Bacterial Blight of Kiwifruit in California

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ABSTRACT

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Flower bud rot, blossom blight, and leaf spot of kiwifruit (*Actinidia deliciosa*) have been observed in numerous commercial orchards in California since 1986. As the diseased flower buds develop, petals become yellow-orange, then brown, and the entire bud falls from the vine. Fruit may be small, misshapen, or fail to set. The bacterial pathogens *Pseudomonas syringae* and *P. viridiflava*, in addition to the saprophyte *P. fluorescens*, consistently have been isolated from both symptomatic and asymptomatic vines throughout the spring. In pathogenicity tests, *P. viridiflava* caused significantly more flower bud rot and blossom blight, but not leaf spot, than *P. syringae*. *P. fluorescens* was not pathogenic.

In New Zealand (12), *Pseudomonas viridiflava* (Burkholder) Dowson, and in France (8) both *P. viridiflava* and *P. syringae* pv. *syringae* van Hall produce diseases of reproductive and vegetative tissues of kiwifruit, *Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson. This plant has been grown commercially in California since the mid-1960s and is currently planted on 3,640 ha (1). A bud rot and blossom blight of kiwifruit has been observed in five California counties since 1986. In California, disease outbreaks have been associated with late spring rains or when overhead sprinkler irrigation was used. The disease has not been observed in flood-irrigated vineyards. A bacterial canker disease has been reported in California (caused by *P. syringae* [9]), Japan (*P. syringae* pv. *morsprunorum* (Wormwald) Young et al [11]), and Italy (*P. syringae* [10]).

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In April, disease symptoms consisted of brown, sunken lesions on the sepals that enclose the floral buds. During bud expansion, the petals exhibited a yellow-orange discoloration that later became necrotic (Fig. 1A and B), or they failed to mature and abscised. The inner tissues of abscised buds were completely decayed.

Diseased female flowers opened more slowly than healthy flowers or did not open completely, with the sepals failing to bend backward (Fig. 1C and D). Generally, within the flowers, anthers and filaments turned orange-brown, and the styles were stunted and necrotic.

Leaf spotting occurred only after summer rains or when overhead irrigation was used. The leaf spot phase occurred along the leaf margins and began as small, yellow halos (1–2 mm), the center of which became necrotic. Later the spots enlarged and coalesced to form large irregular patches of necrotic tissue (Fig. 2).

The objectives of this study were to identify the etiological agents of the bud rot, blossom blight, and leaf spot diseases of kiwifruit in California.

MATERIALS AND METHODS

Sampling, isolation, and identification. During 1989, five diseased orchards were sampled throughout the spring and early summer months. Asymptomatic dormant twigs and buds were collected initially in March. Twigs, green stems, immature leaves, and immature floral buds were sampled from late March to late April. During bloom (mid-May to early June), when disease symptoms became evident, symptomatic blossoms were collected. A final sampling of mature leaves with leaf spot symptoms was done in July.

Each collection was maintained at 5 C for 24 hr and processed using the modified isolation methods of Gross et al (2). Asymptomatic tissue pieces were placed in separate, individual flasks containing 100 ml of 0.01 M potassium-phosphate buffer + 0.1% peptone, and shaken on a rotary shaker (300 rpm) for 2 hr. Aliquots of 0.1 ml from fivefold serial dilutions were spread in triplicate on the surface of King's medium B agar (KB) (4) and incubated at 25 C for 24–48 hr. Colonies with a green, diffusible fluorescent pigment were subcultured and stored on KB.

Symptomatic blossoms were dissected, and a few milligrams of pistils, stamens, petals, and sepals were separately comminuted in 10 ml of 0.01 M potassium-phosphate buffer + 0.1% peptone; 0.1-ml aliquots from fivefold serial dilutions were spread onto KB and processed as described above. Leaf spot

tissues were handled in a like manner.

Single colony strains were identified according to the LOPAT determinative scheme (5).

Pathogenicity tests. In greenhouse experiments, two strains each of *P. syringae* (L4-10 and B1-2-1), *P. viridiflava* (SC3-1 and 86-26), and *P. fluorescens* (Trevisan) Migula (L4-4 and SC3-2), all derived from blighted blossoms, were each used to inoculate 25 dormant buds, 12 blossoms, and 10 leaves. Inoculum consisted of a 24-hr-old colony grown on KB and adjusted with a spectophotometer to 1×10^8 colony-forming units (cfu) per milliliter of distilled water. Tenfold dilutions gave the desired inoculum levels.

Healthy canes were collected during April from an orchard with no previous disease history, and buds with attached pedicels were removed. Bud inoculation was done by injecting 0.1 ml (1×10^4 or 1×10^6 cfu/ml) of inoculum into the side of unopened buds using a syringe and 23-gauge hypodermic needle. Similar injections of sterile distilled water served as controls. The inoculated buds were placed in moist chambers (100% relative humidity) and incubated for 5 days at 25 C, after which buds were cut in half and rated for disease severity.

