

Effect of Leaf Removal and Plant Pruning on the Development of Stem Canker Disease Caused by *Phytophthora citricola* on *Persea americana* and *Persea indica*

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Abstract

The removal of leaves from the upper part of avocado plants (mostly young leaves), located between 20 cm and 100 cm from the top of the plant, did not affect the rate of canker development caused by the inoculation with *P. citricola* compared to the control. However the removal of leaves from the lower area of the plant (mostly mature leaves), located between the soil line and 100 cm above it, resulted in significant ($P < 0.01$) increase in rate of canker development after inoculation with *P. citricola*. When the plant was pruned by cutting off the upper 100 cm, the rate of canker development was significantly ($P < 0.01$) higher than the control. After plant topping, the rate of canker development was significantly ($P < 0.05$) higher compared to plants inoculated with *P. citricola* after mature leaf removal.

Introduction

Avocado trunk canker disease which is commonly known as "citricola canker" is caused by *Phytophthora citricola* Sawada. This disease was first described by Fawcett (1916) and Barrett (1917). Subsequently several reports have appeared in the literature redescribing the disease (15, 23) but it was not until 1973 that Zentmyer *et al.* (1973) identified the pathogen as *Phytophthora citricola*. In recent years *P. citricola* has caused increasing losses in avocado groves throughout California (3). *P. citricola* affects the crown, lower trunk, and sometimes the main structural roots (8, 9, 24). The typical symptoms of the disease include bark cracking and the exudation of a white, sugary material usually near the base of the trunk. Scraping the bark reveals a blackened, necrotic lesion in the inner bark and phloem. In advanced stages, defoliation and twig dieback occur and, if the canker encircles the trunk, the tree will die. *P. citricola* can be isolated from feeder roots, cankers, and soil near infected avocado trees.

Pruning of avocado trees has been used by growers as a cultural practice for removing damage caused by frost, surviving drought conditions, eliminating shading effects due to overcrowding, and for shaping trees. The immediate effect of pruning and defoliation on the nutritional status is to reduce the contribution of carbon from photosynthesis to the carbon economy of the plant (6, 4). This results in a readjustment of plant metabolism for promotion of new leaf area expansion and re-establishment of the photosynthetic capacity. These adjustments include changes in the allocation of

photosynthates (18) and a reduction in the respiration rate of the remaining tissues (5). Root growth decreases following the disruption of the photosynthetic supply caused by defoliation or by pruning the shoots.

The effect of the nutritional status of the host plant might be a factor that influences resistance to infection by *Phytophthora* spp., especially horizontal resistance (13). Treatments that lowered the concentration of amino acids reduced the susceptibility of potatoes to infection by *P. infestans* (22, 12, 11). Hoitink *et al.* (1986) showed a direct correlation between the nitrogen concentration in leaf tissue of the rhododendron cultivar Roseum Elegans and susceptibility to *Phytophthora dieback*. Warren *et al.* (1973) reported that the levels of carbohydrates in potato plants also played a role in susceptibility to infection by *Phytophthora* spp. El-Hamalawi and Menge (1994a) indicated a strong positive correlation between the level of free amino acids and total soluble carbohydrates present in the bark of avocado plants and the degree of its colonization by *P. citricola*.

Tree pruning is a commonly used cultural practice in avocado groves; however, field observations indicate that pruning avocado trees may result in rapid expansion of lesions caused by the stem canker pathogen, *P. citricola*. Therefore the objectives of this study were to evaluate the effects of: *i*) removal of young leaves; *ii*) removal of mature leaves; and, *iii*) pruning of plants on the severity of avocado stem canker caused by *P. citricola*.

Materials and Methods

Plant material. Avocado seedlings *Persea americana* cv. Topa Topa were grown from seed in the greenhouse at 24°±2°C. The seeds were individually planted in 0.5-L plastic sleeves with perforated bases for drainage. The sleeves were filled with UC-mix #4 (16). Plants of *Persea indica* were grown from seed in flats containing sand in the greenhouse. After six weeks, seedlings were transplanted into 20-L pots containing UC-mix #4, watered with dilute (14%) Hoagland's solution (21) as needed. Plants were maintained in the greenhouse for 1.5 years before being used in the study.

