Xanthomonas campestris Associated with Avocado Canker in California

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ABSTRACT

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Canker symptoms were observed on avocado trees in several groves in southern California. The symptoms varied from small, sunken, dark areas with watery, reddish brown tissue beneath the bark to large, watery areas up to 10 cm in diameter, with the bark split open and a white powdery exudate on the surface. Multiple cankers on trunks and branches were usually connected by reddish brown necrotic streaks beneath the bark and often in the wood. Yellow mucoid bacteria, identified as *Xanthomonas campestris*, were isolated from 25 canker samples from five counties. Inoculation of the bacteria to stems of avocado plants resulted in a spreading internal reddish brown necrosis, accompanied by multiplication of the bacterium. No symptoms were produced by inoculation of avocado leaves.

Cankers have been observed on trunks and branches of avocado (*Persea americana* Miller) trees in southern California groves for many years, but the occurrence has generally been infrequent and the cause not determined. In 1980, similar canker symptoms were observed on avocado trees in South Africa, and a report of the disease was published in 1982 (8). The symptoms of canker in both countries are very similar. Lesions first appear as slightly sunken dark areas on the bark, with a necrotic, watery pocket underneath the sunken area. In more developed cankers the bark splits, and fluid oozes out and dries; this leaves a powdery white residue around the periphery and sometimes over the lesions. Cankers are typically in the range of 2–10 cm in diameter and usually appear first at the base of the tree and spread mostly upward, often in a straight line on one side of the trunk or branch. Necrotic streaks extend from the necrotic areas underneath cankers both above and below the lesions. Necrotic streaks between cankers are usually in the xylem, sometimes toward the center of branches or trunks.

The causal agent of the canker disease in South Africa was identified as *Pseudomonas syringae* (4,5,9). In 1990, investigation of the disease in California was begun to determine whether it was also caused by *P. syringae*.

MATERIALS AND METHODS

Samples were obtained from avocado groves in San Diego, Orange, Los Angeles, Ventura, and Santa Barbara counties in southern California. Excised cankers, branches, or tree cores taken through cankers were brought back to

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the laboratory for isolation of bacteria. Isolations were usually made from the margins of necrotic areas beneath the cankers or along the necrotic streaks that extended above and below cankers. Such tissue was macerated in sterile water and streaked onto agar medium. Some bacterial isolations were made directly from ooze emanating from the canker surface. Several selective and non-selective media were evaluated in initial isolations, but for most samples three media were used: yeast extract-dextrose-calcium carbonate (YDC) agar (10), a semiselective Tween agar medium (7),

