

# The Role of Snails and Ants in Transmitting the Avocado Stem Canker Pathogen, *Phytophthora citricola*<sup>1</sup>

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**ABSTRACT.** The sugary exudate appearing on bark lesions of *Persea americana* Miller and *Persea indica* plants after infection with *Phytophthora citricola* contained viable oospores and hyphal fragments in the field and in the greenhouse. This sugary exudate was a source of inoculum and dispersal of the pathogen within and between avocado plants. Spraying water onto lesions moved inoculum from the sugary exudate to wounds below. Water from sprinkler irrigation washed propagules into the soil around the plants. Viable propagules of *Phytophthora citricola* were identified in the feces of snails (*Helix aspersa*) that had fed on infected bark tissues. When these snails were moved to healthy plants, they made wounds on succulent tissue, and the infectious feces induced cankers. Ants (*Iridomyrmex humilis*) were attracted to the sugary exudate and also transmitted infectious propagules to wounds on avocado stems and to the soil. Control strategy for the avocado stem canker disease should consider control of vectors.

In California, a severe stem canker disease of avocado (*Persea americana*), caused by *Phytophthora citricola* Sawada, has spread to all major avocado growing areas (Coffey, 1987). *Phytophthora citricola* affects the crown, lower trunk, and sometimes the main structural roots (Coffey, 1987; Zentmyer et al., 1973; Zentmyer et al., 1974). *Phytophthora citricola* acts mainly as a wound pathogen (El-Hamalawi and Menge, 1994a, 1994b) that usually invades wounded host tissue at or near the soil line and causes a canker in the inner bark tissue, which may be lethal to the tree, if not arrested. In advanced stages, defoliation and twig dieback occur. *Phytophthora citricola* attacks mature trees and also causes collar rot in young nursery trees (Coffey and Cohen, 1984).

Understanding the sources of inoculum and the dispersal mechanism of any pathogen is important to develop effective control measures. Little is known about either the short

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or long-range spread of the stem canker disease of avocado or the dispersal of its causal agent. Dispersal mechanisms of most *Phytophthora* species such as *Phytophthora palmivora*, causing black pod of cocoa, *Phytophthora nicotianae*, causing black shank of tobacco, *Phytophthora infestans*, causing potato late blight, and *Phytophthora cinnamomi*, causing avocado root rot, are closely or directly related to the movement of water.

Insects are not usually viewed as vectors of *Phytophthora*. However, transmission of *Phytophthora* species has been reported for a number of insects such as *Sciara* flies (Murphy and McKay, 1927), *Drosophila* (Hunter and Buddenhagen, 1969), the weevil *Scyphophorus interstitialis* Gyll., and ants (McGregor and Moxon, 1985; Newhook and Jackson, 1977; Okaisabor, 1974; Peregrine, 1969; Taylor and Griffin, 1981; Turner, 1972). Snails have also been shown to be vectors of *Phytophthora* (Kueh and Khew, 1982; Turner, 1964).

Sugary exudates are associated with stem canker development in avocado trees that are infected with *Phytophthora citricola*. Snails, ants, *Drosophila*, and other insects are common in avocado groves and frequently are observed to be in contact with infected trunk cankers of avocado trees. There is little attempt at insect control in most California avocado orchards. Chemical control of mites, thrips, and loopers may be attempted, but biological control is more frequently practiced. The brown garden snail (*Helix aspera* Muller) is often a pest in avocado orchards and can be controlled with copper painted in bands around the base of the trees or by the introduced predatory decollated snail, *Ruminia decollata* Linn. The objective of this study was to determine if the sugary exudates from avocado stem cankers serve as a source of inoculum in avocado groves and the possible role of snails and ants as dispersal vectors of *Phytophthora citricola* to avocado plants.