Preparation of inoculum and inoculation method. The isolate of *P. citricola* (cc-6) used in this study was recovered originally from a canker on an avocado tree near Temecula, CA. The stock culture was maintained on slants of clarified V8C agar medium (per liter: Campbell®V8 juice clarified by centrifugation, 200 ml; CaCO₃, 2 g; agar, 15 g; 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. *Phytophthora citricola* was reisolated monthly from colonized bark tissue of avocado plants to maintain its virulence and the identity of *P. citricola* was confirmed microscopically using the revised key of Stamps *et al.* (1990).

Stems were inoculated 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 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. The area of *P. citricola* cankers was evaluated 5, 10, 15, 20, 25, 30, and 40 days after

inoculation as described below. Samples from inoculation sites and surrounding tissues of plants were transferred to *Phytophthora*-selective PARPH medium (17) to verify infection with *P. citricola*.

Disease assessment of stem canker. Disease incidence and canker development were assessed by measuring lesion area in square centimeters. Lesions 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.

Leaf removal and pruning of *Persea americana* and *Persea indica* plants. Forty 1.5-year-old *Persea americana* and *Persea indica* plants (210-250 cm in height) were divided into 4 groups of 10 plant replicates each as follows: group 1, the leaves located in the upper part (mostly young leaves) of the plant between 20 cm and 100 cm below the plant top were removed, group 2, all leaves in the area located between the soil line and 100 cm above it (mostly mature leaves) were removed, group 3, the top 100 cm of the plants was cut off and, group 4, control plants had no leaf removal or plant pruning. Five days after leaf removal or plant pruning, all plants were inoculated with *Phytophthora citricola* at 50 cm above soil line as described above. The disease development was assessed by measuring the canker size as described above. The experiment was repeated once using new sets of plants and the data were combined.

Statistical analyses. The effect of leaf removal and plant pruning on the rate of development of stem canker caused by *P. citricola* on *Persea americana* and *Persea indica* was statistically evaluated using Waller-Duncan's k-ratio t-test. Linear regression analysis was performed on the data and the significance levels (P) of differences between the slopes of linear regression lines were evaluated using Analysis of Variance with SAS statistical software version 6.02 (SAS Institute, Gary, NC).

Results and Discussion

The removal of leaves from the upper part (mostly young leaves) of *Persea americana* and *Persea indica* plants located between 20 cm and 100 cm from the top of the plant did not affect the rate of canker development ($P > 0.05$) caused by the inoculation with *P. citricola* compared to the control (Tables 1 and 2). However the removal of leaves from the lower area of the plants (mostly mature leaves) located between the soil line and 100 cm above it, resulted in a significant ($P < 0.01$) increase in rate of canker development after inoculation with *P. citricola* compared to the control (Tables 1 and 2). When the plants were topped by cutting off the upper 100 cm, the rate of canker development was significantly ($P < 0.01$) higher than the control (Tables 1 and 2). After plant pruning, the rate of canker development was significantly ($P < 0.05$) higher when compared to plants which had mature leaf removal (Table 2). All rates of canker development were linear over a period of 40 days after inoculation of *Persea americana* and *Persea indica* with *P. citricola* (Figures 1A and 1B). *P. citricola* was recovered from all cankers developed on the avocado plants.

Table 1. Effect of leaf removal and plant pruning on the rate of development of stem canker caused by *P. citricola** on *Persea americana* and *Persea indica*.

Treatment	Rate of canker development (cm ² /day)	
	<i>Persea americana</i>	<i>Persea indica</i>
Control	0.585 A	1.901 A
Young leaf removal	0.623 A	2.040 A
Mature leaf removal ^o	1.259 B	4.027 B
Plant topping ^d	1.687 C	5.798 C
LSD	0.244	0.445

^a After 5 days of leaf removal or plant pruning, plants were inoculated with *P. citricola*. Canker size was measured after 5, 10, 15, 20, 25, 30 and 40 days following plant inoculation. Each value is the mean of two experiments with 10 replicates each. Values in each column followed by the identical letters are not significantly different (P=0.05) according to Waller-Duncan's k-ratio t test.