During May, healthy canes were collected from the same orchard, and the blossom-pedicel units were removed. Inoculum was prepared as above. Newly opened blossoms were atomized with 1 ml of 1×10^4 or 1×10^6 cfu/ml of inoculum or sterile distilled water per blossom using an airbrush (model 200-1-IL, Badger Air-Brush Co., Franklin Park, IL). Treated blossoms were incubated in moist chambers (100% relative humidity) for 3 days at 25 C before evaluating.

Healthy leaves were collected in June, and the lower sides of leaves were pressure-infiltrated with inoculum from a needleless syringe (0.1 ml of 1×10^4 or 1×10^6 cfu/ml of inoculum per leaf). Leaf petioles were submerged in distilled water, leaves were incubated in moist chambers (100% relative humidity for 5 days) at 25 C, and lesion diameters were measured. Control leaves were sprayed with sterile distilled water and handled similarly.

All inoculated and control tissues were subjected to reisolation assays to verify presence or absence of the suspect pathogens. Fisher's protected LSD was used to compare treatments in an analysis of variance (7).

RESULTS

Isolation and identification. A small variety of bacteria were isolated from dormant and actively developing tissues. The vast majority of the colonies fluoresced green or blue-green under ultraviolet (UV) light. Likewise, isolations from symptomatic buds, blossoms, and leaves yielded colonies that fluoresced green or blue-green. Approximately 50% of the strains were identified as *P. fluorescens,* 40% as *P. syringae,* and the remainder as *P. viridiflava. P. fluorescens* strains fluoresced green under UV light, were positive for cytochrome oxidase and arginine dihydrolase, were negative for potato soft rot, did not produce a hypersensitive response in tobacco inoculated with 10^6 cfu/ml after 24 hr incubation, and were variable for levan production. *P. syringae* strains fluoresced blue-green under UV light, were positive for levan production, and caused a hypersensitive reaction in tobacco; however, they were negative for cytochrome oxidase, potato soft rot, and arginine dihydrolase. *P. viridiflava* strains fluoresced blue-green under UV light, produced a hypersensitive response in tobacco and soft rot of potato pieces, and were negative for cytochrome oxidase, arginine dihydrolase, and levan production.

Pathogenicity tests. Both strains of *P. syringae* and of *P. viridiflava* were pathogenic on kiwifruit buds, blossoms, and leaves, whereas *P. fluorescens* strains were not. However, in dormant buds and blossoms, both inoculum levels of each strain of *P. viridiflava* caused more severe disease and thus received significantly higher disease ratings than both inoculum levels of each strain of *P. syringae* ($P < 0.01$) (Table 1). *P. viridiflava* caused extensive destruction of the entire floral tissues, whereas *P. syringae* affected primarily the pistils and stamens. Additionally, in bud inoculation tests, higher inoculum levels of *P. viridiflava* (1×10^6 versus 1×10^4 cfu/ml) led to significantly higher disease ratings ($P < 0.01$). Buds and blossoms inoculated with strains of *P. fluorescens* or sterile distilled water appeared healthy ($P > 0.01$). The respective bacterial species were recovered only from their

