Effect of Wound Age and Fungicide Treatment of Wounds on Susceptibility of Avocado Stems to Infection by *Phytophthora citricola*

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ABSTRACT

Bark wounds of avocado (*Persea americana*) stems of the cultivar Topa Topa became resistant to infection by *Phytophthora citricola* within 12-14 days after injury. The size of cankers declined significantly as the time interval between wounding and inoculation increased. Wounds that were re-injured after 2 mo of aging became susceptible to infections similar to those of wounds made on previously unwounded stems. These results suggest that chemical treatment applied to fresh bark wounds must protect against *P. citricola* for at least 14 days to be effective. Fungicide treatments for curing as well as protecting avocado stems against trunk canker disease caused by *P. citricola* were investigated. Wounds on intact and excised avocado stems were treated with fosetyl Al, fosetyl Al + Tree Seal, metalaxyl, Bordeaux mixture, Tree Paint, or Tree Seal after infection with *P. citricola*. The development of cankers was suppressed only by treatments including fosetyl Al. Wounds were protected by fosetyl Al through the period required for the wound to become resistant to infection. Brief washing of wounds treated with fosetyl Al 24 hr after treatment did not reduce protection of the wound site.

Avocado trunk canker disease caused by *Phytophthora citricola* Sawada currently causes severe losses in the production of avocado (*Persea americana* Miller) fruit in California (6). *P. citricola* colonizes and kills the phloem after cankers form in the crown, lower trunk, and sometimes the main structural roots (6,9,32). If the canker encircles the stem, the tree dies. Studies by El-Hamalawi and Menge (9) indicated that wounding is necessary for infection. Wounds created by gofers and voles, pruning, sucker shoot removal, staking, and winter injury are the major routes for entry of *P. citricola* into avocado bark (personal observations).

Researchers have demonstrated that wounds on several hosts other than avocado become less susceptible to infection by fungi with age (2,3,5). Wound healing is an active process, and the formation of the periderm, as well as suberin accumulation, are associated with induced resistance mechanisms in plants (13,29). A number of wound-induced reactions in plants are enhanced by microbial elicitors during resistance expression (4), suggesting that processes occurring during wound closure could be augmented by biological or chemical agents.

Systemic fungicides, including fosetyl Al and metalaxyl, are the most efficacious means of controlling *Phytophthora*-related diseases in established orchards after infection has occurred (7,10,14-16, 18,21,25,28,30,31). Phosphonates are highly water soluble and are amphoteric. Since phosphonates are transported in the phloem, application of the fungicides can be timed to coincide with the phenology of the host so that the target organ of the plant is a metabolic sink when the fungicides are applied. The evidence to date suggests that phosphonate activity can be explained by a combination of both fungitoxicity to *Phytophthora* spp. and the elicitation of a defense reaction in the host (11). The contribution of each depends on the sensitivity of the pathogen to direct inhibition by phosphonate in the specific environment under consideration, and on the strength of the host plant's defense response in the tissue under attack (11). Metalaxyl is translocated readily only in the xylem. It is active as a protectant, and to some extent, as a curative fungicide (11).

Bordeaux mixture and other copper-based fungicides are protectants which have demonstrated efficacy in controlling *P. parasitica* on citrus (26). However, copper-based fungicides were ineffective for control of collar rot caused by *P. cactorum* in apple trees (19,23).

The standard chemical and cultural techniques for controlling *P. cinnamomi*, the causal agent of avocado root rot, have not been effective for controlling the avocado trunk canker disease caused by *P. citricola*. Since *P. citricola* is present in the soil of most avocado groves in California (6), wounds are the main court of infection for *P. citricola*, and their occurrence is unavoidable (9), the objectives of this investigation were: 1) to determine the susceptibility of avocado plants to infection with *P. citricola* during the process of wound-aging; 2) to develop chemical treatments that would protect wounds against trunk canker disease and cure established stem cankers; 3) to compare the effect of chemical treatments in controlling stem canker disease on intact avocado plants and on excised stems to evaluate the possible involvement of intact-plant physiology; and 4) to evaluate the use of an excised-stem technique in determining the efficacy of chemical treatments to control stem canker pathogens.

MATERIALS AND METHODS
Chemicals used. The following chemicals were utilized: fosetyl Al (Aliette 80W), metalaxyl (Ridomil 2E), Tree Seal (asphaltum 45%, siliceous material 15%, water 40%), Tree Paint (copper naphthenate 10%, asphaltum 40%, petroleum naphtha 50% = Tree Seal + copper), and Bordeaux mixture (8-8-100; 12.5% Cu). Chemical stock solutions or suspensions were prepared immediately before use.

