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Susceptibility to *Phytophthora citricola* of Certain Avocado Rootstock Cultivars Known to be Tolerant to *P. cinnamomi*

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Abstract. **Various isolates of *Phytophthora citricola* Sawada showed different degrees of virulence on avocado. As a stem canker pathogen, *P. citricola* was, in general, more pathogenic than *P. cinnamomi* Rands on many common avocado cultivars. The clonal rootstock cultivars Thomas, Barr Duke, and Martin Grande (G755C), all moderately resistant to *P. cinnamomi,* were susceptible in varying degrees to stem, crown, and/or root infection by *P. citricola.* Thomas, the least tolerant cultivar, was even more susceptible than Topa Topa to *P. citricola.* Duke 7 and Toro Canyon clonal cultivars showed the highest degree of tolerance to *P. citricola.* Avocado rootstock cultivars which possess high degrees of resistance to both *P. citricola* and *P. cinnamomi* are urgently needed.**

Phytophthora canker (or crown rot, or collar rot) on the bark of avocado *(Persea americana* Mill.) was first detected in California perhaps more than 70 years ago (Zentmyer *et al.,* 1974; Coffey *et al.,* 1988). *Phytophthora citricola* Sawada as the causal organism of the disease on avocado, however, was not confirmed until 1974 by Zentmyer *et al.* (1974) who also thoroughly described the fungus. The disease, now commonly known as citricola canker, affects the growth and yield of avocado trees and has been found in every avocado-growing county in southern California (Coffey and Cohen, 1984; Coffey, 1989). It was estimated in 1988 that about 20 percent of avocado groves in California were affected by this disease and, in some cases, trees were killed in large numbers every year (Coffey *et al.,* 1988). The fungus has also been detected in soils, in feeder roots and main roots of avocado (Coffey and Cohen, 1984; Oudemans and Coffey, 1987), and has been reported to produce collar rot symptoms.

Materials and Methods

Plant material. The main avocado cultivars used in our greenhouse experiments included the six clonal rootstocks: Barr Duke, Duke 7, G6, Martin Grande (G755C), Thomas, and Toro Canyon. The clonal plants were propagated by the etiolation technique developed by Frolich and Platt (1971). All six clonal cultivars chosen in our study have been reported to possess moderate to high degrees of tolerance or resistance to avocado root rot caused by *P. cinnamomi* (Coffey and Guillemet, 1987; Gabor *et al.,* 1990). Seedlings of cultivars G6 and Topa Topa were also used in some experiments for comparison purposes. Plants used were grown in planting media of sand or vermiculite, or peat/vermiculite (1:1) or peat/perlite (3:1), all of which received

complete fertilizers when watered. When used in inoculation experiments, the plants were generally 5 to 12 months old, but were uniform in age among replicates.

Phytophthora isolates. Six isolates of *P. citricola* were used in experiments involving inoculation of avocado plants. They were: cc-1 (= P3911), the type isolate of the species, from citrus fruit in Taiwan; cc-2 (= P1273), from avocado trunk in Vista, California; cc-3 (= P1946), from avocado trunk in Nipomo, California; cc-4 (= P3053), from avocado root in Goleta, California; cc-5 (= P3711), from avocado root in Santa Barbara, California; and cc-6 (= AA-1), from avocado root in Temecula, California. In addition, a standard *P. cinnamomi* isolate, cm (=T139, or Pc40), from avocado root in Carpinteria, California, was included in most inoculation experiments for the purpose of comparing the relative pathogenicity of the two *Phytophthora* species on the avocado cultivars tested. All cultures were maintained and grown on V8-$CaCO_3$ agar medium (per liter, Campbell V8 juice 200 ml, $CaCO_3$, agar 15g).