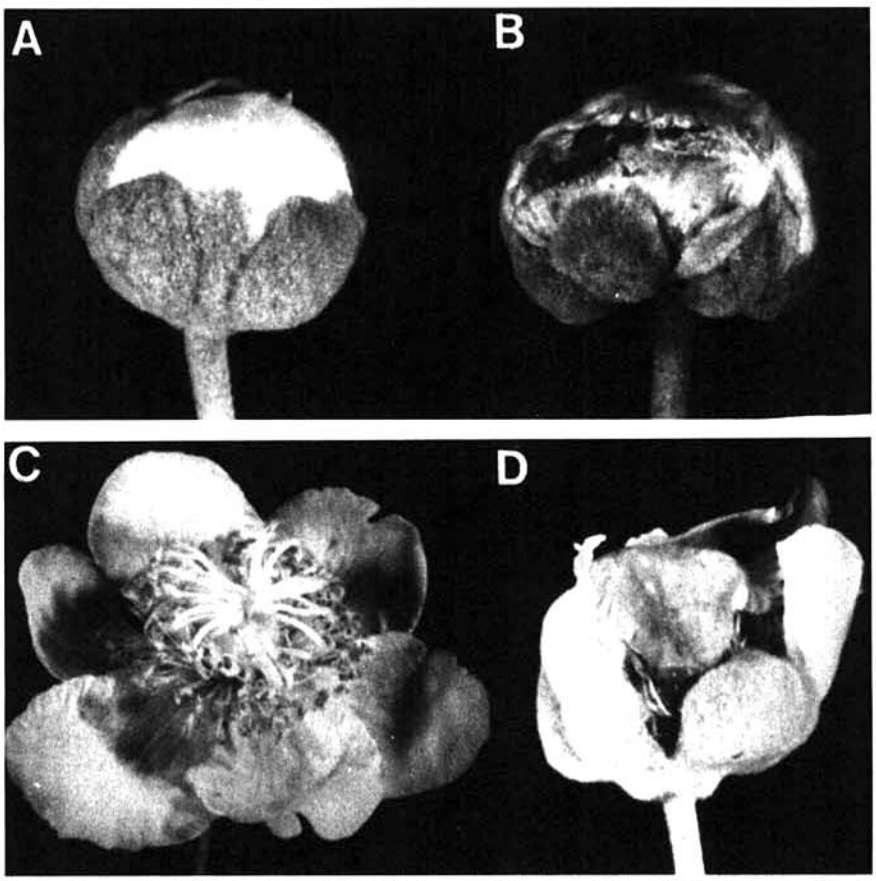

Fig. 1. Female floral buds affected with bud rot and blossom blight: **(A)** healthy floral bud, **(B)** affected floral bud, **(C)** healthy blossom, **(D)** affected blossom. Note failure of blossom to open, with sepals failing to bend backward.

Fig. 2. Kiwifruit leaf with necrotic leaf spots surrounded by yellow halos.

Table 1. Severity of disease development on kiwifruit buds, blossoms, and leaves inoculated with *Pseudomonas syringae, P. viridiflava*, or *P. fluorescens*

Isolate (strain)	Inoculum concentration (cfu/ml)	Bud rot severity[w]	Blossom blight severity[x]	Leaf spot lesion diameter[y] (cm)
P. viridiflava (SC3-1)	10^6	4.3 a[z]	3.7 a	7.0 ab
	10^4	3.6 bc	3.8 a	6.5 ab
P. viridiflava (86-26)	10^6	4.0 ab	4.5 a	6.8 ab
	10^4	3.2 c	3.9 a	7.3 a
P. syringae (B1-2-1)	10^6	2.2 d	2.5 b	6.3 ab
	10^4	2.0 d	1.9 b	5.0 b
P. syringae (L4-10)	10^6	1.8 d	2.5 b	6.0 ab
	10^4	1.8 d	2.4 b	5.5 ab
P. fluorescens (SC3-2)	10^6	0.9 e	0.5 c	0.0 c
	10^4	0.9 e	0.5 c	0.1 c
P. fluorescens (L4-4)	10^6	0.7 e	0.5 c	0.1 c
	10^4	0.7 e	0.4 c	0.0 c
Sterile distilled water	...	0.5 e	0.3 c	0.9 c
LSD (0.01)		0.4	0.9	2.1

[w] Disease severity ratings: 0 = no discoloration; 1 = wound discolored; 2 = partial discoloration of immature pistils and stamens; 3 = total discoloration of immature pistils and stamens; 4 = total discoloration or rotting of immature pistils and stamens, partial discoloration or rotting of petals and sepals; 5 = total discoloration and rotting of entire bud. Figures are means of 25 replications.

[x] Disease ratings: 0 = no discoloration; 1 = slight discoloration of petals but not of pistils, stamens, and sepals; 2 = slight discoloration of pistils, stamens, petals, and sepals; 3 = >50% discoloration of pistils, stamens, petals, and sepals; 4 = complete discoloration of pistils, stamens, petals, and sepals, <50% of tissues rotten; 5 = total discoloration and rotting of entire blossom. Figures are means of 12 replications.

[y] Figures are means of 10 replications.

[z] Means in a column followed by the same letters are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

inoculated tissues.

P. viridiflava and *P. syringae* also caused leaf spot symptoms that varied in disease severity ($P < 0.01$) (Table 1). *P. fluorescens* and sterile distilled water controls remained healthy. The three species of bacteria were reisolated on KB.

DISCUSSION

Overall, *P. fluorescens* occurred in higher frequency than *P. syringae* or *P. viridiflava* in California kiwifruit plantings. Even so, both *P. syringae* and *P. viridiflava* were recovered from dormant and asymptomatic tissue during the spring, suggesting that the pathogens overwinter near, on, or within kiwifruit vines. Based on reports describing the epiphytic phase of the life cycle of *P. syringae* on nonhost plants (3), recovery from asymptomatic tissues may have resulted from contamination of the vines from other source plants. *P. syringae* was recovered from various weed hosts in kiwifruit orchards from March through July (W. D. Gubler, *unpublished*). In California, *P. syringae* apparently is widespread. An earlier study showed this species to be the dominant ice nucleation-active bacterium on plants (6).

Our pathogenicity tests showed that *P. syringae* and *P. viridiflava,* but not *P. fluorescens,* caused disease of the various plant parts of kiwifruit and confirms the report from France (8). However, *P. viridiflava* caused more severe disease on inoculated buds and blossoms than *P. syringae*. The pectolytic activity of *P. viridiflava* (5) might have contributed to relatively higher disease on buds and blossoms.

Whether bacterial blight of kiwifruit is associated with bacterial canker of kiwifruit (9) is unclear, because periodic isolations onto KB from cankered, bleeding trunks of young vines yielded only *P. fluorescens*.

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