^b All leaves in the area located between 20 cm and 100 cm from the plant top were removed (young leaves).

^o All leaves in the area located between the soil line and 100 cm above it were removed (mature leaves).

^d The top 100 cm of the plant was cut off.

Depending on the severity of the defoliation, the resulting deficit in photosynthetic carbon production can be met partly by the mobilization of reserve carbohydrates and nitrogen compounds to sustain both new growth and existing tissue (5, 6, 4). Following the disruption in photosynthesis, storage carbohydrates (starch) were immediately utilized causing a rapid decrease in starch and a concomitant increase in free sugar concentrations to compensate for sugar losses due to disruption in photosynthesis (1). Stored carbohydrates from roots and crowns of Lucerne (19) were claimed to contribute directly to shoot growth. The total carbohydrates in the fine roots were less in pruned than unpruned citrus trees (7). Culvenor *et al.* (1989) showed that the mobilization of nitrogenous compounds can be of equal or greater importance than carbohydrates after severe plant defoliation. Loss of protein exceeded that of total non-structural carbohydrates. The roots were the major source of mobilized nitrogen during a period when root growth had ceased. Loss of carbohydrates and nitrogen from roots and branches of subterranean clover lasting 5-9 days was observed in case of severe defoliation. The response time before there is diminished root growth varies among species, ranging from 24 hours to 2 weeks (1). Pruning citrus trees did not only reduce root growth but also resulted in the death of at least 20% of the roots at a soil depth of 9-35 cm thirty days after pruning. However, nine to eleven month after pruning, total biomass of leaves and fine roots to a depth of 1 m was similar in pruned and unpruned citrus trees (7).

Pruning and leaf removal results in high levels of nutrient translocation from the root system to the stem and leaves through the phloem to meet the demand for new growth. High levels of nutrients in the bark tissues provide favorable conditions for pathogen establishment and stem canker development. Since *P. citricola* primarily colonizes the bark tissues of avocado plants, it seems logical that the amounts of both free amino acids and total soluble carbohydrates in the bark tissue might reflect the physiological condition that would affect the degree of colonization. El-Hamalawi and Menge (unpublished data) found a significant positive correlation between the level of free

amino acids ($r=0.89$) and total soluble carbohydrates ($r=0.45$) present in the bark of avocado plants and the degree of its colonization by *P. citricola*. The increase in concentration of nitrogen in the juvenile foliage of the rhododendron cultivar Roseum Elegans was also significantly and positively correlated with size and number of lesions caused by *P. cactorum* (14). Low numbers of small lesions were produced on nitrogen-deficient rhododendrons.

Table 2. Significance levels (P) of differences between the slopes of linear regression lines for the effect of leaf removal and plant pruning on the development of stem canker caused by *P. citricola*^a on *Persea americana* and *Persea indica*.

Effect of Treatment	<i>Persea americana</i>	<i>Persea indica</i>
Control vs young leaf removal	N.S. ^e	N.S. ^e
Control vs mature leaf removal ⁰	<0.01	<0.01
Control vs plant topping	<0.01	<0.01
Young leaf removal vs mature leaf removal	<0.01	<0.01
Young leaf removal vs plant topping	<0.01	<0.01
Mature leaf removal vs plant topping	<0.05	<0.01

^a After 5 days of leaf removal or plant pruning, plants were inoculated with *P. citricola*. Canker size was measured after 5, 10, 15, 20, 25, 30 and 40 days following plant inoculation. Each value is the average of two experiments with 10 replicates each.

^b All leaves in the area located between 20 cm and 100 cm from the plant top were removed (young leaves).

^c All leaves in the area located between the soil line and 100 cm above it were removed (mature leaves).

^d The top 100 cm of the plant was cut off.