Plant material. Avocado plants cv. Topa Topa were grown from seed in UC No. 4 soil mix (17) in plastic liners (6 x 12 cm) with perforated bases for drain-age. After 6 wk of growth in the greenhouse at 24 ± 2 C, seedlings were transplanted into 4-L pots containing the same soil mix. One-year-old plants and stem cuttings (20 cm) from 1-yr-old Topa Topa plants were used in the study. *Persea indica* (a close relative of avocado which is more susceptible than *Persea americana* to *P. citricola*) plants were grown from seed in a flat containing sand in the greenhouse. Forty-five days after sowing, seedlings were transplanted individually into 4-L pots containing UC No. 4 soil mix and kept in the greenhouse for 6 mo before being used in the curative treatment of stem cankers. Plants were watered with dilute Hoagland's solution (27) as needed.

Preparation of inoculum and inoculation method. The isolate of *P. citricola* (cc-6) used in these studies was recovered originally from a canker on avocado near Temecula, California. The stock culture was maintained on slants of clarified V8C agar medium (per liter: V8 juice clarified by centrifugation, 200 ml; CaCO₃, 2 g; agar, 15 g; and deionized water, 800 ml) and stored in the dark at 18 C. Fresh cultures were grown on V8C agar dishes and incubated at 24 C in the dark. *P. citricola* was reisolated monthly from colonized bark tissue of avocado plants to maintain its virulence. The identity of *P. citricola* was confirmed microscopically using the revised key of Stamps et al (24).
Intact or cut stems were inoculated by removing a 4-mm-diameter disk from the bark with a cork borer to expose the cambium and placing a V8C agar plug of similar size containing mycelium of *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.

**Effect of wound age.** Avocado plants were wounded by removing a 4-mm-diameter disk from the bark with a cork borer to expose the cambium. Wounds were inoculated as described above each day, or on alternate days, for 14 days after wounding. Wound susceptibility to infection by *P. citricola* was assessed 2 wk after inoculation by measuring the area of the canker. Cankers were traced on transparent adhesive tape and transferred to a white sheet of paper. The area of the canker was determined by tracing the outline using a compensating polar planimeter. The size of the inoculation site was subtracted to obtain the canker size. Complete wound healing was judged by the absence of the fungus from the wound site and its adjacent tissues. The presence of *P. citricola* in the tissues 2 wk after inoculation was verified by transferring plugs of bark from the inoculation site or the margin of the canker after the outer periderm had been removed and incubating them on PARPH medium (20) at 22 C.

A second wound-aging experiment was conducted in which wound sites were sprayed with water using a micro-sprinkler system attached to a timer set to spray water for 3 sec every hour, delivering 100 ml during the time interval between wounding and inoculation. Water spraying ceased at the time of inoculation. Plants used as controls were sprayed with a similar amount of water not directed to the wound site. Within each experiment, each treatment included 10 replicate plants. Each experiment was repeated twice.

**Reinjury of aged bark wounds.** Bark wounds (4 mm diameter) were made on the stems of avocado plants with a cork borer. Wounds were allowed to heal and age for 2 mo. Wound periderm covering former wounds was removed using a 5-mm-diameter cork borer, and wounds were inoculated with *P. citricola* as described above. As controls, fresh bark wounds and uninjured but aged bark wounds were also inoculated with *P. citricola*. Within each experiment, each treatment included 10 replicates. Each experiment was repeated twice.

**Chemical treatments after or before inoculation.** Eighteen hours after inoculation, one of the following preparations (0.3 ml) was applied with a brush over and 5 mm around wounds inoculated with *P. citricola*: fosetyl Al (0.4 g a.i./ml), fosetyl Al (0.4 g a.i./ml) followed by application of Tree Seal after the fosetyl Al preparation had dried, metalaxyl (0.22 g/ml), Bordeaux mixture (0.12 g/ml), Tree Seal (0.9 g/ml), Tree Paint (0.8 g/ml), a preparation containing fosetyl Al (0.4 g a.i.) + Tree Seal (0.2 g) + water (0.8 ml), or a mixture containing fosetyl Al (0.4 g a.i.) + Tree Seal (0.5 g). Experiments were conducted with both intact avocado plants and stem cuttings. In control treatments, plants and stem cuttings were not treated with any chemical.