## Materials and Methods

**PLANT MATERIAL.** 'Topa Topa' avocado (*Persea americana*) plants were grown from seed in UC no. 4 soil mix (Matkin and Chandler, 1957) in plastic liners (6 x 12cm) with perforated bases for drainage. After 6 weeks of growth in a shaded greenhouse, seedlings were transplanted into 4-L pots containing the same soil mix. Plants of *Persea indica* Spreng., a close relative of avocado, which is more susceptible to *Phytophthora citricola* than *Persea americana*, were grown from seed in a flat of sand in the greenhouse. Forty-five days after sowing, seedlings were transplanted individually into 4-L pots containing UC no. 4 soil mix. One-year-old *Persea americana* and *Persea indica* plants and stem cuttings (20 cm) from 1-year-old *Persea indica* plants were used in the study. Greenhouse temperatures fluctuated between 18 and 30 °C. Daytime photosynthetically active radiation averaged 157  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Two-year-old 'Hass' avocado (*Persea americana*) grafted on 'Barr Duke' or 'Topa Topa' rootstocks was used in lathhouse experiments. Lathhouse conditions closely resembled outdoor conditions with regard to temperature (average 27 °C day/ 17 °C night) and day length, although daytime light intensity was reduced 60% to about 104  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Trees in the greenhouse and lathhouse were irrigated with dilute (14%) Hoagland's solution (Tuite, 1969) as needed.

**PREPARATION OF INOCULUM AND INOCULATION METHOD.** The isolate of *Phytophthora citricola* (cc-6) used in these studies was recovered originally from a canker on avocado near Temecula, Calif. The stock culture was maintained on slants of clarified V8C agar medium (per liter: Campbell V8 juice clarified by centrifugation, 200 mL; CaCO<sub>3</sub>, 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 *Phytophthora citricola* was confirmed microscopically using the revised key of Stamps, et al. (1990).

Avocado plants were stem-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 *Phytophthora citricola* on the exposed cambium. The wound was moistened with a drop of water after inoculation and wrapped with a strip of Parafilm to prevent drying. Stem cuttings were inoculated at the middle section of each cutting and were incubated at 20 °C in a moist chamber (35 x 22 x 15 cm). Two weeks after inoculation, samples of the infected bark were removed and incubated on PARPH agar (Mitchell et al., 1986) to detect *Phytophthora citricola*.

**SUGARY EXUDATE AS A SOURCE OF *PHYTOPHTHORA CITRICOLA* INOCULUM.**

Following inoculation of 2-year-old trees with *Phytophthora citricola* (above the graft union) at about 10 cm above the soil line in the lathhouse, an exudate of sugary material was associated with developing cankers. At the early stages of infection, the sugary material appeared as a viscous liquid (Fig. 1),



Fig. 1. Sugary material exuded as a viscous liquid from canker on avocado plants caused by *Phytophthora citricola* at the early stages of infection.

which dried with time to form a powdery residue (Fig. 2). The liquid and dry sugary exudates were examined microscopically for *Phytophthora citricola* propagules. Samples of the liquid and the dry sugary exudate were placed on PARPH agar to detect *Phytophthora citricola*. Ten healthy greenhouse-grown avocado plants each of *Persea americana* and *Persea indica* and 10 stem cuttings of *Persea indica* were wounded (4-mm-diameter wound) as described above, and each wound was inoculated with 0.1 g of

dry sugary exudate, moistened with 2 drops of water, and wrapped with a strip of Parafilm.



Fig. 2. Powdery sugary material formed after the drying of the sugary liquid exuded during canker development on avocado plants following infection by *Phytophthora citricola*.

In a second experiment, 40 greenhouse-grown *Persea americana* plants were wounded and inoculated with *Phytophthora citricola* at about 130 cm above the soil line. Four weeks after inoculation, the cankers were covered with sugary exudates. Twenty of the infected plants were wounded again at about 10 cm above the soil line. In ten wounded and infected plants, stems above the sugary exudate sites were sprayed with sufficient water to run down the stems over the sugary exudate to the stem wounds. Ten wounded, infected plants were used as controls without water spray. The cut wounds were periodically examined for infection and the presence of *Phytophthora citricola* was verified on PARPH agar. Ten infected plants were watered using sprinklers directed towards the sugary exudate areas and another group of plants were watered by drip irrigation. Three soil samples collected from around the base of each plant of all groups were plated on PARPH agar to detect *Phytophthora citricola* using

the soil dilution technique (Mitchell et al., 1986). All experiments were performed three times.

**TRANSMISSION OF *PHYTOPHTHORA CITRICOLA* BY ANTS.** Ten *Persea americana* trees grown in the lathhouse were wounded and inoculated with *Phytophthora citricola* at about 130 cm above the soil line. Ants (*Iridomyrmex humilis* Mayr.) were attracted to the sugary material exuded by the cankerous tissue of the infected avocado trees. New wounds were made on the stems of ten *Persea americana* avocado trees, at about 10 cm above the soil line, along the path of the ants as they move downward away from the sugary exudate. For the control pots, 10 wounded, infected avocado trees were placed in a tray containing water to keep ants away from the plants. The formation of new cankers in the wound sites was determined. Fifteen to twenty ants that were collected with a sterile tweezer as they were moving away from the sugary exudate area were placed on PARPH agar to detect *Phytophthora citricola*. Twenty ants collected with a sterile

tweezer were placed on wounds made on the stem of ten healthy *Persea americana* avocado plants (two ants/wound) and wrapped with Parafilm. Two weeks later, the inoculation sites were evaluated for canker development.

