Overwintering and Distribution Pattern of *Pseudomonas syringae* pv. *papulans* and pv. *syringae* in Apple Buds

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ABSTRACT

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Pseudomonas syringae pv. *papulans* (PSP) and pv. *syringae* (PSS) were isolated from dormant Mutsu, Golden Delicious, and Empire apple buds showing visible internal necrosis. PSP was detected more frequently from Mutsu buds than from Golden Delicious or Empire. PSP was often associated with the necrotic bud tissue and was consistently recovered from a higher percentage of necrotic buds than PSS. Both pathovars were also recovered from apparently healthy (no visible necrosis) Mutsu, Golden Delicious, and Empire buds. A characteristic distribution pattern of both bacteria was found in Mutsu buds. Neither bacterium was detected on the outer bud scales, and the highest populations were generally associated with the central bud tissues. PSP was generally recovered at higher populations from Mutsu buds, whereas PSS was predominant in Golden Delicious.

Diseases of apple caused by *Pseudomonas syringae* pv. *syringae* (PSS) and pv. *papulans* (PSP) have been reported from many areas of North America (3,7,11–14). Apple leaf scars have recently been identified as one overwintering site for PSP, the cause of blister spot (1). We reported previously that PSP was detected frequently in apple buds, which are a major overwintering site for this pathogen (5). Knowledge of the location of the pathogen within the buds may aid in development of control procedures aimed at eliminating the overwintering bacteria. This paper reports the relative occurrence and spatial distribution of PSP and PSS in apple buds and the association of PSP with internal bud necrosis.

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MATERIALS AND METHODS

During preliminary sampling, it was observed that the internal tissues of some Mutsu buds were necrotic (Fig. 1). Microscopic examination showed that masses of bacteria were associated with the necrotic tissues. Macerate from the necrotic tissues was plated on *P. syringae* medium as described previously (5). After 3 days of incubation at 28 C, numerous oxidase-negative, fluorescent pseudomonads were recovered from the necrotic tissues.

Isolations from buds. Terminal apple buds were collected from Mutsu (four) and Golden Delicious (one) orchards from 20 January to 13 February 1980. Five buds were collected from each of 10 trees per orchard, placed in plastic bags, transported to the laboratory in an ice chest, and processed within 24 hr. Individual buds were cut longitudinally and inspected for visible necrosis before grinding with a sterile mortar and pestle in 1 ml sterile distilled water. A loopful of the macerate was then streaked onto *P. syringae* medium.

In a second experiment, buds from Empire, Delicious, and Golden Delicious orchards were collected on 11 February, 12 February, and 11 March 1980, respectively. Five 10-bud samples of symptomless buds (no visible necrosis) from each orchard were ground in 5 ml

sterile distilled water and serial water dilutions were plated on *P. syringae* medium.

In a third experiment, samples of 50 buds were also taken from additional Mutsu, Empire, and Delicious orchards. Each bud was cut longitudinally to inspect for internal necrosis. Necrotic tissues were macerated and plated on *P. syringae* medium.

All *P. syringae* medium bud isolation plates were incubated for 3 days at 28 C, at which time counts of PSP and PSS colonies were made. Representative colonies were selected from isolation plates, and positive identification was made using methods reported previously (3).

Distribution pattern in buds. Terminal buds of Mutsu and Golden Delicious were collected 11 times from five orchards from 14 November 1980 to 26 January 1981. Buds were collected in plastic bags, transported to the laboratory in an ice chest, and processed within 24 hr. Each 10-bud sample was dissected by sequentially removing bud scales with a sterile scalpel and forceps. All bud scales from the same relative position from each of the 10 buds were pooled (outermost scales, second-outermost scales . . . central bud tissues), and isolations were made after washing groups of 10 bud scales each in 10 ml sterile distilled water for 15 min, with periodic agitation on a vortex stirrer. The central bud tissues, consisting of differentiated and undifferentiated shoot and leaf tissues, were also washed as one sample. Serial dilutions (0.1 ml) from each washing were plated in triplicate on *P. syringae* medium and incubated at 28 C for 3 days, then colony counts of PSP and PSS were made.

RESULTS

Bud isolations. PSP and PSS were recovered from up to 40 and 18%, respectively, of the total buds from four Mutsu orchards (Table 1). From 8 to 36% of the Mutsu buds had necrotic centers and yielded both PSP and PSS. PSP was found in 50–86% of the necrotic buds in all four samples, whereas PSS was recovered from 17% of the necrotic buds in only one of four samples. Both bacteria were also detected from apparently healthy buds that had no visible necrosis. PSP was recovered from 15–33% of the symptomless Mutsu buds, whereas PSS was recovered from 5–19% of the buds. PSP but not PSS was recovered from necrotic and healthy Golden Delicious buds sampled in January 1981.