In another series of experiments, the chemical treatments used in the postinoculation study described above were applied prior to inoculation. Chemically treated plants and stem cuttings were divided into two equal groups. In one group, the chemically treated wounds were rinsed daily with running water for 10 sec, and the second group was not rinsed with water. Water from the washing was prevented from dripping into the soil. Chemically treated wounds were inoculated with V8C agar plugs containing mycelium of *P. citricola*. Untreated control plants and stem cuttings were inoculated at the same time as treated plants. Stem cuttings were incubated in moist chambers at 24 ± 2 C in the dark.

The area of *P. citricola* cankers was evaluated after 2 wk of inoculation as described above. Samples from inoculation sites and surrounding tissues of plants with no symptoms were transferred to *Phytophthora*-selective PARPH medium to verify infection with *P. citricola*. Within each experiment, each treatment included 10 replicate plants or stem cuttings. Each experiment was repeated twice.

**Curative treatment of stem cankers with fungicides.** The stems of 6-mo-old *Persea indica* plants were wounded and inoculated as described above. After stem canker had established (5 days after inoculation), the waxy cuticle layer of the canker was abraded with a razor blade, and one of the following fungicides was applied with a brush over the canker: fosetyl Al (0.4 g a.i./ml), fosetyl Al (0.4 g a.i.) + Tree Seal (0.2 g) + water (0.8 ml), or fosetyl Al (0.4 g a.i.) + Tree Seal (0.5 g) + water (0.5 ml). About 0.2 ml of the fungicide was applied directly to the canker and the surrounding tissues (5 mm around the borders of the canker) on each plant. Nontreated control plants were wounded and inoculated as above. The size of each canker was measured daily. Isolations from the canker and surrounding tissues were carried out on PARPH medium as described previously to verify infection with *P. citricola*. Each treatment included 10 replicates, and the experiments were repeated twice.

**Statistical analyses.** Data were analyzed by analysis of variance using Waller-Duncan's *k*-ratio *t* test. Correlation analysis was conducted to determine the relationship between wound age and canker size using data from days 1 to 14. All analyses were performed with SAS (SAS Institute, Cary, NC; release 6.0 for personal computer) at *P* ≤ 0.05.

**RESULTS AND DISCUSSION**

Canker size declined as the time interval between wounding and inoculation increased (Fig. 1). The bark wounds of avocado stems of cv. Topa Topa became resistant to infection by *P. citricola* within 12–14 days after injury. The canker size declined from 17.8 cm² in fresh wounds to 0 cm² when 14-day-old wounds were inoculated. Frequent, brief

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**Fig. 1.** Effects of age of stem wounds and water spray on wounds on the susceptibility of 1-yr-old Topa Topa avocado plants to infection by *Phytophthora citricola*.

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**Table 1.** Effects of age of stem wounds and water spray on wounds on the susceptibility of 1-yr-old Topa Topa avocado plants to infection by *Phytophthora citricola*.
wetting of the wounds did not affect their susceptibility to infection significantly compared to nonsprayed wounds (Fig. 1). There was high correlation between the canker size and the age of wounds. The correlation coefficients were as follows: \( r = 0.93 \) when the wounds were not sprayed with water, and \( r = 0.80 \) when the wounds were sprayed with water. Bostock and Middleton (3) reported that bark wounds in 2-year-old almond trees became resistant to infection by Ceratocystis fimbriata within 10–14 days after injury, and the frequency and size of cankers declined significantly as the time interval between wounding and inoculation increased. They related the resistance of the 14-day-old wounds to the changes observed histochemically. By 14 days, all wounds had a suberized and lignified wound periderm several cell layers thick that appeared to extend from the outer periderm to the cambium. Our results (Fig. 1) also indicated that most of the decline in wound susceptibility to P. ciriola occurred during the first 24 hr after wounding, followed by a more gradual decline. Doster and Bostock (8) reported that an increase in lignin accumulation could be detected in almond bark wounds as early as 1–2 days after wounding, and accumulation substantially increased by 6 days. Accordingly, this rapid accumulation of lignin formed very soon after wounding may explain the rapid decline in wound susceptibility of avocado stems to P. ciriola.