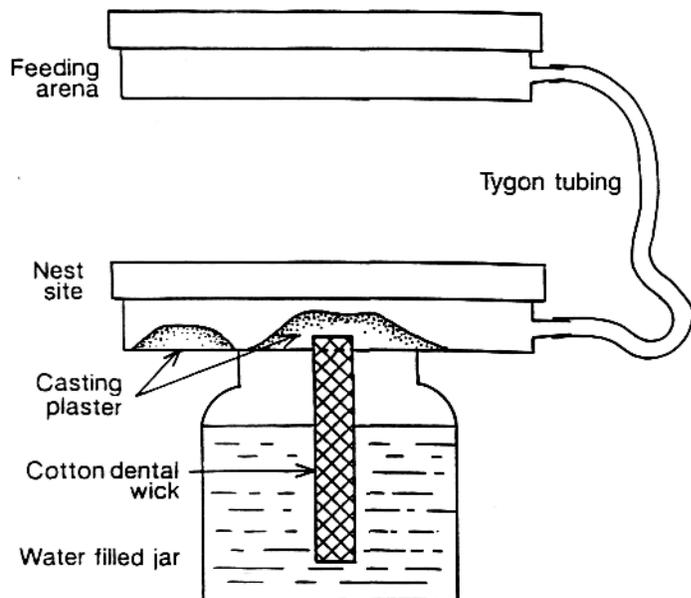


Fig. 3. An artificial ant colony that consisted of two 150-mm-diameter x 30 mm plastic petri dishes connected by a piece of Tygon tubing (30.5 cm x 2.4 mm inside diameter). One petri dish served as a nest site and the other was a feeding arena. Ants moved freely between the two dishes.

About 50 ants (*Iridomyrmex humilis*) were collected from *Phytophthora*-infected avocado plants in the lathhouse by brushing them into containers with an artist's brush and were placed in an artificial colony. The artificial colony consisted of two 150-mm-diameter x 30-mm plastic petri dishes connected by Tygon tubing (30.5 cm x 2.4 mm inside diameter). One petri dish served as a nest site and the other was a feeding arena (Fig. 3). Ants moved freely between the two dishes. The sugary exudate was placed in the second dish for feeding. After feeding on the sugary exudate, ants were transferred to a second artificial colony containing five wounded *Persea indica* stem

cuttings in the feeding dish of the colony. Two artificial colonies were used for each experiment. Replicates containing five wounded *Persea indica* stem cuttings without ants served as the control. Wounds on the stem cuttings were examined for canker development and *Phytophthora citricola* was detected by isolation on PARPH agar. All experiments were performed three times.

**TESTING SNAILS AS VECTORS OF *PHYTOPHTHORA CITRICOLA* ON AVOCADO PLANTS.** *Persea indica* stem cuttings were inoculated with *Phytophthora citricola* as described above. After the cankers developed and covered the stem cuttings (about 10 days after inoculation), 10 brown garden snails (*Helix aspersa*) were placed for 5 days in each moist chamber. Five moist chambers with 10 stem cuttings each were used in the study. Snails fed on non-inoculated avocado stem cuttings were used as controls. After snails fed, they were washed twice with distilled water, dried, and placed in cages for collection of feces. Smears of the fresh fecal material were examined microscopically and samples were placed on PARPH agar to isolate and detect *Phytophthora citricola*. Samples of the moistened feces (about 0.2 g/wound) were also used to inoculate 10 *Persea americana* avocado plants in the greenhouse and 20 stem cuttings of *Persea indica* in moist chambers.

After inoculating 10 *Persea americana* avocado plants with *Phytophthora citricola*, cankers were left for a 2-week period to develop. Six brown garden snails (*Helix aspersa*) were placed in contact with each infected plant. Other snails were placed in contact with non-inoculated plants. The pot and the lower 25 cm portion of the plant

were covered with a plastic bag (perforated with small holes to allow aeration) to confine the snails that fed on the plants for 2 days. Snails fed on infected and non-infected plants were later removed, washed with distilled water, wiped dry with a paper towel, and confined in contact with 10 unwounded, non-inoculated *Persea americana* plants (three snails/plant) and 20 non-inoculated *Persea indica* stem cuttings (two snails/cutting). Stem cuttings and potted plants were examined for the presence of cankers. Sections of cankers that formed on the bark were placed on PARPH agar to detect *Phytophthora citricola*. Three soil samples collected from around each plant were placed on PARPH agar to isolate and detect *Phytophthora citricola*. All experiments were performed three times.

