Pseudomonas syringae pv. *syringae* Associated with Apple and Pear Buds in South Africa

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ABSTRACT

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Starkrimson and Granny Smith apple buds from orchards with a history of blister bark and Packham's Triumph pear buds from orchards with a history of blossom blast were examined for the presence of *Pseudomonas syringae* pv. *syringae*. The bacterium was detected inside apparently healthy buds during growing and dormant seasons over a 2-yr period. Individual bud scales were colonized, and the greatest populations of *P. s.* pv. *syringae* were evident during fall and spring. Epiphytic populations of the pathogen occurred on Starkrimson buds at the green-tip growth stage and on Packham's Triumph at the budbreak stage. No or few bacteria were detected on buds of cultivar Granny Smith. More pear than apple budwood contained *P. s.* pv. *syringae*. The organism probably overwinters in dormant buds. Apple blister bark or pear blossom blast is therefore likely to develop if symptomless buds from infected trees are grafted onto rootstocks in new plantings.

Additional key word: epidemiology

Pseudomonas syringae pv. *syringae* van Hall causes bacterial blister bark of apple (17) and blossom blast of pear (18) in South Africa. The pathogen also has a resident phase on the foliage of the hosts in this country (E. L. Mansvelt and M. J. Hattingh, *unpublished*). Less is known of its association with buds and how this relates to development of the two diseases. Buds are regarded as major overwintering sites of *P. s.* pv. *syringae* (13). Leben et al (14) proposed that pathogens multiplying on the surfaces of leaflets within expanding buds account for the resident phase established on the first spring leaves. In New York State, *P. s.* pv. *syringae* and *P. s.* pv. *papulans* are more commonly present inside buds than on the outer scales (3). However, the *P. s.* pv. *syringae* isolates were not shown to be pathogenic to apple tissue.

We report the association of pathogenic *P. s.* pv. *syringae* with apparently healthy buds of different cultivars obtained from orchards with a history of apple blister bark or pear blossom blast. The presence of the pathogen in budwood material also was investigated.

MATERIALS AND METHODS

Sampling, isolation, and characterization of bacteria. Buds or twigs were collected from commercially maintained apple and pear orchards in the south-western Cape Province of South Africa. Apple blister bark or pear blast occurred in all orchards both during the sampling period and during previous seasons. Fruit tree cultivars, sampling dates, and frequency of sampling are reported with the results.

Samples were kept in plastic bags at 4 C, and the presence of bacteria was determined within 24 hr by plating or streaking suspensions onto King's medium B (MB) (10). Plates were incubated for 3 days at 27 C. Suspect colonies were selected and purified by restreaking on MB. Oxidase-negative fluorescent strains were characterized as described previously (17,18), by biochemical tests and ability to elicit the hypersensitive reaction (HR) in tobacco leaves. Representative HR-positive strains were tested for pathogenicity on apple (17) or pear (18) tissue. Tests to distinguish between *P. s.* pv. *syringae* and *P. s.* pv. *papulans* were not included because the latter is not known to occur in South Africa (17).

Bacteria inside buds. Terminal twigs were cut at random from the host plants on each sampling date (Fig. 1). Leaves were removed and the twigs were surface-disinfested with 70% ethanol and flamed. Fifty randomly selected fruit and leaf buds were aseptically excised from twigs of each cultivar in each orchard, cut longitudinally, and individually suspended in 2 ml of buffered saline as described previously (18). After 1–2 hr, loopfuls of suspension were streaked in duplicate onto MB.

Budwood survey. During 1983–1984, fruit and leaf buds of seven apple and nine pear cultivars, obtained from orchards maintained for the production of budwood, were monitored regularly (Table 1) for the presence of *P. s.* pv. *syringae*. Isolation procedures were as described in the previous section.

Surface populations on buds. Apparently healthy fruit buds were randomly collected at the beginning of two growing seasons (Table 2). Each sample, comprising 200 buds, was suspended in 1 L of sterile peptone solution (19) in a 2-L Erlenmeyer flask. The flask was agitated on an NBS Gyrotory shaker (New Brunswick Scientific Company, NJ) for 4 min at 30-min intervals. After 4 hr, serial dilutions of wash fluids were plated in duplicate onto MB.

Distribution pattern in buds. Terminal fruit buds were collected on six occasions, from full leaf drop (end of May) until bud burst in spring (September), from three cultivars (Table 3). Bud scales and primordium tissue of each sample (consisting of 10 buds) were dissected, and each corresponding scale or primordium was pooled in 10 ml of buffered saline as described by Burr and Katz (3). After 1–2 hr, serial dilutions from each suspension were plated in duplicate on MB.

