Pseudomonas Canker of Kiwifruit

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ABSTRACT

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A new disease of kiwifruit (*Actinidia chinensis*) has been observed in California. Symptoms appear in early spring as wilt and cane blight. Cane cankers with deeply shriveled and dried bark appear later. Internal canker tissue appears red-rusty brown. Cane cankers are usually found adjacent to pruning wounds. A fluorescent *Pseudomonas* sp. that produces a hypersensitive response on tobacco is commonly isolated from canker tissues. Further characterization has shown most *Pseudomonas* isolates to be positive for syringomycin production, potato rot, sucrose utilization, and levan production and negative for oxidase, arginine, and D(−)tartrate utilization. Inoculated peach and kiwifruit shoots have shown injuries similar to field symptoms when subjected to low temperatures. Koch's postulates have been satisfied using seedling plants grafted to cultivar Chico-Hayward.

A canker disease of kiwifruit (*Actinidia chinensis*) has been observed in California. The first incidence was seen on nursery stock in spring of 1980. Last season, a similar problem was noticed in three mature vineyards. Other workers have observed this problem in Butte, Yuba, Sutter, Stanislaus, Merced, Santa Clara, and Santa Cruz counties.

The first symptoms appear in early spring after established plants begin to leaf out. Young vigorous canes showed tip crosier, leaf wilt, and cane blight similar to fire blight. External cane symptoms are deeply shriveled and dried bark. When the bark is removed, a distinct margin of bright green healthy tissue and red-rusty brown tissue is usually found. In most cases, a pruning wound is found close to the shriveled cane. In some cases, the major cordon is involved and the plant is killed back past the bud union almost to the soil line. When these plants resume growth in spring, a rusty red exudate is seen at the canker margin and prolific suckering occurs from the rootstalk. When isolations were made from active margins of diseased tissues, a fluorescent *Pseudomonas* sp. was consistently isolated. The disease syndrome is very similar to bacterial canker or sour sap of stone fruits incited by *Pseudomonas syringae* (15). Blossom blight of kiwifruit incited by *P. viridiflava* has been reported by workers in New Zealand (1). The purpose of this paper was to isolate and identify the cause of the canker disease.

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MATERIALS AND METHODS

Isolation of the pathogen. After bark was cut away from cankered canes, small chips of vascular tissue and pith were removed from the canker margin and macerated in 2 ml of sterile distilled water with a mortar and pestle, then the macerate was streaked on plates of King's B medium (7) and incubated for 48 hr at 25 C. Test isolates of bacteria from kiwifruit cankers as well as comparison isolates were maintained on nutrient agar during the study.

Bacterial cultures used for comparative studies. Three unknown bacteria, 1592, 1652, and 1997, were originally isolated from infested kiwifruit canes collected from a vineyard in Suisun Valley at different times during spring 1982. Pathogenic isolates of *P. syringae* from lilac, magnolia, and philadelphus were provided for comparison from the collection of M. Lai.

Characterization of bacterial isolates. Test isolates from kiwifruit and comparison isolates were characterized by the following tests: ability of the bacteria to rot onion bulbs, carrot roots, and celery stems in moisture chambers at 25 C; tobacco hypersensitivity (8); oxidase reduction (11); production of fluorescent pigment (7); 2-ketogluconate reduction (11); presence of lipase (12); starch hydrolysis (6); presence of arginine dihydrolase (13); levan production (3); nitrate reduction (11); presence of DL-phenylalanine deaminase (12), lecithinase (5), peroxidase (11), and pectinase (4); liquefaction of gelatin (11); growth in 6% (w/v) NaCl in nutrient broth; growth at 40 C in nutrient broth; production of syringomycin (2); and ice-nucleation ability (10). Flagella arrangement as well as size and shape of bacteria were determined by transmission electron microscopy (TEM) after growth for 16 hr in nutrient broth.

Pathogenicity tests. Pathogenicity of three kiwifruit isolates was compared with three known isolates of *P. syringae*. Isolates were grown for 48 hr on nutrient agar at 24 C. Suspensions were prepared by flooding plates with 10 ml sterile distilled water. Five-day-old cowpeas (California Blackeye 3) were dusted with carborundum and inoculated by rubbing a cotton swab dipped in the appropriate bacterial suspension over the leaf surfaces (9); the plants were maintained on a greenhouse bench under moist conditions for 3 days. Detached peach shoots of cultivar Red Haven, detached kiwifruit shoots of cultivar Chico-Hayward, and intact kiwifruit plants grafted with Chico-Hayward were inoculated individually with the three unknown and three known isolates of *P. syringae*. Detached shoots were surface-sterilized in 0.01% NaOCl and 95% ethanol. Basal ends were sealed with melted wax and shoots placed in sterile test tubes with moistened blotter paper as a modification of a technique used by Weaver (14). Distal ends were cut with sterile pruning shears and a drop of bacterial suspension applied with a sterile swab before the tubes were capped and sealed with Parafilm. Intact plants were inoculated by making wedge-shaped wounds (2–3 mm) at seven equally spaced sites on each scion. A drop of each bacterial suspension was applied to the appropriate wound site using a swab applicator. Masking tape was used to cover each wound site. Detached shoots and intact plants were incubated at 6 C for 7 days, then exposed to −5 C for 2 hr and incubated another 7 days at 6 C. A replicate group of inoculated shoots and intact plants was held at 6 C during the entire incubation period.