In the field, snails were collected from around the base of the trunk of trees infected with *Phytophthora citricola*. Snails were washed with distilled water and placed in cages, and the feces were collected for microscopic examination. Samples of the feces were placed on PARPH agar to detect *Phytophthora citricola*. Samples of the feces were also used for inoculation of 10 *Persea americana* avocado plants in the greenhouse. Experiments were performed three times.

## Results

**SUGARY EXUDATE AS A SOURCE OF *PHYTOPHTHORA CITRICOLA* INOCULUM.** Microscopic examination of the liquid sugary exudate associated with the canker development caused by *Phytophthora citricola* (Fig. 1) revealed the presence of fungal oospores and hyphal fragments (Fig. 4), whereas the dry sugary (Fig. 2) material contained only oospores (Fig. 4). The identity of *Phytophthora citricola* was confirmed, since 100% of the isolations on PARPH agar yielded *Phytophthora citricola*. Placing the sugary exudate on stem wounds of *Persea americana* and *Persea indica* plants and wounded stem cuttings of *Persea indica* resulted in infection and canker formation in 90%, 100%, and 100% of the sites inoculated, respectively. *Phytophthora citricola* was also identified in samples of the cankerous tissue. The sugary material that washed downward following spraying with water caused canker formation in 60% (18 out of 30 plants used in three experiments) of the wounded plants. After spraying infected plants with water or by using sprinkler irrigation, *Phytophthora citricola* was detected in 44% (80 out of 180 samples) of the soil samples. *Phytophthora citricola* was detected in 10% (18 out of 180 samples) of the soil samples of the nonsprayed and drip-irrigated plants. Over all experiments, replicate experiments were nearly identical.

**ROLE OF ANTS IN TRANSMITTING *PHYTOPHTHORA CITRICOLA* IN AVOCADO PLANTS.** Sixty percent (18 out of 30 in the three experiments) of the wounded sites made in avocado plants on which ants traversed between the sugary exudate of the canker and the soil line became infected with *Phytophthora citricola*. All of the ants feeding on the sugar exudate yielded *Phytophthora citricola* when they were plated on PARPH. In the absence of ants, wounds on the control plants did not become infected with *Phytophthora citricola*. When ants collected from the stems of infected avocado plants in the lathhouse were used as inoculum, 67% (20 out of the 30 plants used in the three experiments) of wound sites on avocado stems became infected. Ants that fed on the sugary exúdate in artificial colonies carried *Phytophthora citricola* and caused 73% (22 out of 30 stem cuttings used in three experiments) of the wounded stem cuttings to develop cankers. No cankers developed in wounded stem cuttings without ants. All

isolations from cankers on PARPH agar confirmed the presence of *Phytophthora citricola*.

**ROLE OF SNAILS AS DISPERSAL VECTORS OF *PHYTOPHTHORA CITRICOLA* TO AVOCADO PLANTS.** Oospores and hyphal fragments were microscopically detected in the feces of snails that fed on cankered avocado stem cuttings. Feces of the snails that were fed on either infected plants in the greenhouse or infected stem cuttings in moist chambers tested positive for the presence of *Phytophthora citricola* on PARPH agar. Placing feces of snails that were fed on cankered stem cuttings onto wounds caused infection and canker development in 77% (23 out of 30 plants in three experiments) of the *Persea americana* plants and 85% (51 out of 60 stem cuttings used in three experiments) of the *Persea indica* stem cuttings.

Snails which fed on infected *Persea americana* plants caused infection and canker development in 40% (12 out of 30 plants used in the three experiments) of the *Persea americana* plants and 55% (33 out of 60 stem cuttings used in the three experiments) of the *Persea indica* stem cuttings. *Phytophthora citricola* was detected in 100% of the newly developed cankers using PARPH agar. Eighty percent (72 out of 90 samples in three experiments) of the soil samples collected from the area around the trunks of *Persea americana* plants inoculated with *Phytophthora citricola* via snails yielded *Phytophthora citricola* on PARPH agar (Table 3).

