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Pathogenicity of Isolates of *Phytophthora citricola* from Different Hosts on Unripe Fruit of Avocado

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The two most devastating diseases of avocado in California are root rot, caused by *Phytophthora cinnamomi* Rands, and crown rot and trunk canker caused by *P. citricola* Sawada (1). *P. cinnamomi* primarily attacks the young feeder roots, while *P. citricola* affects the crown, lower trunk, and sometimes the main structural roots (1, 18). As with *P. cinnamomi, P. citricola* has a wide host range (2, 3, 4, 8, 9, 10, 11, 14, 15, 17), and in California has been reported from English walnut *(Juglans regia* Jeps.), sweet cherry *(Prunus avium* L.), white fir *[Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.], and red fir *(A. magnifica* A. Mum). *P. citricola* has also been reported to cause a fruit rot of citrus in Australia (2) and guava *(Psidium guajava* L.) in Hawaii (8).

In California, fungal decay of unripe avocado fruit is quite rare due to unfavorable environmental conditions for the pathogen and natural resistance of the Hass fruit (5, 6, 12, 13). However, in the spring of 1986 and 1987, unripe fruit (cy. Hass) growing on the lower branches of trees in a grove in north Santa Barbara County exhibited fruit rot symptoms consisting of dark circular lesions externally and brown discoloration internally (Figure 1). A preliminary study showed *P. citricola* to be the causal agent (7). The rot affected approximately 5 % of the fruit in this grove, and occurred in the spring after heavy rains, indicating that the inoculum was probably splashed up from the ground, with a disease cycle similar to brown rot of citrus caused by *P. citrophthora.* In addition, *P. citricola* was also isolated from the soil, roots, and cankers of infected trees in the same grove (7). The purpose of this work is to further investigate *Phytophthora* fruit rot of avocado and to determine the relative pathogenicity *of P. citricola* isolates from other hosts, especially citrus, on unripe avocado fruit. This will increase our understanding of the potential of *P. citricola* to cause a fruit rot of avocado in California, should this fungus be introduced into avocado groves from other sources.