In our study, when the wound was allowed to age for 14 days prior to inoculation, by 15 days after inoculation P. ciriola could not be isolated from the wound and surrounding tissues. This confirms the inability of the fungus to penetrate aged wounds. The increased resistance with age of wounds to infection results, not only from suberin and lignin accumulation, but also from a number of wound-induced biochemical changes occurring during wound healing (13,29). The biochemical changes observed in or adjacent to wounded plant tissues include evolution of ethylene and ethane; increased activity of peroxidases, polyphenol oxidase, and phenylalanine ammonia-lyase; accumulation of phenols and oxidized phenols, and dramatic changes in isoprenoid metabolism; and deposition of lignin, suberin, and hydroxy-proline-rich glycoproteins in cell walls (1,4,13,22,29). Many of the products of these reactions have antimicrobial activity or function as a barrier to pathogen ingress by conferring impermeability to host tissues. However, the results of the current study showed that the changes occurring during wound aging of avocado did not prevent the infection by P. ciriola when the plant was reinfected after 2 mo of aging. Re-infected, aged bark wounds that were inoculated with P. ciriola developed an average canker size of 17.1 ± 2.1 cm² 2 wk after inoculation, which did not differ significantly (\( P = 0.05 \)) from those on fresh wounds 17.8 ± 1.9 cm². Uninjured aged bark wounds were not infected, and P. ciriola was not recovered from the inoculation sites.

Results (Tables 1 and 2) obtained from these studies suggest that fungicidal treatments applied to fresh bark wounds need to be effective for a minimum of 14 days to prevent infection without hindering formation of defensive barriers. All chemical treatments (fosetyl AI, metalaxyl, Bordeaux mixture, Tree Paint, and Tree Seal) applied to wounds on the stems of intact or excised Tora Tora avocado plants prior to inoculation provided protection against infection by P. ciriola for 14 days, the period required for the wound to become resistant to the fungus. Although wound healing in woody plants may progress at a slower rate under field conditions than under greenhouse conditions, most fungicides are effective for longer than 14 days. For example, we found that treatments with fosetyl AI at the rate used in this study protected stem wounds for a 5-mo period (unpublished data). The chemical treatments not only prevented the establishment of the fungus, but also rendered the inoculum inviable, as indicated by the failure of recovery of P. ciriola from inoculation sites and surrounding tissues (Tables 1 and 2). The daily water rinse of chemically treated wounds, started 24 hr after fungicide application, did not affect the efficacy of the treatment against P. ciriola. All controls, intact and excised, were severely infected with P. ciriola, as shown by canker development.

A recent study demonstrated that wounds on the stem of avocado plants are the main infection court for P. ciriola (9). Wounds created by sucker shoot removal, pruning, or by any other cultural practice should be covered by one of the chemicals used in this study to prevent infection with P. ciriola, which is often present in the soil and on roots in many avocado groves. It is also evident from this study that accidental wetting of the wound covered by the chemical 24 hr after application should not affect the efficacy of the treatment.

When chemical treatments were applied 18 hr after inoculation, only treatments containing fosetyl AI were completely effective in controlling the bark canker disease in avocado plants (Table 1, Fig. 2). Stem canker was not observed after fosetyl AI treatment of inoculated plants, and P. ciriola was not recovered from the inoculation site or surrounding tissues. Metalaxyl, Bordeaux mixture, Tree Paint, and Tree Seal were less effective than fosetyl AI for controlling stem canker if applied after inoculation.

### Table 1. Effect of chemical treatments of intact Tora Tora avocado plants on stem canker caused by Phytophthora ciriola

<table>
<thead>
<tr>
<th>Chemical treatment and rate/ml</th>
<th>Canker size (cm²)</th>
<th>Recovery of P. ciriola</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosetyl AI (0.4 g a.i.)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>Fosetyl AI (0.4 g a.i.)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>followed by Tree Seal (0.5 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>Fosetyl AI + Tree Seal (0.4 g a.i. + 0.5 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>Fosetyl AI (0.4 g a.i.)</td>
<td>0.0 d</td>
<td>-</td>
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<tr>
<td>+ Tree Seal (0.2 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>+ H₂O₈ (0.8 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>Metalaxyl (0.22 g a.i.)</td>
<td>4.3 c</td>
<td>+</td>
</tr>
<tr>
<td>+ Bordeaux mixture (0.12 g)</td>
<td>2.0 b</td>
<td>+</td>
</tr>
<tr>
<td>Tree Paint (0.8 g)</td>
<td>6.0 c</td>
<td>+</td>
</tr>
<tr>
<td>Tree Seal (0.9 g)</td>
<td>11.2 b</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>17.8 a</td>
<td>+</td>
</tr>
</tbody>
</table>

* Of each chemical, 0.3 ml was applied with a brush directly on canker and surrounding bark tissues 18 hr after inoculation.
* Canker size was assessed 2 wk after inoculation. Each value is the mean of three experiments with 10 replicates each. Values followed by identical letters are not significantly different (\( P = 0.05 \)) according to Waller-Duncan's k-ratio t test.
* A sample taken from treated bark area and surrounding tissues of each plant replicate either with or without symptoms was placed on Phytophthora-selective PARPH medium to determine the survival of P. ciriola. 
  = Absence of the fungus in all 30 replicate samples tested; + = presence of the fungus in all 30 replicate samples tested.