Inoculation procedures. Stems of test plants were inoculated with *Phytophthora* by the method reported by Farih *et al.* (1981). Briefly, stem tissue about 1 5 cm above the soil line. was first surface-sterilized with ethanol and then wound-inoculated with a 4-mm-diam. mycelium-agar disc of *Phytophthora,* followed by covering it with a piece of wax paper and then wrapping with adhesive tape. Each uninoculated control plant received a *Phytophthora*-free agar disc. Five replicate plants of each treatment were incubated in randomized blocks in a greenhouse and harvested at 15 days in most experiments. The bark was removed and the lesion on the cambium was traced, transferred to paper, and its size measured with a planimeter. After subtracting the size of the inoculation area (13 mm^2, i.e., the 4-mm-diameter agar disc), the lesion size data were calculated and analyzed.

The crown region of the plant, 5 to 10 cm below the soil line, was wound-inoculated in some experiments with a procedure similar to that for stem inoculation above the soil, except the additional requirement of temporary excavation of the soil, covering the inoculated site with parafilm, and weekly heavy watering ('waterlogging') with the aid of a saucer under the pot (Tsao and Garber, 1960).

Results and Discussion

Relative pathogenicitv and virulence of the *Phytophthora* isolates. The six *P. citricola* isolates showed different degrees of virulence in causing stem canker on avocado. In general, isolate cc-6 was the most virulent, followed by cc-3, cc-4, cc-2, cc-5, and cc-1, the last of which is a citrus isolate from Taiwan. *P. cinnamomi* (isolate cm) produced the smallest lesions on the stems of all of the avocado cultivars used, and was the least pathogenic among the seven isolates tested. For example, the lesion size on stems of cultivar Thomas for isolates cc-6, cc-3, cc-2, cc-5, cm, and the uninoculated control was 2095, 1890, 1780, 1148, 568, and 18 mm^2, respectively. In a separate experiment when cultivar Duke 7 was tested, the stem lesion size for isolates cc-6, cc-3, cc-4, cc-2, cc-5, cc-1, cm, and the uninoculated control was 444, 425, 320, 265, 185, 146, 96, and 11 mm^2, respectively. Additional experiments using cultivars Barr Duke, Martin Grande,

and Toro Canyon produced results showing a similar trend for these isolates, i.e., isolate cc-6 was the most virulent of all *P. citricola* isolates and *P. cinnamomi* (cm) the least virulent.

The consistent observations that the highly virulent *P. citricola* isolates (e.g., cc-6 and cc-3) produced much larger stem lesions than *P. cinnamomi* on some of the popular commercial rootstock cultivars (e.g., Thomas and Martin Grande), which have been reported by other workers to be resistant to P. cinnamomi, strongly indicate that there might be a difference in the tolerance of these clonal rootstock cultivars to the two *Phytophthora* species. The results of specific experiments designed to produce such answers are reported below.

Relative degrees of resistance or tolerance of clonal rootstock cultivars to *P. citricola* and *P. cinnamomi.* Six clonal rootstock cultivars were compared for their resistance to *P. citricola* by stem or crown inoculation. Their relative degrees of tolerance were then assessed by comparing their lesion size. In the first experiment involving stem inoculation with isolate cc-2, five clonal cultivars were compared along with one seedling cultivar (Topa Topa). Results recorded at 10 days showed that cultivars Thomas, Barr Duke, Martin Grande, Topa Topa, G6, and Duke 7 had average lesion size of 1732, 959, 905, 833, 642, and 467 mm^2, respectively. Duke 7 was among the most tolerant cultivars to *P. citricola;* the lesion size of 467 mm^2 was significantly ($P = 0.05$) different from those of Martin Grande, Barr Duke, and Thomas. Thomas, which is widely known as highly tolerant to *P. cinnamomi,* exhibited significantly ($P \leq 0.05$) larger *P. citricola* lesions (at 1732 mm^2) than those on all other cultivars. It was, surprisingly, even more susceptible to *P. citricola* than the seedling Topa Topa (at 833 mm^2) which is widely known as highly susceptible to *P. cinnamomi.*

In another experiment in which five *Phytophthora* isolates were used, Thomas, Martin Grande, and Barr Duke were compared for their relative tolerance to stem infection by *P. citricola* and *P. cinnamomi.* The three cultivars, while all being somewhat tolerant to *P. cinnamomi* infection, exhibited different degrees of susceptibility to *P. citricola* (Fig. 1). Thomas, again, was the most susceptible cultivar to *P. citricola.* The differences in lesion sizes between Thomas and Barr Duke were highly significant ($P \leq 0.05$) when inoculation was made with the more virulent isolates (cc-6, cc-3, and cc-2) (Fig. 1).