^e N.S. = not significant ($P > 0.05$).

Young leaves are considered nutrient sinks rather than nutrient sources; therefore, their removal from avocado plants did not disturb the nutrient balance in the bark tissue. This could explain the insignificant difference in extent of *P. citricola* colonization of plants with young leaf removal compared to control plants. Pruning and mature leaf removal causes disruption of the photosynthetic process which resulted in carbohydrate and nitrogen mobilization from roots and stems, resulting in increased levels of carbohydrates and nitrogen in the phloem of the avocado stem. That explains the increase in extent of colonization by pruning and mature leaf removal. Pruning of avocado plants caused the highest *P. citricola* colonization due to higher mobilization of nutrients to meet the demand of new growth triggered by the loss of the apical dominance.

Our results indicate that pruning *P. citricola*-infected avocado trees should be avoided. It was shown that cankers did not form after avocado plant inoculation with *P. citricola* during the period of March-April and August-September, even though the pathogen was recovered from the inoculation sites (unpublished data). The failure of infection during these periods was related to the growth cycle of avocado trees. Therefore if pruning is required, care should be taken to prune avocado trees during periods of minimum susceptibility to *P. citricola*. A recent study demonstrated that wounds on the stem of

avocado plants are the main infection court for *P. citricola* (9). Wounds created by pruning should be covered by a protective paint containing fosetyl-Al (9).

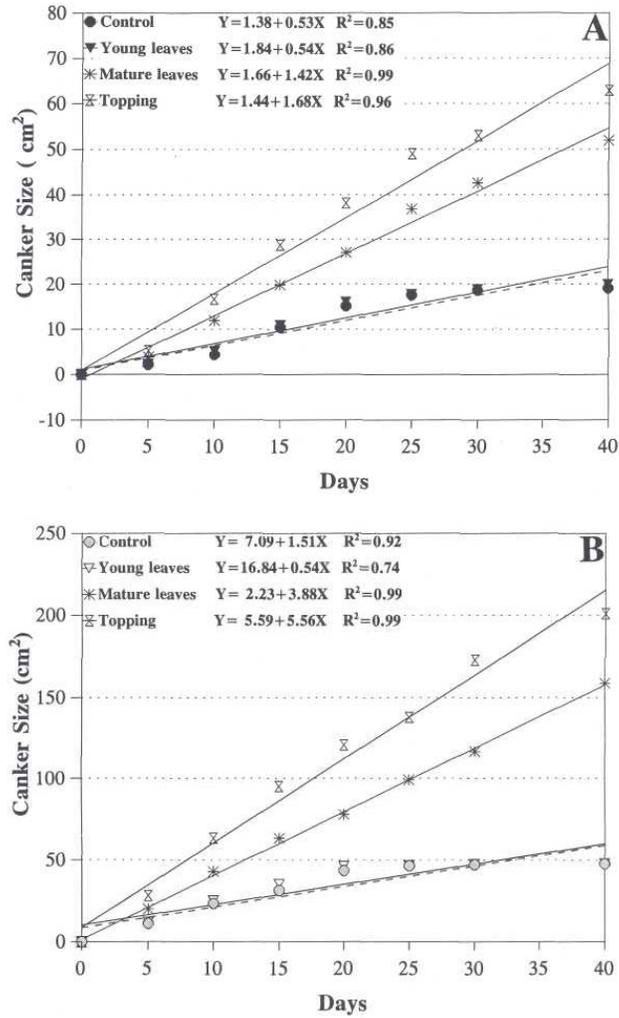


Fig. 1. Linear regression lines for the effect of removal of young and mature leaves and pruning of *Persea americana* (A) and *Persea indica* (B) plants on canker size caused by *P. citricola*. All leaves in the area located between 20 cm and 100 cm from the plant top (young leaves) and leaves in the area located between the soil line and 100 cm above it (mature leaves) were removed in two different sets of 10 plant replicates each. The top 100 cm of the plant was cut off in a set of 10 plant replicates. Each experiment was repeated once and data were combined.

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