RESULTS

Most of the representative isolates of bacteria that produced fluorescent pigments on MB were identified as *P. s.* pv. *syringae*. All isolates identified as such elicited the HR on tobacco leaves. All strains tested were pathogenic to either apple or pear tissue. Colonies of nonfluorescent bacteria, when present in low numbers, were easily distinguished from those of the pathogen.

Bacteria inside buds. Early in the growing season, *P. s.* pv. *syringae* was frequently detected in apparently healthy, symptomless fruit and leaf buds. Colonies of other bacteria usually failed to develop on MB. Later in the season, however, the presence of *P. s.* pv. *syringae* in apple buds was often masked by large numbers of yellow-pigmented bacteria.

In general, higher numbers of buds of all three cultivars contained *P. s.* pv. *syringae* during 1984–1985 than during 1983–1984 (Fig. 1), but the numbers fluctuated considerably during these periods. For example, at bud swell (2 October) in 1984–1985, 22% of Starkrimson apple buds contained *P. s.* pv. *syringae*. On the succeeding nine

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Table 1. Percentage of fruit and leaf buds,[a] sampled at different dates from apple and pear cultivars,[b] with internal detectable levels of *Pseudomonas syringae* pv. *syringae*

Sampling date	Apple							Pear								
	Gloucester '69	Lady Williams	Prima	Red-chief	Smoothee	Star-krimson	Top Red Delicious	Anne's Favorite	Beurre Bosc	Ceres	Clapp's Favorite	El Dorado	Forelle	Packham's Triumph	Star-krimson	Winter Nelis
13 Dec. 1983	0	2	0	NS[c]	2	20	16	4	2	20	2	30	52	14	0	NS
10 Jan. 1984	2	0	0	0	0	0	0	0	6	10	0	0	6	0	0	0
24 Jan. 1984	0	0	2	0	2	2	2	0	8	0	0	4	20	2	0	0
7 Feb. 1984	2	12	14	2	12	8	0	0	6	20	0	4	44	26	2	0
21 Feb. 1984	0	0	0	2	2	0	0	0	32	30	12	16	0	0	0	0
6 Mar. 1984	0	24	10	2	18	18	0	0	4	44	0	18	44	8	2	0
20 Mar. 1984	2	0	22	30	0	4	0	0	22	40	2	0	76	28	0	0
3 Apr. 1984	0	0	44	4	0	32	0	2	46	18	0	0	22	6	0	0
17 Apr. 1984	4	4	0	4	4	20	0	0	20	22	0	0	40	4	4	0
1 May 1984	6	8	2	0	18	8	18	78	64	52	28	58	92	72	28	0
29 May 1984	0	0	12	2	4	2	0	20	26	22	44	4	40	42	2	0
26 Jun. 1984	0	0	0	0	4	2	0	14	24	16	8	16	24	24	20	2
24 Jul. 1984	4	4	0	0	2	0	2	4	16	6	2	22	26	6	8	0
21 Aug. 1984	NA[d]	2	0	0	0	NA	2	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] Fifty buds (cut from single twigs 30–40 cm removed from 10–12 trees) were collected on the sampling date from each cultivar in the different orchards. Buds were processed individually.
[b] Trees in orchards maintained for commercial production of budwood.
[c] NS = not sampled.
[d] NA = not available; cultivars were pruned after previous sampling date.

Table 2. Epiphytic populations of *Pseudomonas syringae* pv. *syringae* on fruit buds of apple (Starkrimson and Granny Smith) and pear (Packham's Triumph) trees in orchards[a] at, respectively, the green-tip and budbreak growth stages

Sampling date	Colony-forming units per 200 buds[b]		
	Starkrimson[c]	Granny Smith	Packham's Triumph
18 Sept. 1984	NS[d]	NS	1.7×10^7
2 Oct. 1984	7.5×10^5	1.0×10^4	NS
5 Oct. 1984	1.9×10^6	NS	NS
16 Sept. 1985	2.5×10^4	ND[e]	3.7×10^5
23 Sept. 1985	3.5×10^5	ND	5.5×10^5
30 Sept. 1985	1.5×10^7	2.0×10^3	NS

[a] Separate orchards of Starkrimson and Packham's Triumph and a third with a mixed planting of Starkrimson and Granny Smith.
[b] Sampled at random from each cultivar in each orchard on each date.
[c] Results obtained with buds from the two separate orchards were combined.
[d] NS = not sampled.
[e] ND = not detected.

