

Comparison of Resistance to *Phytophthora citricola* in Nineteen Avocado Rootstocks under Greenhouse Conditions

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Abstract

Nineteen vegetatively-propagated 8-mon-old avocado (*Persea americana*) rootstock selections (Aguacate mico, Anaheim, Borchard, D9, Duke 6, Dusa, G22, G582, G1033, Golden, Hibbard, Thomas, UC2001, UC2002, UC2003, UC2004, UC2011, Evstro, and Velvick) were evaluated for resistance to *Phytophthora citricola* in the greenhouse. Two weeks after stem inoculation with *P. citricola*, the canker size was determined. The relative resistance of the rootstock selections to infection with *P. citricola* was calculated relative to Hibbard rootstock which demonstrated the highest mean canker size of all rootstocks tested. The rootstocks Thomas and UC2004 demonstrated the second lowest relative resistance among the rootstocks. A group of twelve rootstocks including Borchard, UC2002, UC2003, Dusa, D9, Duke 6, UC2011, G22, Evstro, Aguacate mico, UC2001, and G1033 showed the highest relative resistance (74.5-84.5%) to *P. citricola*. The rootstocks G592, Anaheim, Golden, and Velvick were intermediate in their level of relative resistance compared with other rootstocks.

Introduction

Avocado (*Persea americana* Miller) trunk canker disease, which is commonly known as citricola canker, is caused by *Phytophthora citricola* Sawada. *P. citricola* is causing increasing damage to avocado groves throughout California in recent years (1). *P. citricola* affects the crown, lower trunk, and sometimes the main structural roots (1, 10). The typical symptoms of the disease include bark cracking and exudation of a white, sugary material, usually at the base of the trunk. In advanced stages, defoliation and twig dieback occur. If the canker encircles the trunk the tree will die.

Phytophthora citricola readily produces thick-walled oospores in culture. It also produces sporangia which liberate motile zoospores. It is very likely that because of its ability to produce zoospores, *P. citricola* can establish itself and spread throughout avocado groves or nurseries; and because of its abundant oospores, it is very difficult to control. Oospores are the most resistant spore structure produced by *Phytophthora* spp. and may survive in soil in the absence of a host for more than one year. The fungus has been also detected in feeder roots and main roots of avocado (2).

Currently, the use of clonally-propagated, moderately resistant rootstocks represents an important component of the integrated approach to control *Phytophthora cinnamomi* in avocados (1, 9). This approach has been used to develop avocado rootstocks with field

resistance to *P. cinnamomi* by screening more than 3,000 selections of different *Persea* spp. for root rot resistance (11, 12).

Because of the potential threat of *P. citricola* to the avocado industry in California, the purpose of the work reported here was to screen a number of the common, commercially available avocado rootstock cultivars for high levels of resistance to infection by *Phytophthora citricola*.

Materials and Methods

Rootstock propagation. The seedlings of *P. americana* cv. Lula were grown from seed planted in flats containing sand or vermiculite in the greenhouse at $24 \pm 2^\circ\text{C}$. While still small, seedlings were transplanted individually into paper pots (10-cm diam., 10 cm depth) filled with UC-soil mix. Nineteen rootstock selections (*Table 1*) were propagated vegetatively. Budwood from each clonal selection was tip-grafted to seedlings of *P. americana* cv. Lula as nurse plant. Shoot etiolation and subsequent root production allowed the establishment of clonal plants using a delicate procedure developed by Frolich and Platt (1971). Large clonal rootstocks were transplanted into plastic pots (15 cm diam., 17 cm depth) containing peat/vermiculite (1:1) potting media, one month prior to inoculation. All clonal rootstock plants used in this study were uniform in age (eight months old).

Preparation of inoculum and stem inoculation method. The isolate of *Phytophthora citricola* (cc-6) used in these studies was originally recovered from a canker on an avocado plant. The stock culture was maintained on slants of clarified V8C agar medium (per liter: Campbell® V8 juice cleared by centrifugation, 200 ml; CaCO_3 , 2 g; agar, 15 g; deionized water, 800 ml) and stored in the dark at 18°C . Fresh cultures were grown on V8C agar plates and incubated at 24°C in the dark. Avocado seedlings were inoculated with mycelial discs (4 mm diam.) and the pathogen was re-isolated monthly from colonized bark tissue to maintain its virulence. To confirm that cankers resulted from infection by *P. citricola*, samples of canker tissues were plated on a selective PARPH medium (6) (per liter of corn meal agar: pimaricin [Delvoicid] 0.02 g, ampicillin 0.25 g, rifampicin 0.01 g, pentachloro-nitrobenzene 0.10 g, and hymexazole 0.0075 g). Plates were incubated at 24°C in the dark. Discs of *P. citricola* on PARPH were subcultured on V8C agar plates and incubated at 24°C in the dark to allow production of oospores and the identity of *P. citricola* was confirmed microscopically using the revised key of Stamps *et al.* (1990).