Both PSP and PSS were detected in the symptomless 10-bud samples taken from the Empire and Golden Delicious but not from the Delicious orchard. PSP was recovered from five of five and one of five bud samples from Empire and Golden Delicious, respectively, whereas PSS was recovered from one of five 10-bud samples from both cultivars.

When 50 buds were inspected per orchard, five, two, and two buds were necrotic from Mutsu, Empire, and Delicious orchards, respectively. PSP was recovered from four of the necrotic Mutsu buds but not from those of the other samples.

Distribution pattern. A characteristic distribution pattern of PSP and PSS was found in Mutsu buds (Table 2). In all 11 samples, neither pathogen was detected on outer bud scales and the highest populations were generally associated with the central tissues. In six of the nine Mutsu samples, populations of PSP were higher than PSS; however, PSS was higher in both Golden Delicious samples. None of the buds sampled in this experiment contained visibly necrotic tissues.

Table 1. Recovery of *Pseudomonas syringae* pv. *papulans* (PSP) and pv. *syringae* (PSS) from individual terminal apple buds in 1981[a]

Date	Location[b]	Cultivar	Percent necrotic buds	Percent recovery from: Total buds PSP	Total buds PSS	Necrotic buds PSP	Necrotic buds PSS	Symptomless buds PSP	Symptomless buds PSS
20 Jan.	CO	Mutsu	36	38	18	50	17	31	19
28 Jan.	VA	Mutsu	10	32	14	80	0	27	16
30 Jan.	SM	Mutsu	8	18	8	50	0	15	9
13 Feb.	RJ	Mutsu	14	40	4	86	0	33	5
21 Jan.	SM	G. Delicious	4	14	0	100	0	10	0

[a] Fifty buds were collected randomly from an orchard, each cut longitudinally, and inspected for visible necrosis before being macerated in 1 ml sterile distilled water in a mortar and pestle. Macerate was streaked on *P. syringae* medium (5) and incubated for 3 days at 28 C before results were recorded.

[b] CO = Cohn Farm, Wayne County, NY; VA = VanAckers Farm, Wayne County, NY; SM = Smith Orchard, Wayne County, NY; and RJ = Red Jacket Orchard, Geneva, NY.

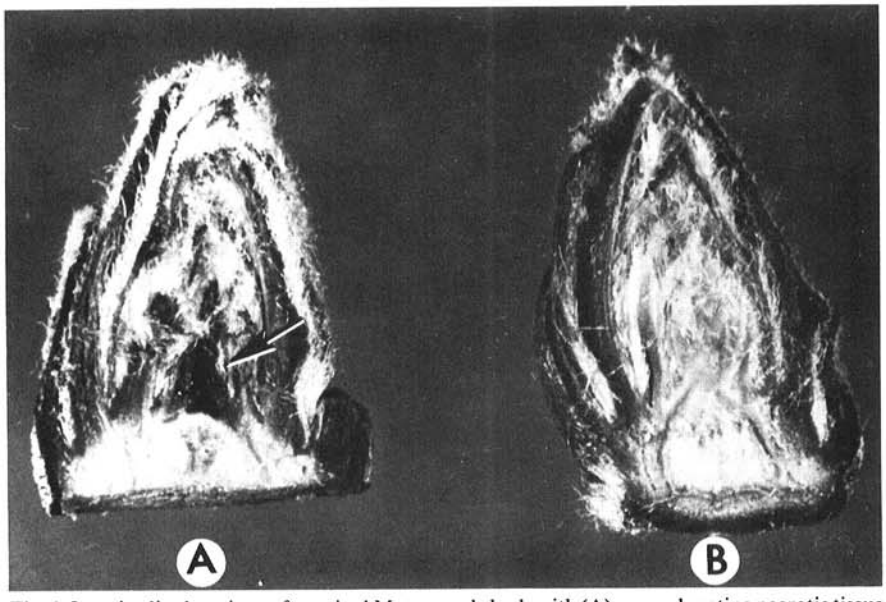

Fig. 1. Longitudinal sections of terminal Mutsu apple buds with **(A)** arrow denoting necrotic tissue and **(B)** symptomless tissues.

DISCUSSION

In New York State, severe blister spot infection caused by PSP occurs only on the Mutsu cultivar (3). Blossom and fruit infections and canker development caused by PSS are rare on all apple cultivars but may develop on pears and are more frequently a problem on sweet cherries. Despite the lack of disease on apple caused by PSS, our results indicate that apple buds can be major overwintering sites for PSS and PSP. Because significant percentages of apple buds are infested with PSS and PSP, they may provide an inconspicuous reservoir from which the pathogens could be disseminated via nursery trees to new orchard sites.