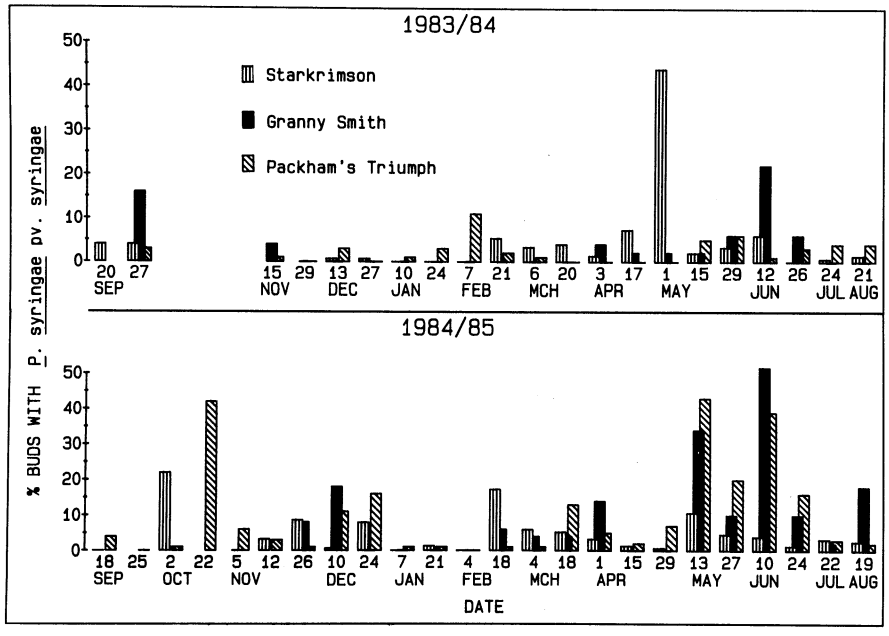

Fig. 1. Percentage of buds of apple (Starkrimson and Granny Smith) and pear (Packham's Triumph) trees harboring *Pseudomonas syringae* pv. *syringae* during two seasons (southern hemisphere). Buds were from two orchards with Starkrimson, two with Packham's Triumph, and a fifth with a mixed planting of Starkrimson and Granny Smith. Fifty buds (cut from single twigs 30–40 cm removed from each of 10–12 trees) were collected on the sampling date from each cultivar in the different orchards. Results obtained with the same cultivar from different orchards were combined.

sampling dates, the pathogen was detected on five occasions and, thereafter, constantly until termination of the survey. With some exceptions, *P. s.* pv. *syringae* was generally more readily isolated from buds of Starkrimson or Granny Smith from fall (April) until the end of winter (August).

During 1984–1985, 42% of newly formed Packham's Triumph pear buds (22 October) contained *P. s.* pv. *syringae*. In contrast, few harbored the pathogen earlier, at bud swell. *P. s.* pv. *syringae* was also recovered from pear buds in late fall (May) and early winter (June), particularly during 1984–1985.

Budwood survey. Higher percentages of fruit and leaf buds carrying *P. s.* pv. *syringae* were detected in budwood cut from several pear cultivars, particularly Beurre Bosc, Ceres, and Forelle, than in buds from apple cultivars (Table 1). Differences were evident among cultivars, and values obtained from one sampling date to the next often fluctuated sharply. With the exception of Winter Nelis, high percentages of buds of all pear cultivars contained the pathogen at early leaf fall (1 May).

Surface populations on buds. At the apple green-tip growth stage, much higher epiphytic populations of *P. s.* pv. *syringae* were found on Starkrimson than on Granny Smith fruit buds (Table 2). The pathogen was not detected on Granny Smith buds collected on two of the four sampling dates. At pear budbreak, the populations on Packham's Triumph buds were similar to the levels found on Starkrimson.

Distribution pattern in buds. *P. s.* pv. *syringae* was detected at least twice per sampling date on each of the fruit bud scales or central primordium tissue of each apple and pear cultivar (Table 3). The pathogen was most consistently present at leaf fall (May). Populations declined later during winter but increased again in spring (September). This was more pronounced on Packham's Triumph

Table 3. Distribution pattern of *Pseudomonas syringae* pv. *syringae* on fruit bud scales of apple (Starkrimson and Granny Smith) and pear (Packham's Triumph) trees in orchards[a]