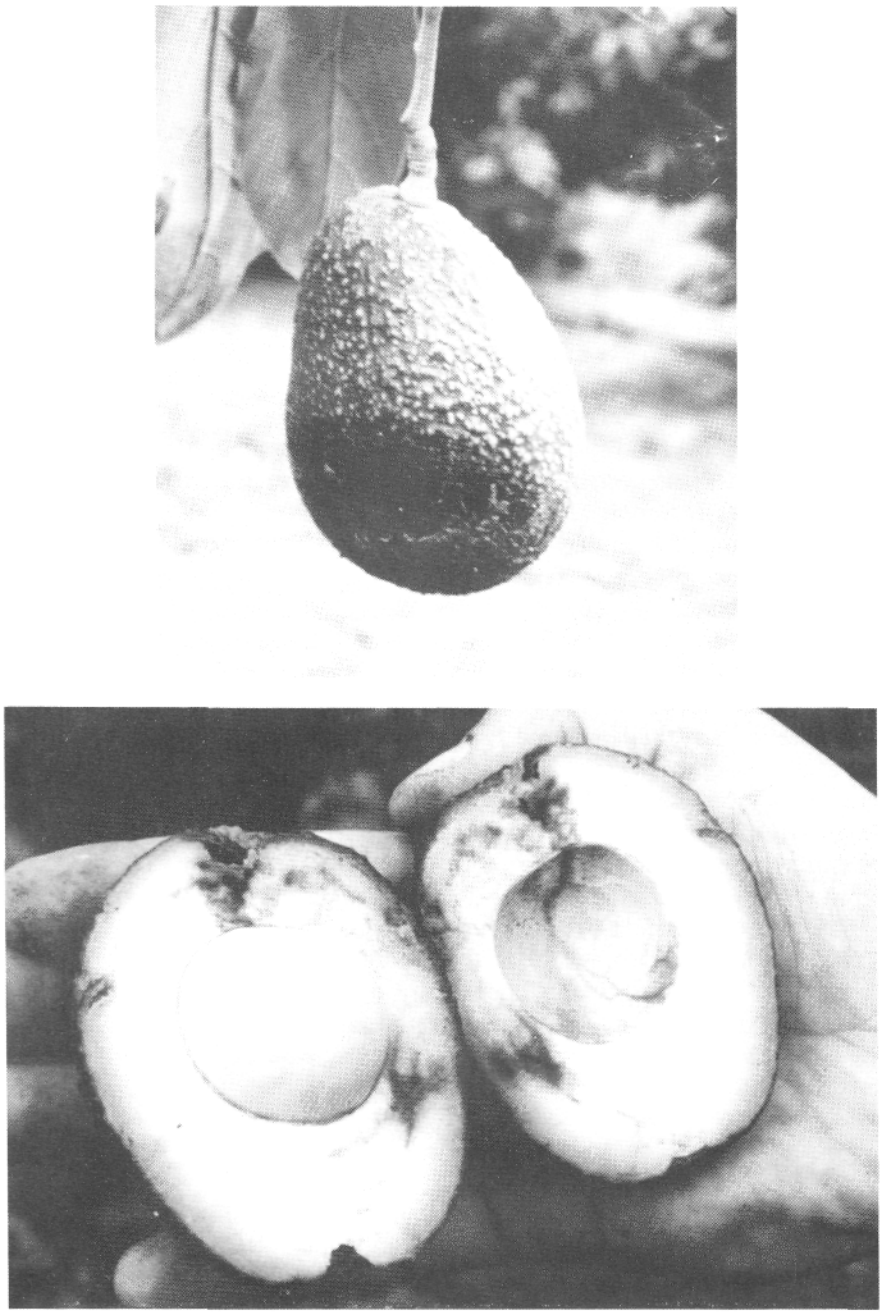

Figure 1. *(A) Exterior rot symptoms caused by* Phytophthora citricola *on unripe Hass fruit. Note dark, circular lesions on peel. (B) Interior of fruit showing extensive rot symptoms that have progressed down through the flesh and into the seed.* Photos taken by Mike Coffey of fruit from an avocado grove located in Santa Barbara County.

Materials and Methods

The *P. citricola* isolates used in this study are shown in Table 1. They were obtained from the Phytophthora collection at the University of California at Riverside (UCR), and included two California avocado isolates which were recently isolated either from rotted

avocado fruit in the field (P3060, Santa Barbara County), or from soil from an avocado grove (P2019, San Diego County).

Table 1. Pathogenicity of nine isolates of *P. citricola* from various hosts on mature, unripe avocado fruit (cv. Fuerte)[a]

Isolate	Host	Origin	% rot symptoms[b]
P1807	cherry	California	0
P1802	walnut	California	0
P1711	citrus	Australia	0
P1814	citrus	S. Africa	0
P1815	citrus	S. Africa	0
P1595	citrus	Australia	0
P705	hops	England	100
P3060	avocado	California	100
P2019	avocado	California	100

[a]Four fruit were wound-inoculated at four sites with 1 x 10^4 zoospores and incubated at 24 C for 96 hours. The experiment was repeated once and the data pooled for the two experiments

[b]Rot was defined as brown discoloration at least 1 centimeter in diameter 96 hours after inoculation.

To determine if wounding was necessary for consistent infection of unripe fruit, isolates of *P. citricola* (P3060, P2019) were used. Unripe fruit (cv. Fuerte) was collected from a grove at UCR and surface-sterilized with 10% bleach, rinsed with running tap water for 30 minutes, and allowed to air dry. Initially, fruit from the cv. Hass were used because the disease was initially reported on this fruit. However, the peel of Hass fruit is much darker than that of Fuerte fruit upon ripening, which makes visualization of rot symptoms more difficult. Preliminary studies showed that the effects of wounding and differential pathogenicity were the same regardless of whether Hass or Fuerte fruit were used. Eight fruit were placed on their side in plastic dishes for support and four sites on each of four fruit were inoculated with either 1 x 10^4 zoospores or an agar plug taken from the edge of an actively growing mycelial colony on V-8 agar for a total of 16 inoculation points per isolate. The fruit was either left unwounded or was wounded by pricking the peel with a sterile needle to a depth of approximately 0.5 millimeters. The fruit was sealed in plastic containers, incubated at 24 C for 96 hours, and observed for rot symptoms.

