissue of G755 and G1033 were significantly less than those recorded for G6 or D7 at 72 hr after inoculation (Table 1). Lesions were least severe in G755 and were typically restricted to the point of zoospore inoculation. Lesions in G1033 were characterized by slight tissue browning that extended several millimeters beyond the point of inoculation, but further lesion expansion was arrested within 48 hr. Lesions in G6 and D7 girdled the shoot section and continued to expand throughout the experiment. The lower

percentage of shoot sections yielding fungal colonies after plating on selective medium reflected the smaller lesions observed for G755 and G1033. Colonies could be recovered from all tissues showing lesions. Hyphal activity was usually detected less than 4 mm in advance of the lesion front.

Root tissue inoculation. Gross root system morphology differed among vegetatively propagated selections. Juvenile roots of G755, G6, and G1033 were succulent and less dissected than the fibrous D7 root system. Comparisons of root performance were therefore limited to roots of similar diameters, usually less than 3 mm.

Of the four selections tested by in situ root dipping (Table 2), G755 showed the highest degree of resistance to *P. cinnamomi* infection as measured by lesion length and severity. Lesions in G755 roots were usually restricted to the root tip only. Root lesion lengths of G1033 and G6 could not be significantly differentiated; however, a lower percentage of G1033 roots was infected and infected roots were less severely diseased than those of G6 or D7. The percentage of G755 and G1033 roots infected with *P. cinnamomi* did not differ significantly.

A similar pattern of infection was observed in root drench experiments (Fig. 1). Selection G755 showed very low levels of root infection by *P. cinnamomi* at inoculum densities of 1×10^5 and 5×10^5 zoospores per plant. Selection G1033 yielded a higher percentage of infected roots after challenge than G755 but outperformed D7 at the lower inoculum densities. At the highest level of zoospore inoculum, G1033 and D7 did not differ significantly in performance, whereas G755 performed slightly better.

Lesions developed on all excised roots of every selection with both root sample diameters (Table 3). Lesions developed at the root/water-surface interface of all selections by 24 hr after incubation in the zoospore suspensions. However, 1-mm-diameter roots of G755 performed significantly better than the other selections, and 3-mm-diameter root pieces appeared very resistant to infection produced by this method of inoculation.

DISCUSSION

Necrotic lesions developed within 12 hr of inoculation of *P. cinnamomi* zoospores on etiolated shoots of G6 and D7, and these lesions continued to expand, eventually causing necrosis of entire stem sections. On shoots of G1033, lesions showed only limited expansion that ceased within 48 hr. Lesions on etiolated shoots of G755 were restricted to the point of zoospore inoculation. Restricted lesions on G755 were characterized by slight tissue browning under the area covered by the inoculum droplet. Under conditions of the laboratory screen, the behavior of etiolated shoots could be divided into two groups: G6 and D7, which responded in a susceptible manner to infection, and G755 and G1033, which showed significant resistance to fungal invasion.

The use of etiolated stems for predicting rootstock resistance to *P. cinnamomi* is based on the premise that root tissue is generated directly from etiolated tissue during vegetative propagation and may therefore show a similar response to invasion by *P. cinnamomi*. We have clearly demonstrated that G755 showed the highest level of resistance in both etiolated tissue and root tissue screening and that G6 and D7 performed in a consistently susceptible manner. Selection G1033, though not performing quite as well as G755 in stem or root tests, was far superior to both G6 and D7 in its resistance. Challenging juvenile tissues, either stem or root, with high levels of zoospore inoculum constitutes a severe test for resistance to *P. cinnamomi*. A rootstock with field tolerance, based on other attributes such as high root growth potential (7), would be overlooked in such a test. Consequently, laboratory results based on this etiolation test should be interpreted in conjunction with the behavior of rootstocks growing in soil infested with *P. cinnamomi* under both greenhouse and field conditions.

