Bacterial Blister Bark and Blight of Fruit Spurs of Apple in South Africa Caused by *Pseudomonas syringae* pv. *syringae*

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ABSTRACT

Mansvelt, E. L., and Hattingh, M. J. 1986. Bacterial blister bark and blight of fruit spurs of apple in South Africa caused by *Pseudomonas syringae* pv. *syringae*. Plant Disease 70:403-405.

Green fluorescent, oxidase-negative bacteria were consistently isolated from margins of lesions on trunks, stems, and blossoms of diseased apple (*Malus pumila*) trees in South Africa. Strains were characterized by colony morphology on *Pseudomonas syringae* agar medium and by tests for gelatin liquefaction, aesculin hydrolysis, tyrosinase activity, and tartrate utilization. *Pseudomonas syringae* pv. *syringae* (*P. s.* pv. *syringae*) and intermediate forms predominated. Typical blister bark symptoms developed after inoculation of *P. s.* pv. *syringae* into apple trees.

Blister spot of apple (*Malus pumila* Mill.) fruit caused by *Pseudomonas syringae* pv. *papulans* (*P. s.* pv. *papulans*) (Rose) Dhanvantari (8) occurs in North America (2,6,19,20) and Italy (1). The pathogen is also associated with a canker disease of apple trees in the United States (19) and England (13). *P. syringae* pv. *syringae* (*P. s.* pv. *syringae*) Van Hall (8) causes a blight and canker disease of apple in New York State (17); however, Burr and Katz (5) recently stated that blossom and fruit infections and canker development caused by *P. s.* pv. *syringae* are rare on all apple cultivars.

In 1927, Hopkins (10) described a serious canker disease of the bark of apple trees growing in the Grabouw District of South Africa, affecting up to 80% of the trees in some orchards. His strains appeared to resemble *P. s.* pv. *papulans*. Bark diseases of apple trees caused by *P. syringae* were ignored in South Africa until 1982, when unusual symptoms were noticed in virtually all Starkrimson plantings. During the spring of 1983, fruit spurs were severely blighted and trees died back in some orchards. Pseudomonads were consistently isolated from diseased trees.

This report is concerned with the etiology of the disease in South Africa and the identity of the causal organism.

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MATERIALS AND METHODS

Sampling and isolation. Diseased material was taken during spring and summer of 1982–1983 (southern hemisphere) from apple cultivars Golden Delicious, Granny Smith, Red Chief, Smoothee, Starking, Starkrimson, Top Red, and the rootstock Merton 793. Preliminary microscopic examination showed masses of bacteria in this material. Tissue from margins of lesions and blighted blossoms was surface-disinfested with 70% ethanol, flamed, cut into small pieces, and transferred to 20-ml screw-cap bottles containing 10 ml of sterile buffered saline (18). After 1–2 hr, loopfuls of suspensions were streaked in duplicate onto medium B (MB) of King et al (11). Agar plates were incubated at 26 C for 3 days. Representative colonies of bacteria that fluoresced were purified by repeated restreaking on MB. Relatively pure cultures of these bacteria were isolated early in the growing season, but the ratio of secondary invaders increased considerably after that.

Reference strains. Strains of *P. s.* pv. *syringae* (PDDCC 397, PDDCC 409, PDDCC 457, and PDDCC 514) and *P. s.* pv. *papulans* (PDDCC 4046) obtained from the Plant Disease Division Culture Collection, Auckland, New Zealand, were included in the investigation.

Characterization of strains. Strains that fluoresced were tested for cytochrome oxidase activity with Difco oxidase differentiation disks. Oxidase-negative strains were streaked onto Difco nutrient agar supplemented with 5% sucrose (NSA) (15) and *P. syringae* agar medium (PSM) (4). Strains with colonies resembling those of the reference strains on these media were subjected to the GATTa tests (14) for gelatin liquefaction (G), aesculin hydrolysis (A), tyrosinase activity (T), and tartrate utilization (Ta). They were also tested for the production of acid from salicin (7), proteolysis of casein and action in litmus milk (7), ice-nucleation activity (16), and production of syringomycin (9).

Uninoculated control media were included in all tests, which were performed twice on different days. Inoculated media were incubated at 26 C.

