

Canker of Loquat Caused by *Pseudomonas* sp.

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ABSTRACT

A species of *Pseudomonas* that incites stem cankers on loquat trees was found in California for the first time in 1969. The bacterium, which apparently infects only loquats, is a green fluorescent, arginine dihydrolase-negative, oxidase weak-positive, and lipase-producing pseudomonad. The bacterium is serologically unrelated to *Pseudomonas syringae* and *P. savastanoi*, and cannot be identified with *P. eriobotryae*. *Phytopathology* 61:248-249.

Cankers not previously reported in California were recently observed on several loquat trees (*Eriobotrya japonica* [Thunb.] Lindl.) growing at Chico, Calif. Symptoms were similar to the canker phase of a loquat disease described in Japan and reported to be caused by a bacterium, *Pseudomonas eriobotryae* (Takimoto) Dows. (2, 5, 6, 10). We isolated the causal organism, and studied its physiological and biochemical activities on several different media and its relationship to *P. syringae* and *P. savastanoi*.

The noticeable feature and only symptom of the disease observed is the presence of numerous stem cankers of various sizes. Cankers apparently start on relatively young growth and increase in size as the stem enlarges with age. A canker first becomes evident when the bark over the initial lesion shrinks, becomes shredded, and sloughs away, producing an open wound. As the canker enlarges, callus tissue forms a tan to brown margin and eventually develops an irregular-shaped "lip" around the edge (Fig. 1). The exposed and affected wood within the canker appears as a charcoal-black, concentrically depressed cavity. Cankers on large limbs may become 3-6 inches long and may girdle the limb.

No parasitic fungus was observed and isolated. Initial attempts to isolate bacteria during the summer were not successful, but they were readily isolated from cankers in late fall and midwinter on nutrient agar (Difco), EMB agar (Difco), and *Pseudomonas* agar F (Difco). With a sterile needle, the isolated bacteria were inoculated in late fall into the stems of loquat seedlings in cans. The seedlings, with inoculation sites wrapped with moist sterile cotton pads, were maintained in a moist chamber for 4 days in the greenhouse. Canker lesions developed 5 weeks after inoculation. A bacterium, designated SL 404, was reisolated from the cankered loquat seedlings. Cultural characteristics were similar to those of the originally-isolated bacterium. In a preliminary screening test for phytopathogenicity, tobacco leaves (*Nicotiana tabacum* L.) exhibited a hyper-

sensitive reaction to the bacterium after 24-hr incubation. *Pseudomonas syringae* SL 414 and *P. savastanoi* SL 409 were not pathogenic on loquat.

Morphological and cultural characteristics of isolate SL 404 are: gram-negative rods $0.7-0.9 \times 1.2-3.0 \mu$, motile by 1-3 polar flagella, no capsule, no spores; colonies on nutrient agar round, rough, white, and glistening, the substratum becoming light brown in 3 days; on *Pseudomonas* agar F, the organism produces a greenish fluorescent pigment, and the substratum turns dark brown in 1 week; on Tween 80 (polyoxyethylene sorbitan monooleate) agar, for a lipase test (8), the substratum turns dark brown in 5 days.

Physiological characteristics of isolate SL 404 are: aerobic; gelatin not liquefied; milk, alkaline, not coagulated, peptonized slowly; nitrites not produced from nitrate; indole not produced; lipase produced; hydrogen sulfide produced; starch not hydrolyzed; Usinsky's solution turns greenish; acid but no gas from arabinose, glucose, galactose, mannitol, levulose, salicin, and xylose; no acid or gas from aesculin, cellobiose, sorbitol, and sucrose; negative for 2-ketogluconate (3), and arginine dihydrolase (11); weak-positive for oxidase (9); positive for β -glucosidase (1); minimum temp 6 C, optimum temp 25 C, maximum temp 32 C, and thermal death point 36 C in 3 days.