The susceptible Thomas and the tolerant Duke 7 were again compared in a separate experiment in which the crown inoculation method was used involving *P. cinnamomi* (cm) and two isolates (cc-2 and cc-6) of *P. citricola.* While both cultivars exhibited small lesions caused by *P. cinnamomi,* Thomas was highly susceptible, and Duke 7 highly tolerant, to both isolates of *P. citricola.* The lesion produced at 15 days by cm, cc-2, and cc-6 on Thomas was 198, 1538, and 2053 mm^2, respectively, and on Duke 7 was 120, 254, and 290 mm^2, respectively. The cultivar Toro Canyon, which was also included in this experiment, reacted to the three *Phytophthora* isolates in a highly tolerant fashion similar to Duke 7. The lesion produced by cm, cc-2, and cc-6 on Toro Canyon was 139, 300, and 212 mm^2, respectively.

The susceptibility of Thomas and tolerance of Duke 7 to *P. citricola* were again verified in another crown inoculation experiment in which Barr Duke, G6, and Martin Grande were also included. These latter three cultivars showed varying degrees of susceptibility which ranged in between Thomas and Duke 7. More experiments are needed, however, to ascertain their true degree of tolerance to *P. citricola.*

Several other experiments, not reported here, have been conducted in which a number of other avocado cultivars (e.g., Borchard, Rollie, etc.) were tested and additional inoculation methods were used to test the reaction of these cultivars to root infection by P. *citricola.* Thomas continued to show high degrees of susceptibility to *P. citricola* in all experiments including those involving root inoculation (Fig. 2). The results of these experiments will be reported in detail in other papers.

Citricola canker, which is causing increasing damage to avocado in California, can be best controlled by the use of resistant cultivars. Our findings that many rootstock cultivars may react in the opposite way to the infections by *P. cinnamomi* and *P. citricola* are of great significance to the efficient planning of disease control strategies and to the future of the avocado industry. Identification of rootstock selections, in the near future, that possess high degrees of resistance to both of these *Phytophthora* species will be of great value to all avocado growers, and especially to those who are confronted with a replant problem in an already *P. citricola*-infested grove.