Hypersensitive reaction and pathogenicity. Inoculations were performed with cultures 48–72 hr old grown on NSA. The ability of each strain to cause a hypersensitive reaction (HR) was tested on White Burley tobacco leaves (12).

Representative HR-positive strains were tested for pathogenicity to woody stems, buds, and fruit of apple. Test strains were suspended in sterile water and adjusted to 10^6 colony-forming units per milliliter. Stems were inoculated by the method of Latorre and Jones (14), and buds were inoculated by injection with a hypodermic syringe fitted with a 26-gauge needle. Results were recorded 1 mo after inoculation. Fruit on trees in orchards were inoculated at different growth stages. Each test fruit was surface-disinfested with 70% ethanol, stab-inoculated, and covered with a plastic bag for 1 day. Results were recorded after 20 days.

RESULTS

Symptoms on naturally infected trees. Circular raised tan areas appeared on the bark of apple trees in the spring (Fig. 1A). The areas ranged from 4 to 5 mm in diameter to complete girdling of the trunk. The outer tan epidermal layer of the blister covered spongy bark tissue composed of greenish cells. The blister eventually dried and flaked off, exposing underlying necrotic tissue. There was no sap or gum exudation. Necrosis usually developed at the junction of twigs and branches, at buds, and at pruning wounds. Severely affected branches often died back, whereas others survived even though lesion development was extensive. If the trunk became girdled, the tree often died within weeks.

Lesions were not detected on mature leaves or fruit of trees in orchards; however, during the spring of 1983, blight of fruit spurs occurred in some orchards. Dead blossoms were dark brown, but surrounding bark appeared unaffected (Fig. 1B). Blister bark lesions extending along branches and the main trunk developed later at the beginning of summer.

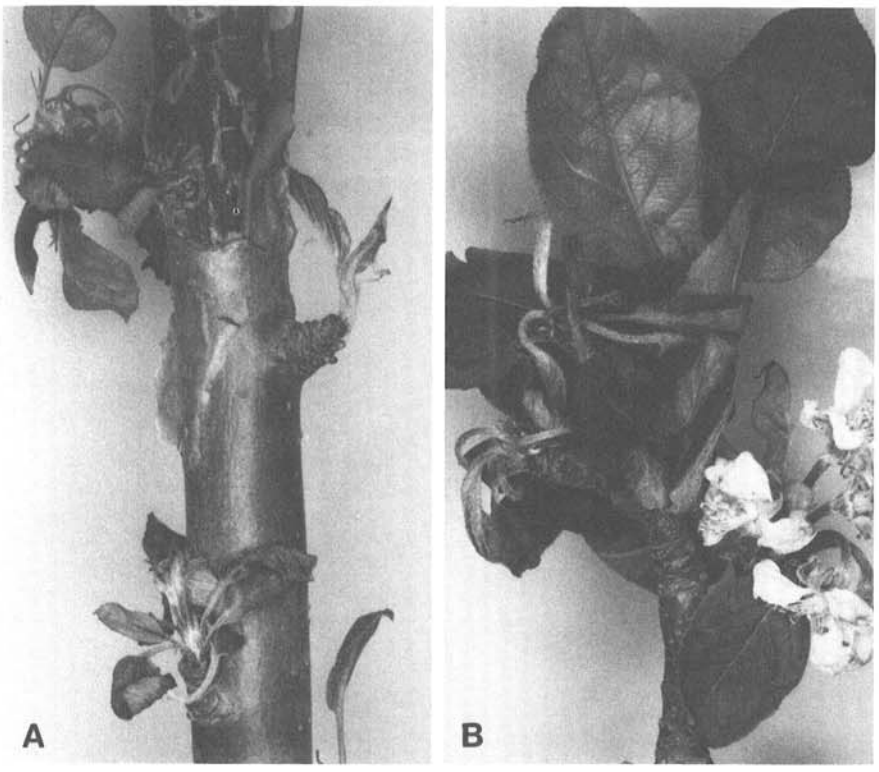

Fig. 1. Blister bark and blight of fruit spurs of apple caused by *Pseudomonas syringae* pv. *syringae*. **(A)** Raised tan area on bark and dried out epidermal layer that flakes off to expose underlying necrotic tissue. **(B)** Blight of fruit spurs.