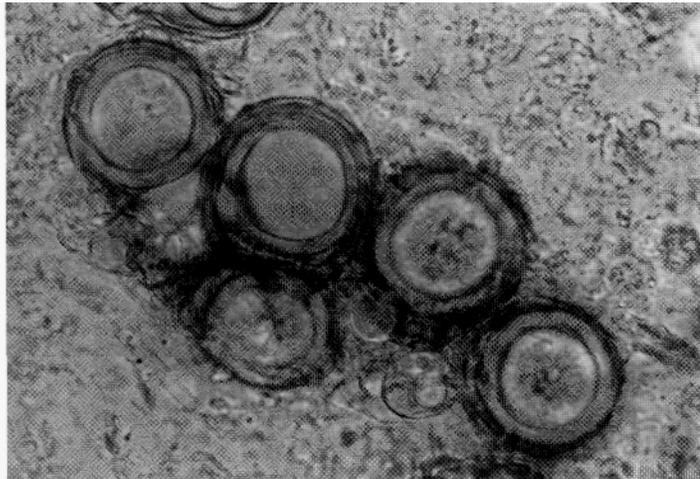


Fig. 4. Oospores and hyphal fragments of *Phytophthora citricola* found in the sugary material exuded during canker development after infection of avocado stems.

Feces from snails collected from around the base of the trunk of *Phytophthora citricola*-infected avocado trees in the field tested positive for *Phytophthora citricola* on PARPH and were found to contain oospores and hyphal fragments of *Phytophthora citricola*. Inoculating *Persea americana* in the greenhouse with feces material from snails collected from field trees infected with *Phytophthora citricola* resulted in infection of 60% (18 out of 30 plants used in the three experiments) of the *Persea americana* plants

## Discussion

The sugary material exuded by the *Phytophthora citricola* infected stems of avocado trees (Figs. 1 and 2) was an important source of inoculum since it contained viable fungal oospores and mycelial fragments. The sugary exudate can be disseminated by rain or sprinkler irrigation to the soil or other wounded parts of the tree (El-Hamalawi and Menge, 1994a). Also, the dry sugary material was frequently observed to drop off the stem cankers and mix with the soil around the base of the tree, which contaminates the soil with *Phytophthora citricola*. Zoospores formed from oospores in water films can be transmitted throughout the tree, to other avocado trees, or to the soil surface by

surface water after rain or sprinkler irrigation. Application of the fungicide fosetyl-AI as a paint on the canker is an effective method to prevent the sugary exudate from being an inoculum source for disease transmission. Recent results indicate that fosetyl-AI could cure the canker disease and eradicate *Phytophthora citricola* in the plant tissues (El-Hamalawi and Menge, 1994b).

Our observation in the field and the lathhouse indicated that ants (*Iridomyrmex humilis*) were attracted to the sugary exudate produced on developing cankers caused by the infection of avocado plants by *Phytophthora citricola*. Since the sugary exudate contained viable propagules such as oospores and hyphal fragments, these could be readily transmitted from one part of the tree to another. Ants may also transmit the inoculum from tree to tree or to the soil.

The feces of the snails collected from the field contained viable oospores and hyphal fragments of *Phytophthora citricola*, which indicates the possible importance of snails in spreading the stem canker disease in avocado groves. The presence of oospores and hyphae in the feces also confirmed that propagules of *Phytophthora citricola* are resistant to the digestive secretion of the snail. The fungus does not only survive inside the snails, but the inoculum potential may increase as it passes through the alimentary canal (Gregg, 1957). The passage of oospores of *Phytophthora cactorum*, *Phytophthora erythroseptica* and *Phytophthora megasperma* through the snails *Planorbarius corneus* (Linn.), *Helix aspersa* (Muller), and *Eopania vermiculata* (Multer) resulted in a high percentage of oospore germination (Gregg, 1957; Shaw, 1967). The passage of oospores of *Phytophthora palmivora* through the snail *Cryptozona semirugata* Beck, also resulted in a high percentage of oospore germination (Dutta et al., 1984; Kumari et al., 1988).

Since a considerable amount of inoculum was carried by snails and ants, these organisms may be important factors for the dispersal of *Phytophthora citricola* in avocado orchards and should not be ignored. It is possible that other insects and arthropods also may disperse inoculum of *Phytophthora citricola* in a similar manner in the field. Controlling snails and ants in the avocado groves should be included in the strategy for controlling the stem canker disease.

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