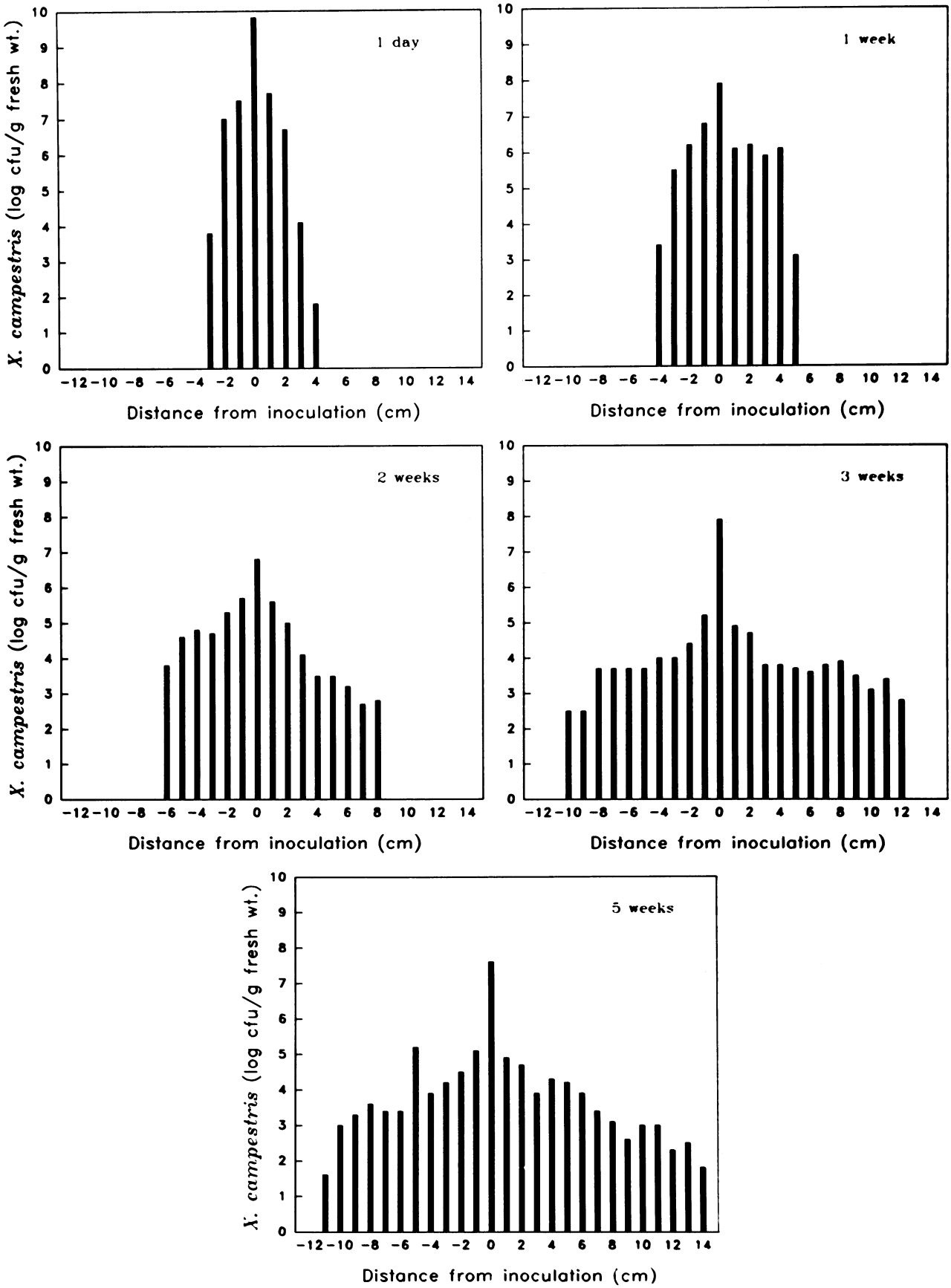

Fig. 1. Population levels of *Xanthomonas campestris* strain 06903 in avocado stems from 1 day to 5 wk after inoculation.

and Bacto Pseudomonas Agar F (Difco Laboratories). Bacterial colonies were restreaked to single colonies several times and frozen at −80 C in 40% glycerol for routine use and lyophilized in 20% powdered milk for permanent storage.

Bacteria were inoculated to *P. americana* cv. Hass plants by making a single 5-mm-long wound with a razor blade about 15–20 cm above the graft union and placing 20 μl of a bacterial suspension (1.5×10^{11} colony-forming units [cfu] per milliliter) onto the wound. For the study, two plants per strain were inoculated with five different strains of *X. campestris*. A total of 21 plants were inoculated with strain 06903 for the more detailed multiplication study (Fig. 1), and three plants were harvested at each sampling period. The 1-yr-old plants were obtained from a commercial nursery. Plants were maintained on a greenhouse bench with overhead misting every 10 min for 2.5 sec for 24 hr/day. The temperature-controlled greenhouse ranged from 19 C at night to 30 C during the day with natural lighting only. To monitor the spread and multiplication of the bacteria in plants, the stems of three plants at each sampling period were surface-sterilized with 95% ethanol and cut into 1-cm sections. The sections were weighed and diced in a known volume of sterile water. The suspensions were dilution-plated onto the Tween medium to calculate cfu per gram of tissue. Inocu-