Table 1. Number of *Pseudomonas syringae* pv. *syringae* (*P. s.* pv. *syringae*) and intermediate-form strains[a] isolated from diseased apple trees positive for different characteristics

Characteristic	Group	
	P. s.* pv. *syringae **(60 strains)**	**Intermediates (15 strains)**
Levan production	56	8
Fluorescence on PSM[b]		
Pale	28	4
Intense	2	5
Acid production from salicin	0	0
Casein hydrolysis	52	11
Reaction in litmus milk		
Alkaline	56	14
Acid	2	1
Ice-nucleation activity	49	11
Syringomycin production	38	3

[a] As defined by Latorre and Jones (14).
[b] *P. syringae* agar medium of Burr and Katz (4).

Characterization of strains. Seventy-seven strains that produced diffusible green pigments fluorescing blue under ultraviolet light (2,600 Å) on MB were obtained from lesions in bark and from blighted spurs from different apple orchards. Two strains that showed cytochrome oxidase activity were discarded.

On MB, colonies of the strains were convex, umbillicate or umbonate, smooth, and glistened with a vitreous appearance against the light. On PSM and NSA, most isolates produced mucoid, convex, umbonate or pulvinate, transparent or opaque colonies. Colonies on the three media were circular with entire or lobate margins.

Results of the GATTa tests indicated that the oxidase-negative fluorescent strains were *P. s.* pv. *syringae* (60 isolates) or intermediate forms (15 isolates) as defined by Latorre and Jones (14). None of the strains was identified as *P. syringae* pv. *morsprunorum* (Wormald) Young, Dye, & Wilkie (8). Higher ratios of the *P. s.* pv. *syringae* isolates produced levan, hydrolyzed casein, showed ice-nucleation activity, and produced syringomycin than the intermediate forms (Table 1). The two groups reacted similarly in litmus milk. None of the isolates produced acid from salicin. Although all isolates fluoresced on MB, this property was not consistent on PSM.

Hypersensitive reaction and pathogenicity. Fifty-two of the 60 *P. s.* pv. *syringae* strains and five of the 15 intermediate form strains induced an HR on tobacco plants. Of those tested, all strains of *P. s.* pv. *syringae* that induced the HR caused typical blister symptoms on bark of apple stems and necrosis of buds. Bacteria reisolated from lesions induced the HR on tobacco and proved to be *P. s.* pv. *syringae*. In contrast, the intermediate form isolates caused no symptoms on apple. Similarly, none of the *P. s.* pv. *syringae* strains caused blister spots on fruit, but areas surrounding the inoculation sites did become darkened and the pathogen was reisolated after 20 days.

DISCUSSION

The bark disease on apple in South Africa resembles conditions encountered elsewhere on this host (13,17,19). We propose the name blister bark to refer to the disease caused by *P. s.* pv. *syringae*. Unlike *P. s.* pv. *papulans*, which incites blister spot symptoms on apple fruit (1,2,6,19,20), none of our *P. s.* pv. *syringae* isolates infected fruit even when inoculated at different stages during the growing season, and blister spot was not noticed in any diseased orchard. The failure of *P. s.* pv. *syringae* to cause blister spot on fruit has also been documented elsewhere (3,20).

Blister bark occurs in all localities of South Africa where apples are produced. The disease is of economic importance on the cultivars Starkrimson and Top Red and the rootstock Merton 793. It also affects Golden Delicious, Granny Smith, Red Chief, Smoothee, and Starking. Starkrimson, the most susceptible cultivar, had numerous, extensive lesions in virtually all orchards.

We were unable to confirm the claim by Hopkins (10) that *P. s.* pv. *papulans* occurs on apples in South Africa. Two groups of oxidase-negative fluorescent pseudomonads were isolated from diseased apple trees, but pathogenicity to the host was confined to *P. s.* pv. *syringae*. However, the *P. s.* pv. *syringae* strains did not make up a homogeneous group, as indicated by their characterization in different tests (Table 1). Positive results were not recorded for all 60 strains in any of the tests. Furthermore, on PSM agar, these strains not only produced colonies and fluorescence typical of *P. s.* pv. *syringae* but some colonies appeared to be intermediate between those of *P. s.* pv. *syringae* and *P. s.* pv. *papulans*. We conclude that different phenotypes of *P. s.* pv. *syringae* are capable of causing blister bark of apples in South Africa.

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