### Table 2. Effect of chemical treatments of excised stems of Tora Tora avocado plants on control of stem canker caused by Phytophthora ciriola

<table>
<thead>
<tr>
<th>Chemical treatment and rate/ml</th>
<th>Canker size (cm²)</th>
<th>Recovery of P. ciriola</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosetyl AI (0.4 g a.i.)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>Fosetyl AI (0.4 g a.i.)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>followed by Tree Seal (0.9 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>Fosetyl AI + Tree Seal (0.4 g a.i. + 0.5 g)</td>
<td>2.1 c</td>
<td>+</td>
</tr>
<tr>
<td>Fosetyl AI (0.4 g a.i.)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>+ Tree Seal (0.2 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>+ H₂O₈ (0.8 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>Fosetyl AI (0.4 g a.i.)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>+ Tree Seal (0.5 g)</td>
<td>0.0 d</td>
<td>-</td>
</tr>
<tr>
<td>+ H₂O₈ (0.5 g)</td>
<td>20.6 b</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>32.0 a</td>
<td>+</td>
</tr>
</tbody>
</table>

* Of each chemical, 0.3 ml was applied with a brush directly on canker and surrounding bark tissues 18 hr after P. ciriola inoculation.
* Disease development was assessed 2 wk after inoculation. Each value is the mean of three experiments with 10 replicates each. Values followed by identical letters are not significantly different (\( P = 0.05 \)) according to Waller-Duncan's k-ratio t test.
* A sample taken from treated bark area and surrounding tissues of each plant replicate either with or without symptoms was placed on Phytophthora-selective PARPH medium to determine the survival of P. ciriola. 
  = Absence of the fungus in all 30 replicate samples tested; + = presence of the fungus in all 30 replicate samples tested.
Fig. 2. Eighteen hours after inoculation of *Persea americana* cv. Topa Topa with *Phytophthora citricola*, one of the following preparations (0.3 ml) was applied over and around the inoculated wounds with a brush: Bordeaux mixture (0.12 g/ml), Tree Seal + Copper (Tree Paint 0.8 g/ml), metalaxyl (Ridomil, 0.22 g a.i./ml), and fosetyl Al (Aliente, 0.4 g a.i./ml). Fosetyl Al was the most effective fungicide in controlling the stem canker on avocado plants.

subsequently, fosetyl Al is the fungicide of choice as a curative agent against *P. citricola* on avocado stems. While there is a general agreement that copper sprays applied to the trunk are of questionable value in controlling crown or root rot, a few successes with these sprays have been reported in controlling crown rot caused by *Phytophthora* in apple trees (19, 23). Copper is usually effective as a protective but not as a curative treatment, since it is not absorbed into the plant tissue.

Our results indicate that treating established cankers of *P. citricola* on *Persea indica* plants with fosetyl Al was effective in curing avocado stem cankers. Treatment of cankers (average size 3.2 ± 0.3 cm²) 3 days after inoculation and applying fosetyl Al resulted in cessation of the canker expansion after 1–2 days of the chemical application. Ten days after treatment, *P. citricola* was not recovered from canker sites or surrounding tissues. In control plants, the cankers continued to expand until the plant died.

Chemical treatments applied on inoculated excised stems gave similar results to those found on intact plants (Table 2) except in the case of fosetyl Al + Tree Seal (1:1), which was not effective on excised stems. Since the fosetyl Al + Tree Seal preparation was prepared without the addition of water, it was highly viscous. Therefore, the movement of the fungicide into the plant tissue of the excised stems was probably slower than in intact plants and was not sufficient to inhibit fungal establishment. Intact plants required a lower level of fosetyl Al to control the disease than did excised stems (Tables 1 and 2). This observation could add to the evidence that changes in the host response to infection are involved in the mode of action of fosetyl Al (12). Consequently, it should be noted that even though the use of excised plant material could be useful in screening chemicals for disease control, the method still has certain limitations.

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LITERATURE CITED