Results from greenhouse studies using container-grown D7 and G6 rootstocks in naturally infested soil have demonstrated that both rootstocks support high populations of *P. cinnamomi* (7). Our results with shoot and root tissue verify the susceptibility of these rootstocks to infection by *P. cinnamomi*. Kellam and Coffey (7) suggested that the high root rot tolerance observed in D7 during field trials was more likely due to high root regeneration capacities of the rootstock than to inherent resistance of D7 root tissue to infection. Preliminary data from greenhouse container experiments with G755 and G1033 (M. K. Kellam and M. D. Coffey, *unpublished*) show that these rootstocks, compared with G6 and D7, support significantly lower populations

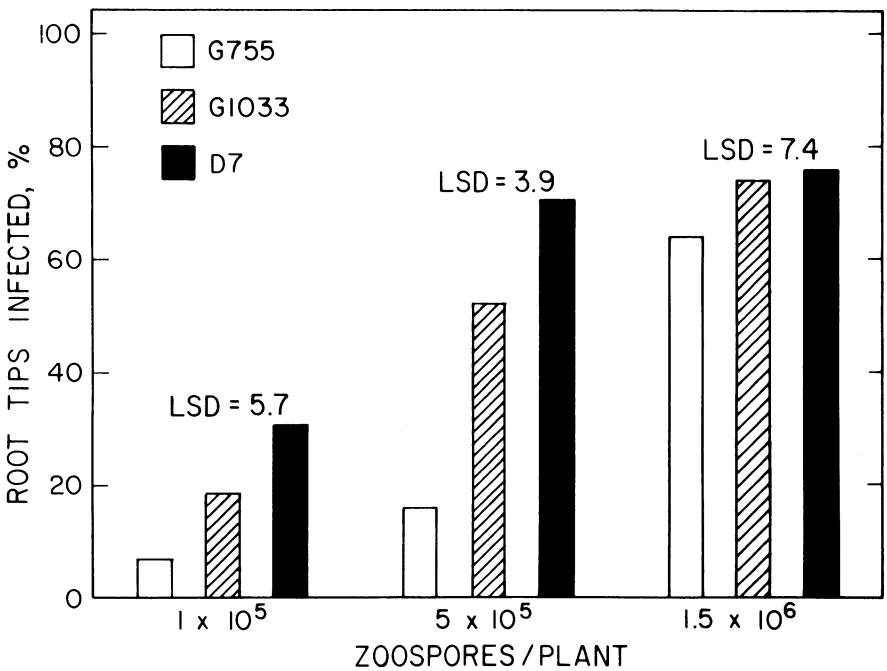

Fig. 1. Percent infection 7 days after three avocado rootstocks were drenched with zoospores of *Phytophthora cinnamomi*. Plants were exposed to three zoospore concentrations.

Table 3. Resistance to *Phytophthora cinnamomi* of excised roots generated from etiolated tissue during vegetative clonal propagation of avocado rootstock selections[y]

Selection	Mean lesion lengths (mm)	
	Root segments 1 mm in diameter	Root segments 3 mm in diameter
G755	14.4 a[z]	3.1 a
G1033	19.3 b	8.5 b
G6	19.2 b	19.5 b
D7	19.0 b	19.7 b

[y] Excised root segments 5 cm long were suspended in zoospore suspensions so that about 1 cm of the root tip was exposed to zoospore infection.

[z] Different letters in each column indicate a significant difference ($P = 0.05$) according to Duncan's new multiple range test.

of *P. cinnamomi* and that significantly fewer feeder roots yield pathogen colonies when plated on selective media. Our data suggest that the lower incidence of root infection, and hence reduced levels of soil populations, are primarily due to a high level of resistance to root rot in G755 and G1033.

After 22 mo in a severe root rot situation, G6 and D7 rootstocks grafted with Hass scions showed average decline ratings of 6.9 and 7.7, respectively, whereas the average rating for grafted G755 trees was only 0.5 (2). Decline ratings were based on an evaluation of aboveground symptoms on a scale of 1–10, where 0 = a healthy tree and 10 = tree death. The resistance data accumulated from greenhouse experiments and limited field trials involving G6, D7, G755, and G1033 rootstocks are in line with the levels of tissue resistance to *P. cinnamomi* infection recorded for etiolated tissues of these selections in our laboratory tests.

Previously, a hypersensitive response in root tissue infected with *P. cinnamomi* has only been described for *Acacia pulchella* (9). Similarly, etiolated tissue or roots of selection G755 responded to *P. cinnamomi* inoculation by restricting pathogen development to the inoculation point. These data suggest that some *Persea* selections may possess high resistance to *P. cinnamomi* infection, in contrast to the range of low to high susceptibilities described for various eucalypts (10).

In summary, the etiolated tissue test shows promise as a screen for assessing potential root rot resistance in new rootstock selections. It is likely that the resistance mechanisms operating in a field-resistant rootstock are complex, and etiolated shoot tissue may afford a means of analyzing the tissue resistance component. Final judgement on the general validity of this screening technique awaits the accumulation of a much larger data base covering the performance of a much wider selection of avocado rootstocks in laboratory tests, greenhouse experiments, and field trials.

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