Our studies showed that PSP is frequently associated with internal bud necrosis. It is still uncertain, however, if the bacteria caused the necrosis or if they merely multiplied on necrotic tissues that were injured by freezing or other means. Some strains of these bacteria also have been shown to produce ice nuclei (10) and, therefore, they may enhance freezing injury. The exact association of PSP with internal bud necrosis and mortality needs further investigation.

Control of blister spot is now achieved

Table 2. Populations of *Pseudomonas syringae* pv. *papulans* (PSP) and pv. *syringae* (PSS) and their distribution pattern in the apple gemmisphere

Date	Cultivar	Location[b]	Bacterium	Bud scale number[a]								
				1	2	3	4	5	6	7	8	9
14 Nov. 1980	Mutsu	RJ	PSP	0[c]	0.3	1.7	13.3	5.3	260.7	220.3	···	···
			PSS	0	0.0	1.7	2.3	7.0	5.3	0.0	···	···
12 Dec. 1980	Mutsu	ST	PSP	0	0.0	0.0	4.0	1.0	0.0	0.0	0.7	···
			PSS	0	0.0	2.3	1.3	1.0	10.0	45.7	9.3	···
20 Dec. 1980	Mutsu	ST	PSP	0	0.0	0.0	4.0	4.3	3.7	130.0	6.7	863.0
			PSS	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 Dec. 1980	G. Delicious	ST	PSP	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			PSS	0	0.1	0.0	0.0	1.0	2.7	0.7	4.0	15.0
15 Jan. 1981	Mutsu	VA	PSP	0	0.0	0.1	0.7	4.7	10.3	26.7	0.0	···
			PSS	0	0.0	0.0	0.0	0.6	0.6	0.3	0.3	···
26 Jan. 1981	Mutsu	VA	PSP	0	0.1	0.7	3.3	3.7	5.0	103.3	490.0	···
			PSS	0	0.0	0.0	2.7	1.3	2.0	10.0	0.0	···
15 Jan. 1981	Mutsu	CO	PSP	0	0.0	0.1	1.0	5.3	0.0	6.7	6.7	···
			PSS	0	0.0	0.1	0.2	1.0	14.0	10.0	63.0	···
26 Jan. 1981	Mutsu	CO	PSP	0	0.0	0.3	0.7	2.7	13.8	13.3	23.3	1.7
			PSS	0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
15 Jan. 1981	G. Delicious	SM	PSP	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
			PSS	0	0.0	0.0	0.0	0.1	4.7	0.0	0.0	0.3
15 Jan. 1981	Mutsu	SM	PSP	0	0.4	24.0	6.0	12.3	47.7	26.7	190.0	···
			PSS	0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	···
26 Jan. 1981	Mutsu	SM	PSP	0	0.0	0.1	0.2	1.3	0.0	0.3	9.3	···
			PSS	0	0.0	0.0	0.0	0.0	13.0	0.0	7.0	···

[a] Ten apple buds were dissected and bud scales of similar position were pooled and washed in 10 ml sterile distilled water for 15 min. Wash dilutions (10^{-1}, 10^{-2}, 10^{-3}) were plated in triplicate (0.1 ml per replicate) on *Pseudomonas syringae* medium (5). Bud scale number one is the outermost scale, and the highest number corresponds to the central bud tissues. Numbers in between are relative to those positions.

[b] RJ = Red Jacket Orchard, Geneva, NY; ST = Orchard at Geneva Experiment Station; VA = VanAcker Farm, Wayne County, NY; CO = Cohn Farm, Wayne County, NY; and SM = Smith Orchard, Wayne County, NY.

[c] Values are average population × 10^3 colony-forming units per bud scale.

by applying three sprays of streptomycin shortly after petal fall (2), when fruit are most susceptible (4). We had hoped that if the bulk of the PSP population was on the external portion of the bud, the pathogen would be susceptible to eradication with dormant sprays. We now feel that this approach to control is unlikely to succeed because the highest populations of the pathogen are at the centers of the buds. A preliminary test using a dormant spray of Bordeaux mixture (8-8-100) failed to reduce epiphytic populations of the pathogen monitored 2 wk after spray application compared with untreated checks (*unpublished*).

Buds have previously been documented as survival sites for several microorganisms, including plant-pathogenic bacteria (6,8,9). Our results illustrate another important example of this phenomenon.

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