Pathogenicity of *P. citricola* isolates from different hosts was evaluated by inoculating wounded fruit as previously described with either mycelium on agar plugs or zoospores

and allowing the fruit to incubate under the same conditions as above. After 96 hours, the fruit was evaluated for rot symptoms. Rooting was defined as soft, brown discoloration at least 1 centimeter in diameter. If rot occurred, the fruit was cut open and samples of the flesh from various levels below the inoculation point to the seed at 5 millimeter intervals were plated out on *Phytophthora*-selectiva medium to determine the extent of fungal development. If there was no rot, a 5 millimeter-square plug of the peel and flesh around the inoculation point was removed with a razor blade, plated on selective medium, and observed daily for the presence of *P. citricola.* In addition, the pathogenicity of the four citrus isolates of *P. citricola* was tested by inoculating four wounded lemon fruit (cv. Eureka) with each isolate (1×10^4 zoospores) at four different points, incubating at 24 C for 96 hours, and assessing the degree of rot. Infected tissue was plated onto selective medium for recovery of *P. citricola.*

Results and Discussion

Wounding was necessary for consistent infection, regardless of the inoculum used. One hundred percent of the wounded fruit became infected after inoculation with both avocado *P. citricola* isolates, but only nine percent of the unwounded fruit became infected. The small percentage of infection occurring on unwounded fruit was probably the result of naturally occurring wounds on the fruit or from accidental wounding incurred during picking, because only very slight wounding was necessary for infection under experimental conditions. This suggests that *P. citricola* lacks the ability to penetrate the waxy layer and cuticle of the peel. The fruit rot systems observed in the field could be due to the splashing of inoculum in soil on wounded fruit by the force of heavy rains. Wounding could be due to insects, wind damage, or abrasion caused by fruit rubbing on soil or branches.

The type of inoculum used greatly affected the pathogenicity of the nine isolates tested on unripe avocado fruit. All isolates caused rot on unripe fruit when mycelium on agar plugs were used as inoculum; but in contrast, only the two avocado isolates caused extensive rot symptoms when zoospores were used as inoculum (Table 1). These avocado isolates infected the flesh down all the way down to the seed. Since zoospores are believed to be the natural infection units, the differential pathogenicity of the isolates obtained on unripe fruit using zoospores as the inoculum represents the more biologically realistic situation.

The rot symptoms caused by *P. citricola* from hops *(Humulus lupulus* L.) were less severe and generally did not extend more than 5 millimeters into the flesh. The other non-avocado isolates were not pathogenic on unripe fruit, and four days after inoculation there was only a small amount of discoloration (1 millimeter), caused by the initial wounding at the inoculation site. The avocado and hops isolates grew from the infected avocado tissue 24 hours after plating onto selective media, while the other non-avocado isolates grew from the fruit tissue only after it had become very soft and discolored. If fruit inoculated with the non-avocado isolates were allowed to ripen completely, rot would then begin, although the symptoms were less severe than with the avocado isolates. All the isolates from citrus, while unable to rot unripe avocado fruit, were able to extensively rot lemon fruit 96 hours after inoculation and were re-isolated

from rotted fruit on selective medium.

Previous researchers have discovered that unripe avocado fruit contains high levels of an antifungal compound in the peel and that this compound disappears as the fruit ripens (5, 6, 13). The compound inhibited spore germination and germ tube elongation of *Colletotrichum gloeosporioides,* a serious pathogen of avocado fruit in other parts of the world. In the case of fruit rot caused by *P. citricola,* the inability of non-avocado isolates to rot unripe fruit might be the result of inhibition of zoospore cyst germination and/or germ tube elongation by induced or preformed antifungal compounds found in the peel, insufficient enzyme production by zoospore germlings to invade unripe fruit, unfavorable nutritional requirements, or a combination of the above factors (16).

The data presented here suggest that there is some specificity among *P. citricola* isolates from different hosts in their ability to rot unripe avocado fruit, and this specificity is only apparent when zoospores are used as inoculum. Matheron *et al.* (9) suggested that *P. citricola* is not host specific in its ability to cause a crown rot of walnut, and that inoculum derived from other hosts might pose disease problems for walnut. Conceivably, the specificity obtained with avocado fruit rot represents a unique situation, in that isolates of *P. citricola* derived from walnut, cherry, and citrus were not pathogenic on unripe fruit but might cause a crown rot or trunk canker of avocados. This possibility still has to be investigated.

As for the practical concerns about the potential for *P. citricola* fruit rot to become more prevalent in the future, we feel at this time that pruning the lower branches and maintaining the leaf litter or mulch should help to prevent inoculum from the soil from reaching the fruit, should *P. citricola* be present in the grove. In the future, if fruit rot does become more of a problem as *P. citricola* becomes more established in avocado orchards, copper-based fungicides such as bordeaux mixture might provide effective control, as they do for brown rot of citrus.

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