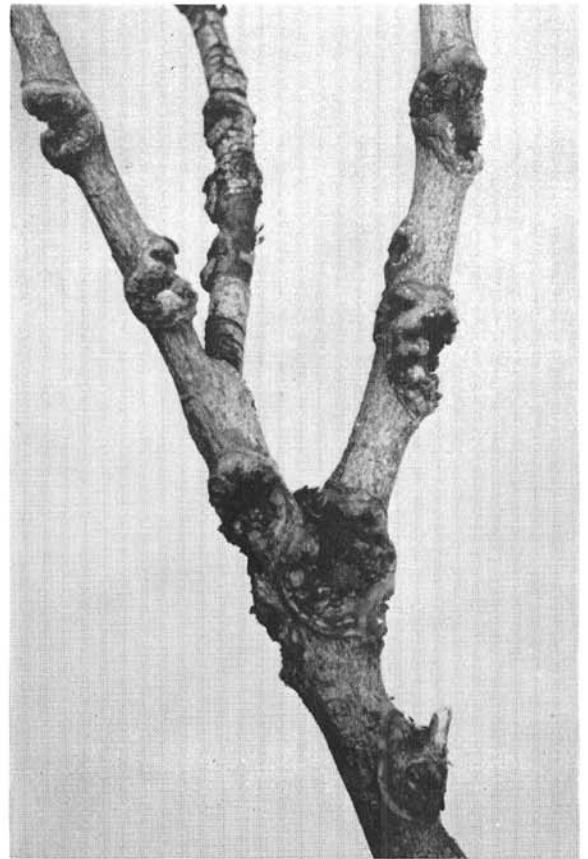


Fig. 1. *Eriobotrya japonica* (Thunb.) Lindl. stem canker caused by *Pseudomonas* sp.

Some of the physiological characteristics of isolate SL 404 are similar to those described for *P. eriobotryae* by Muko (5) and Okabe (6), but their bacterium liquefied gelatin, produced acid from glycerol, and did not produce hydrogen sulfide. Our bacterium showed negative results for these first two tests and positive for the third test. Okabe (6) indicated three strains of *P. eriobotryae* in Japan; our bacterium cannot be identified with any strain of this species.

Misaghi & Grogan (4) reported that of 108 cultures of phytopathogenic pseudomonads representing 11 nomenclatures, all except *P. marginalis* and *P. marginata* do not produce lipase. Our bacterium tested is positive for lipase. Hildebrand & Schroth (1) indicated that the nongall-forming phytopathogenic pseudomonads and the soft-rot group show high β -glucosidase activity, whereas the gall-forming and soil-inhabiting pseudomonads show none. Our isolate SL 404 from loquat canker exhibits strong β -glucosidase activity.

Antisera against isolate SL 404 and *P. syringae* SL 414 were prepared and used for agglutination and agar-gel diffusion tests. Agglutination tests were made with twofold dilution sera in a series of 10- \times 75-mm test tubes using 0.85% sterile phosphate-buffered saline solution (pH 7.0) as diluent. The results of agglutination tests indicate no serological relationship between isolate SL 404, and *P. syringae* SL 414. No agglutination was detected between SL 414 antiserum and SL 404 antigen, nor between SL 404 antiserum and SL 414 antigen.

Agar-gel (0.85% Ionagar and 0.05% sodium azide) tests were performed in patterns of six peripheral wells located 4 mm from a central well. All wells were 4 mm in diam. Isolate SL 404, *P. syringae* SL 414, and *P. savastanoi* SL 409 were tested against isolate SL 404 antiserum. Precipitin bands formed only by the reaction between isolate SL 404 antiserum and its antigen.

On the basis of the above tests, we consider our iso-

late SL 404 from loquat canker in California to be a pseudomonad belonging to the arginine dihydrolase-negative, green fluorescent group (7); it does not appear to be the same species as *P. eriobotryae* reported previously on this host.

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