RESULTS

Isolation and characterization of the pathogen. In all fresh material collected and used for isolation, a fluorescent pseudomonad was found. When isolations were attempted late in the season (after mid-May), numerous contaminant bacteria were obtained and it became increasingly difficult to isolate fluorescent pseudomonads. Results of biochemical and physiological tests used to compare isolates of these fluorescent pseudomonads with known isolates of *P. syringae* indicated the isolates from kiwifruit were similar to *P. syringae* isolated from other plants (Table 1). Isolate 1593 differed only in its ability to rot carrot roots, isolate 1652 was unable to produce levan,

Table 1. Comparison of biochemical and physiological tests of three isolates of fluorescent pseudomonads from kiwifruit (1592, 1652, 1997) with *Pseudomonas syringae* isolated from lilac, magnolia, and philadelphus

Comparative tests	Isolate					
	Actinidia sp. (1592)	*Actinidia* sp. (1652)	*Actinidia* sp. (1997)	*P. syringae* (lilac)	*P. syringae* (magnolia)	*P. syringae* (philadelphus)
Onion bulb rot	+	+	+	+	+	+
Carrot root rot	+	−	−	−	−	−
Celery rot	−	−	−	−	−	−
Tobacco hypersensitivity	+	+	+	+	+	+
Oxidase	−	−	−	−	−	−
Fluorescent pigment	+	+	+	+	+	+
2-Ketogluconate	−	−	−	−	−	−
Lipase	−	−	−	−	−	−
Starch hydrolysis	−	−	−	−	−	−
Arginine dihydrolase	−	−	−	−	−	−
Levan	+	+	+	+	+	+
Nitrate reduction	−	−	−	−	−	−
DL-Phenylalanine deaminase	−	−	−	−	−	−
Lecithinase	−	−	−	−	−	−
Peroxidase	+	+	+	+	+	+
Pectinase	−	−	−	−	−	−
Gelatin hydrolysis	+	+	+	+	+	+
Growth in 6% NaCl	−	−	−	−	−	−
Growth at 40–41 C	−	−	−	−	+/−	−
Presence of syringomycin	+	+++	+	+	?	?
Ice-nucleation ability	−	+	+	+	+	−
Flagella arrangement	Polar 1–3	Polar 1–2	Polar 1–2	Polar 1–2	Polar 1–3	Polar 1–3

and isolate 1997 gave a positive lipase and undetectable syringomycin response. All kiwifruit isolates and known isolates had ice-nucleating potential except isolate 1592 and *P. syringae* from philadelphus.

Pathogenicity tests. All test isolates as well as the known *P. syringae* isolates produced pathogenic reactions on cowpea. Inoculation of detached shoots produced lesions on kiwifruit as well as peach, with no significant differences between the known *P. syringae* isolates and those from kiwifruit. Frosted shoots were more severely affected than nonfrosted shoots. Grafted kiwifruit plants responded similarly, with significantly stronger symptoms on plants subjected to frost conditions and no significant difference in response between known *P. syringae* isolates and those from kiwifruit. Fluorescent pseudomonads were reisolated from plants inoculated with known *P. syringae* isolates and with the test isolates from kiwifruit. In every case, reisolated bacteria produced hypersensitive reactions on tobacco and had negative oxidase and arginine dihydrolase reactions.

DISCUSSION

Fluorescent *Pseudomonas* sp. isolated from kiwifruit cankers in a mature vineyard were identified as *P. syringae* based on biochemical and physiological characterization and pathogenicity tests. Although there were some minor biochemical and physiological characters (Table 1) that were inconsistent among the kiwifruit isolates, this would also appear to be the case when known isolates of *P. syringae* from other hosts were compared. The best evidence that a *P. syringae* is involved in this disease syndrome is that isolates were pathogenic on kiwifruit canes. In addition, kiwifruit isolates were hypersensitive on tobacco, oxidase negative, and were able to produce syringomycin and act as ice nucleators.

We conclude that the fluorescent Pseudomonas isolates from kiwifruit were strains of *P. syringae*. Pathogenic reactions on cowpea indicate the potential for these isolates to be pathogenic over a wide host range. Because peach shoots, kiwifruit shoots, and kiwifruit plants all showed intensified pathogenic reactions when subjected to frost, the activity of an ice-nucleating entity in pathogenicity is indicated. Thus, a ubiquitous organism with a very wide host range has been shown for the first time to incite disease on kiwifruit.

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