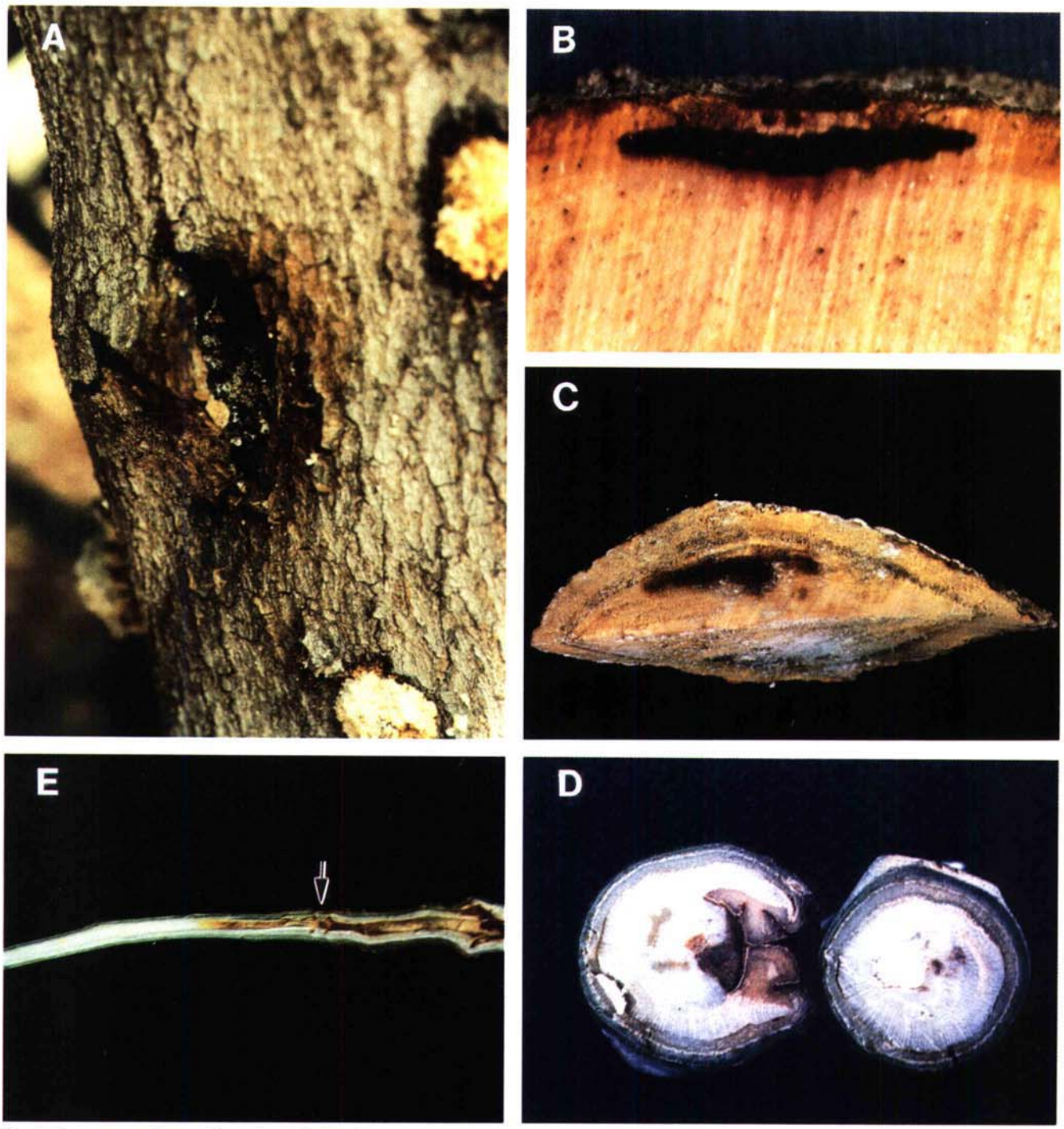

Fig. 2. Symptoms of bacterial canker. **(A)** Canker symptoms on an avocado tree trunk. **(B)** Cross section of canker showing necrotic pocket beneath the bark of a canker. **(C)** Cross section above a canker showing necrotic streaks extending from the canker. **(D)** Cross section of avocado stem 7 wk after inoculation. The section at left was through the inoculation point, and the section at right was 12 cm above the inoculation point, where narrow necrotic streaks were still visible. **(E)** Longitudinal section of an avocado stem 4 wk after inoculation with *Xanthomonas campestris*. The arrow indicates the inoculation point.

lation of avocado leaves on Hass plants was performed by dipping a sterile toothpick into a single 2-day-old bacterial colony on a YDC plate and stabbing the toothpick into the leaf. Plants were maintained under mist conditions as described above. Alternatively, a bacterial suspension (about 10^6 cfu/ml) was infiltrated into sectors of leaves with a syringe that had the needle removed. Detached leaves were surface-sterilized, maintained on water agar, and inoculated as described for citrus bacterial pathogens (1). For each leaf inoculation method, two to six sites per leaf were inoculated with bacteria, and two leaves each of small, medium, and large sizes were inoculated for each of five strains. Each leaf inoculation experiment was done twice.

RESULTS

Symptoms were similar to those described for bark canker of avocado in South Africa (4,5,8,9), except that the necrotic streaks that extended above and below cankers were larger and more extensive than previously described. The necrotic streaks were usually 0.5–1.0 cm in diameter, well below the cortex and into the wood of branches and trunks. Cankers that were separated by 30 cm or more were often connected by continuous necrotic streaks.

As observed by phase-contrast microscopy of tissue sections, rod-shaped bacteria were abundant in the ooze emanating from cankers, in the necrotic areas below cankers, and in the necrotic streaks (Fig. 2) that extended into the wood above and below cankers on branches and tree trunks. Isolation on nonselective media indicated that a wide variety of gram-negative and gram-positive bacterial species inhabited these tissues. Yellow mucoid colonies that resembled *Xanthomonas* were isolated on YDC and the Tween medium from 25 of 69 samples (Table 1). These bacteria were further identified as *X. campestris* (Table 2). Early in the study, *X. campestris* was recovered from a positive sample after 2–3 wk of storage at room temperature. Therefore, samples were normally transported to the laboratory and stored at ambient temperatures before sampling within 1 day.