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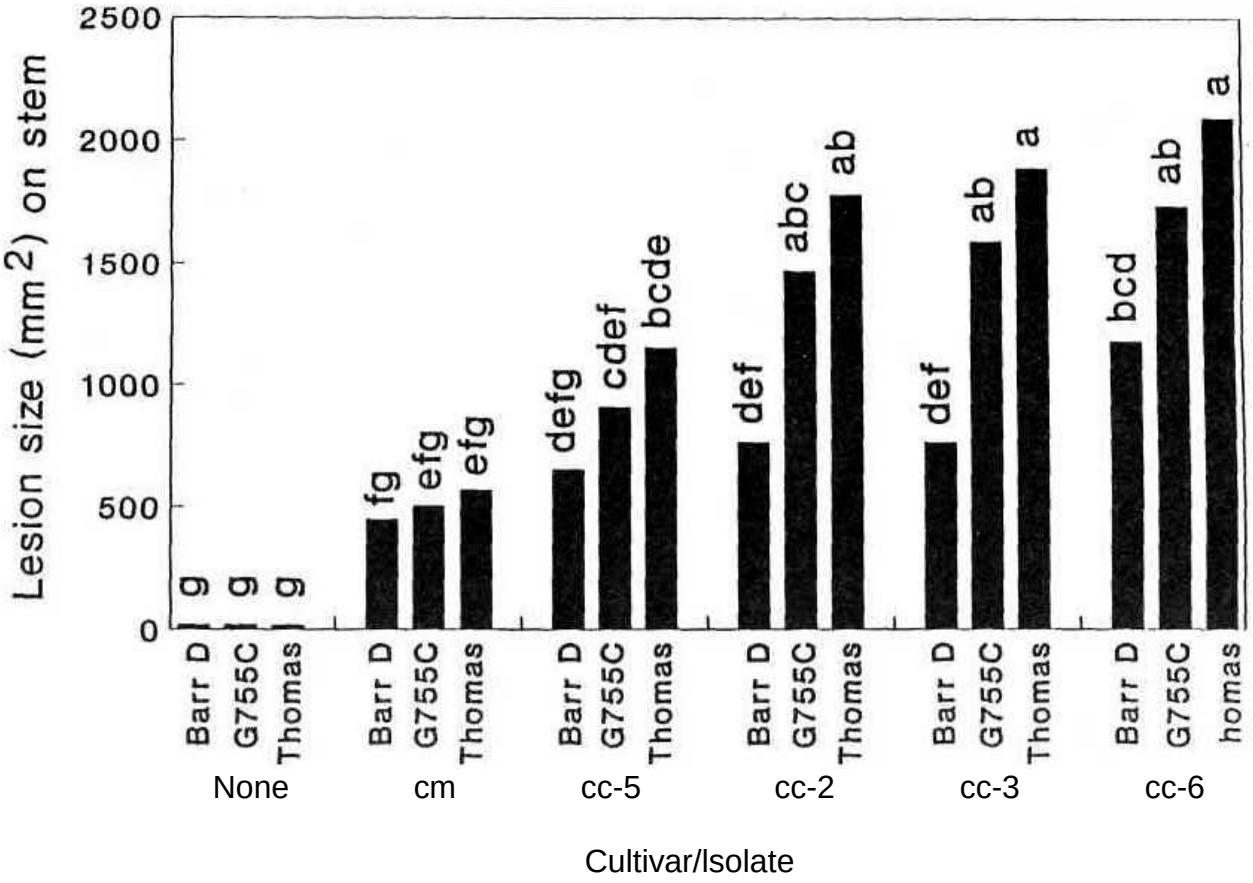

Fig. 1. The relative tolerance or susceptibility of three avocado clonal rootstock cultivars, Thomas, Martin Grande (G755C), and Barr Duke (Barr D), to stem infection by *Phytophthora cinnamomi* (cm) and four isolates of *P. citricola* (cc). Results at 15 days. Each value is a mean of five replicate plants. Values with the same letter do not differ significantly ($P \leq 0.05$) according to FLSD test.

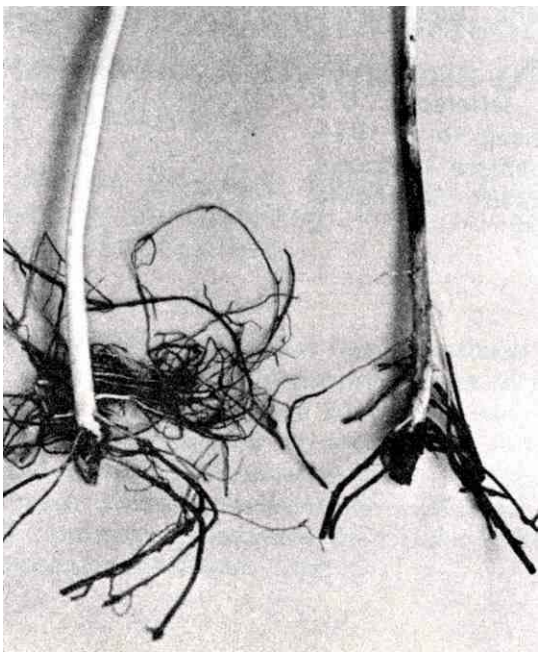

Fig. 2. Lesions on avocado rootstock Thomas produced by *Phytophthora citricola* isolate cc-6 (right). Inoculation was made by adding *Phytophthora*-colonized millet seeds to the crown and root region below the surface of the planting medium. The lesion originated from the roots and crown and extended upward to the stem within 2 wk. Death in some plants often resulted from girdling within one month. The control plant (left) received the non-colonized millet seeds. Photo taken at 8 wk.