Sampling date (1985)	Cultivar[b]	Colony-forming units (×1,000) per 10 bud scales[c]									
		1	2	3	4	5	6	7	8	9	10
17 May	Starkrimson	1.03	0.08	1.13	1.25	0.20	1.48	0.63	0.15	0.38	···
	Granny Smith	ND[d]	12.00	3.25	36.50	3.00	14.50	10.75	ND	0.80	···
	Packham's Triumph	3.98	5.95	12.60	37.20	55.10	130.40	8.18	21.53	0.55	0.95
24 Jun.	Starkrimson	ND	ND	ND	0.05	ND	7.00	9.50	ND	0.30	···
	Granny Smith	1.00	2.00	7.00	0.50	ND	ND	0.30	ND	ND	···
	Packham's Triumph	0.85	2.08	3.73	6.18	21.55	73.00	111.75	137.08	29.95	11.73
22 Jul.	Starkrimson	0.05	ND	ND	ND	ND	ND	ND	ND	ND	···
	Granny Smith	ND	ND	ND	ND	ND	ND	ND	0.65	ND	···
	Packham's Triumph	0.20	1.65	9.30	16.75	22.41	35.65	49.25	78.25	14.08	21.93
19 Aug.	Starkrimson	0.03	ND	0.50	ND	0.53	ND	ND	ND	ND	···
	Granny Smith	ND	ND	ND	0.05	0.20	0.05	0.15	ND	0.05	···
	Packham's Triumph	ND	ND	ND	0.40	2.13	10.53	7.75	16.75	3.30	6.68
16 Sept.	Starkrimson	ND	0.02	0.50	3.70	24.05	0.05	0.01	ND	ND	···
	Granny Smith	1.00	15.00	5.00	2.00	ND	0.20	1.50	ND	ND	···
	Packham's Triumph	0.28	0.03	0.05	0.33	7.50	1.30	17.00	11.08	2.28	0.15
23 Sept.	Starkrimson	ND	ND	ND	ND	ND	ND	ND	10.00	55.00	···
	Granny Smith	ND	ND	0.20	ND	ND	0.70	0.90	0.41	230.00	···
	Packham's Triumph	213.00	187.00	76.00	52.00	74.50	39.00	65.50	210.50	108.00	168.00

[a] One orchard of Starkrimson, two of Packham's Triumph, and a fourth with a mixed planting of Starkrimson and Granny Smith.
[b] Results obtained with two batches of Starkrimson or Packham's Triumph were combined.
[c] On the sampling date scales of 10 buds from each cultivar in each orchard were sequentially removed and separately pooled according to Burr and Katz (3); 1 = outer bud scale, 9 and 10 = primordium of apple and pear, respectively; other bud scales are numbered relative to these positions.
[d] ND = not detected.

than on the two apple cultivars. All buds sampled, including those supporting high populations of *P. s.* pv. *syringae*, appeared healthy.

DISCUSSION

The association of *P. s.* pv. *syringae* with apparently healthy apple and pear buds has important epidemiological implications. Our investigation supports the view that the bud is an overwintering site (2–6,12,20,22). Furthermore, the pathogen appears to be sheltered inside the bud during the hot, extremely dry summer months (November through February) of the southwestern Cape Province.

The pathogen might have been present in many Packham's Triumph buds throughout the year (Fig. 1). However, it would not have been detected if present in fewer than 2% of the buds, in the presence of large numbers of other bacteria, or had populations levels within individual buds been very low. Higher rainfall and lower temperatures experienced during the fall of 1985 apparently favored the pathogen, and the proportions of colonized buds increased sharply (Fig. 1). Furthermore, during that fall, *P. s.* pv. *syringae* was detected on each of the individual bud scales and also on the primordium (Table 3). Population levels on the scales decreased during winter but rose sharply in spring. An epiphytic population detected on buds at budbreak (Table 2) could have originated from pathogen cells within the bud or by the multiplication of *P. s.* pv. *syringae* reported to be present on the surface of dormant fruit buds (2,8,12,15,21,22).

The presence of *P. s.* pv. *syringae* in newly formed buds (e.g., 22 October 1984 in Fig. 1) indicates how rapidly this niche is occupied. This agrees with histological evidence that bacteria are more readily detected in newly formed than in dormant buds (9) and that growth of *P. s.* pv. *syringae* is supported in developing buds (4,14,20).

The assumption that *P. s.* pv. *syringae* cells already present in buds during summer are solely responsible for the high internal fall populations might not be entirely correct. Ice nucleation-active bacteria such as *P. s.* pv. *syringae* increase on fruit trees with the onset of cool, moist weather (7). Subsequent freeze-injury could promote bacterial colonization of damaged host tissue (11,12). This is supported by findings that *P. s.* pv. *syringae* infecting pear leaf and fruit scars during fall passes through vessels to colonize axillary buds (6). *P. s.* pv. *papulans* entering apple leaf scars also becomes established in buds (1).

P. s. pv. *syringae* probably colonizes Starkrimson apple and Packham's Triumph buds in much the same way, but population levels on Starkrimson scales were generally lower (Table 3). In contrast, the pathogen failed to colonize the surface of Granny Smith buds (Table 2). The reason for this is not known.

Deciduous fruit nursery trees are sometimes killed by pathogenic pseudomonads introduced through budwood (16). With peach material, predisposing factors govern expression of bacterial canker symptoms (4). Blister bark or blast is probably more likely to develop if the pathogen is present in the buds grafted onto rootstocks. High percentages of buds of some, particularly pear, cultivars supported the pathogen (Table 1). A comparison of the fate of "low-risk" (e.g., Winter Nelis) and "high-risk" (e.g., Forelle) buds grafted onto similar rootstocks might be worthwhile.

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