Inoculations of Hass avocado plants were initially performed with five strains of *X. campestris* isolated from avocado cankers. No disease symptoms were observed after inoculation of leaves on plants held under mist conditions or after inoculation of detached leaves. Stem inoculations with each strain resulted in a vascular and pith necrosis (Fig. 2E) that spread above and below the inoculation point over time to a distance of 15 cm or more. Such necroses were not observed in control plants similarly wounded and inoculated with water only. Cankers resembling young lesions on trees observed in the field developed in stems at the point of inoculation with *X. campestris*, and some occasionally developed at points distal to the inoculation point.

One strain (06903) was selected for a more detailed study of spread and multiplication of the bacterium in avocado stems over time. Within 5 wk, this strain of *X. campestris* had spread 11 cm below and 14 cm above the inoculation point (Fig. 1). With only one exception, the bacteria in each of the three replicate plants were recovered at the same distance below the inoculation point at each harvest date, but the endpoint of recovery above the inoculation point was more variable (± 1–5 cm) between replications at the later harvest dates. The distances above the inoculation point indicated in Figure 1 reflect the maximum distances at which the bacteria were recovered from the three replicate plants, but the population numbers are averages for the three plants. Populations generally reached 10^3–10^5 cfu/g of stem tissue in newly colonized areas above and below the inoculation point. Necrosis of vascular and pith tissues generally followed the spread of the bacterium. Although *X. campestris* was detected continuously from the inoculation point to the endpoints of detection, the necrosis observed in stem sections was discontinuous along the length of infected stems. Some sections farther from the inoculation point were necrotic, whereas sections closer to the inoculation point were free of necrosis but contained high levels of bacteria. A few plants were smaller than average at the beginning of the experiment, and the inoculation point was closer (8–10 cm) to the graft union than most other plants (15–20 cm). With these smaller plants, the bacterium was never detected below the graft union, suggesting that this might present a barrier to the spread of the bacterium. The internal necrosis resulting from inoculations also stopped at the graft union.

Plants from this experiment with strain 06903 were sampled at 2 and 4 mo after inoculation. No further spread of the bacterium was detected at these later samplings. The bacterium was not detected continuously in stem sections at these later dates, as it had been previously. Instead, *X. campestris* was concentrated in 1- to 6-cm sections of the stems, with gaps of 2–4 cm where the bacterium was not detected. Where detected, the population levels of *X. campestris* after 4 mo were in the range of 10^2-10^4 cfu/g.

Strain 01861 of *X. campestris* pv. *campestris*, isolated from cauliflower, was also inoculated to avocado stems, but this bacterium did not move beyond the 1-cm section at the inoculation point for up to 10 wk after inoculation. In addition, callus tissue developed at the point of inoculation of *X. c. campestris*, rather than the canker tissue that developed at the point of inoculation of the avocado strains.

Table 1. Recovery of *Xanthomonas campestris* from canker samples from five counties in California

County	Grove	Samples: Number tested	Samples: Positive for *X. campestris*
San Diego	A	9	3
Orange	A	2	2
Los Angeles	A	4	2
Ventura	A	3	3
	B	1	1
	C	4	3
	D	9	7
	E	10	0
	F	2	0
	G	3	0
	H	9	2
	I	1	1
Santa Barbara	A	11	1
	B	1	0
Total	14	69	25

Table 2. Comparison of bacterium from avocado canker in California with *Xanthomonas campestris*

Characteristic	Avocado isolate 06903	*X. campestris*[a]
Yellow colonies	+	+
Mucoid growth	+	+
Gram reaction	−	−
Single polar flagella	+	+
Oxidase reaction	−	−
Catalase reaction	+	+
Gelatin hydrolysis	+	+/−
Nitrate reduction	−	−
Arginine dihydrolase	−	−
Growth factor required	+	+

[a] Results as reported by Krieg and Holt (6).

DISCUSSION

Although symptoms of the canker diseases in California and South Africa are similar, the disease in California was associated with *X. campestris* rather than *P. syringae*. Both pathogens caused a similar spreading necrosis in stems of inoculated avocado plants. Cankers resembling early symptoms on mature trees developed after inoculation of young plants with the *X. campestris* strains from avocado in California, but mature trees have not yet been inoculated to observe the development of large cankers.

Both pathogens have been inconsistently isolated from canker samples in this study and elsewhere (5). This is not too surprising, however, considering the high numbers of saprophytic bacteria that invade the necrotic tissues associated with the cankers. In addition, several of the samples from California that we received probably had black streak disease (2,3) instead of bacterial canker.

Neither pathogen appears to be par-

ticularly aggressive or destructive in pathogenicity tests with avocado plants, and no symptoms have been produced by inoculation of leaves or fruit. Although widespread geographically, the percentage of trees showing canker symptoms in groves is usually low. Some heavily infected trees have shown poor growth and yields, however, suggesting that further studies on variation in aggressiveness among strains and the source and spread of these pathogens should be pursued.

ACKNOWLEDGMENT

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