Inoculation of the stems of clonal rootstock was made by removing a 4 mm diameter disc from the bark with a cork borer to expose the cambium and placing a V8C agar plug of similar size containing *P. citricola* on the exposed cambium. The wound was moistened with a drop of water after inoculation and wrapped with a strip of Parafilm to avoid drying (3). The experiment was carried out during the month of June and was repeated during the month of September.

Disease assessment of stem canker. The disease incidence and canker size were assessed two weeks after inoculation by measuring lesion area in centimeters square. The cankers were traced on transparent adhesive tape and transferred to a white sheet of paper. The area was determined by tracing the outline using a compensating polar planimeter (Keuffel & Esser Co., No 39132, Germany). The size of the inoculation site

was subtracted to give the canker size. The relative resistance of the avocado rootstock selections to *P. citricola* was assessed by the stem inoculation technique (4). Data were statistically analyzed using Analysis of Variance according Waller-Duncan's k-ratio t-test to compare the means (P=0.05) of canker size developed two weeks after stem inoculation of rootstocks.

Table 1. Avocado rootstock selections screened for resistance to *Phytophthora citricola* in a greenhouse experiment.

<i>Rootstock selection</i>	<i>Horticultural race</i>	<i>Geographic origin</i>
Aguacate mico	Guatemalan	Guatemala, 1975. Field Collection.
Anaheim	Guatemalan	Anaheim, Calif. 1910. Commercial variety.
Borchard	Mexican	Camarillo, Calif. Field collection, resistant to alkaline soil.
D9	Mexican	Riverside, Calif. Irradiated Duke budwood.
Duke 6	Mexican	Riverside, Calif. Field collection, resistant to <i>P. cinnamomi</i>
Dusa	Mexican	South Africa, Seedlings of Duke 7
G22	Guatemalan	Guatemala. Field collection
G582	Mexican	Guatemala. Field collection
G1033	Guatemalan	Hawaii. Seedling of Guatemalan variety Hayes.
Golden	Mexican	Riverside, Calif. Seedling of Duke 6
Hibbard	Mexican	Pauma Valley, Calif. <i>P. cinnamomi</i> escape tree
Thomas	Mexican	Escondido, Calif. <i>P. cinnamomi</i> escape tree
UC2001	Mexican	Seedling of cultivar Duke 7
UC2002	Mexican	Seedling of cultivar Barr Duke
UC2003	Mexican	Escondido, Calif. <i>P. cinnamomi</i> escape tree
UC2004	Mexican	Nipomo, <i>P. citricola</i> escape tree
UC2011	Mexican	Seedling of Duke Station
Evstro	Guatemalan	Australia. Budwood of seedling resistant to <i>P. cinnamomi</i>
Velvick	Guatemalan	Australia. Budwood of seedling resistant to <i>P. cinnamomi</i>

Results and Discussion

Two weeks after stem inoculation with *P. citricola*, the relative degrees of resistance of the 19 rootstock selections (*Table 1*) were assessed by comparing the canker sizes. All rootstock selections were infected with *P. citricola* but to varying degrees (*Table 2* and *Fig. 1*). Hibbard rootstock was very susceptible to *P. citricola* (mean canker size=23.16 cm²) among tested rootstocks. The relative resistance was calculated based on Hibbard (the highest susceptible rootstock) as having 0.0% relative resistance. Thomas and UC2004 rootstocks were susceptible to *P. citricola* with mean canker size of 15.09 and 11.42 cm²* respectively. Thomas and UC2004 rootstocks demonstrated the second lowest relative resistance, 34.8 and 50.7%, respectively. A group of twelve rootstocks

including Borchard, UC2002, UC2003, Dusa, D9, Duke 6, UC2011, G22, Evstro, Aguacate mico, UC2001, and G1033 had mean canker size ranging from 3.59 (Borchard) to 5.90 cm² (G1033). This group of rootstocks showed the highest relative resistance (74.5-84.5%) to *P. citricola*. The rootstocks G592, Anaheim, Golden, and Velvick had intermediate relative resistance ranging from 66.1 to 68.5% (Table 2).

Six clonal avocado rootstock cultivars including Thomas, Barr Duke, Martin Grande (G755C), Duke 7, G 6, and Toro Canyon were compared for their resistance to *P. citricola* by stem or crown inoculation (8). They also indicated that Thomas rootstock cultivar, which is widely known as highly tolerant to *P. cinnamomi*, exhibited significantly larger *P. citricola* lesions than those on all other cultivars tested. Thomas rootstock cultivar was very susceptible to all *P. citricola* isolates used including cc-2, cc-3, cc-5, and cc-6. These results were in good agreement with our findings concerning the high susceptibility of Thomas rootstock cultivar to *P. citricola*.

The production of avocado rootstocks that express a moderate level of resistance should be considered an important element of the strategy to control the stem canker disease caused by *P. citricola*. Rootstock plants used in this study did not vary in age, propagation technique, and environmental conditions. Accordingly, data presented in this report (Fig. 1) provide important information on the resistance potential of individual selections, prior to field evaluation.

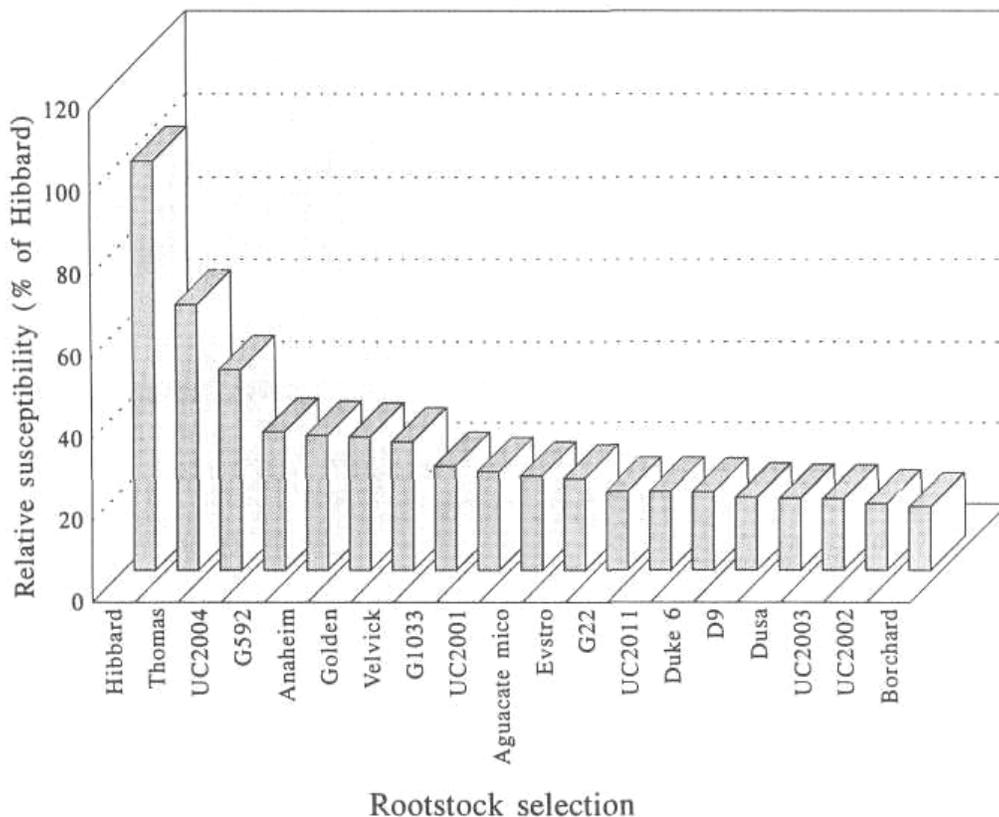


Fig. 1. Relative susceptibility of avocado rootstock selections to the stem canker pathogen, *Phytophthora citricola*. The relative resistance was calculated based on Hibbard as 100% susceptibility.

Table 2. Relative resistance of nineteen avocado rootstocks to the stem canker pathogen *Phytophthora citricola*.

Rootstock selection	Canker size (cm ²) ^x	Relative resistance (%) ^y
Hibbard	23.16	00.0
Thomas	15.09	34.8
UC2004	11.42	50.7
G592	7.85	66.1
Anaheim	7.66	66.9
Golden	7.57	67.3
Velvick	7.29	68.5
G1033	5.90	74.5
UC2001	5.61	75.6
Aguacate mico	5.36	76.9
Evstro	5.19	77.6
G22	4.48	80.7
UC2011	4.46	80.7
Duke 6	4.43	80.9
D9	4.13	82.2
Dusa	4.05	82.5
UC2003	4.03	82.6
UC2002	3.75	83.8
Borchard	3.59	84.5
LSD ^z	2.54	

^x Each value is the mean of two experiments with 10 replicate plants in each experiment. ^z

^y Relative resistance was calculated based on Hibbard as 0.0 resistant.

^z Data were statistically analyzed using Analysis of Variance according to Waller-Duncan's k-ratio t test.

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