Effect of Colonization of Pear Stigmas by *Pseudomonas syringae* pv. *syringae* on Fruit Set

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Abstract. 'Packham's Triumph' (*Pyrus communis* L.) pear blossoms were inoculated with *Pseudomonas syringae* pv. *syringae* van Hall upon emasculation and then hand-pollinated at different times to assess the effects of bacterial colonization on fruit set. The pathogen significantly reduced fruit set by 24%. No lesions developed on pear flowers at any stage of the trials.

Fruit set in the pear-producing areas of the southwestern Cape Province of South Africa is often poor, and in some years >90% of fruitlets abscise. Bacterial blossom blast caused by *Pseudomonas syringae* pv. *syringae* (*P. s. pv. syringae*) (Mansvelt and Hattingh, 1986) contributes to limiting pear production. Dormant buds harbor the pathogen and, at bud swell, open and supply primary inoculum. Flowers are infected in early spring during wet, humid conditions. A previous study has shown that the stigmas are important sites of colonization by the bacterium (Mansvelt and Hattingh, 1987). Occupation of pearl millet [*Pennisetum americanum* (L.) Leeke] stigmas by the ergot fungus restricts pollination (Willingale et al., 1986). This study was undertaken to determine if bacterial colonization of the pear stigma by *P. s. pv. syringae* influences pollination and fruit yield.

Materials and Methods

Full-bearing 'Packham's Triumph' pear trees growing in a commercial orchard at Somerset-West in the southwestern Cape Province were evaluated during Oct. (Spring) 1989-90 and 1990-91 for their ability to set fruit following bacterial inoculation and hand cross-pollination. For each season, a single separate row with 16 (1989-90) or 18 (1990-91) trees was selected. Twenty-five floral clusters at white bud were chosen at random on a tree. One tree represented an experimental unit, and trees were treated with either a suspension of *P. s. pv. syringae* or sterile distilled water. Subsequently, hand-pollinations were performed eight (1989-90) or nine times (1990-91).

Pollen was harvested from 'Clapp's Favourite' flowers at anthesis and stored at -20°C until needed. Inoculum of *P. s. pv. syringae* strain L795 was prepared as described previously (Mansvelt and Hattingh, 1986). Inoculum concentration was adjusted to 2×10^8 colony-forming units (cfu)/ml.

Stigmas of three flowers per cluster were inoculated immediately after emasculation by placing a drop of inoculum suspension on the stigmatic surfaces with a pasteur pipette. Nontreated flowers on the cluster were removed. Inoculated flowers were covered immediately with polyethylene bags humidified with sterile distilled water. Bags were secured with clothes-pegs. Clusters remained bagged until stigmas were hand-pollinated.

Stigmatic surfaces were hand-pollinated with a glass rod that had been dipped into the pollen at 0, 3, 6, 18, 24, 30, 48, and 72 h after inoculation. A 40-h pollination treatment was included for the second season.

To monitor bacterial populations at each

pollination time, one flower per cluster was removed and individually placed in empty, sterile test tubes. Test tubes were stored in a cooler bag in the field and kept at 4°C in the laboratory. Within 24 h, each flower was suspended in 10 ml of sterile water, vortexed, and serial dilutions of wash fluids were made in duplicate onto medium B (King et al., 1954). Plates were incubated for 2 days at 27°C and the number of colonies was counted. Representative isolates were characterized as described previously to confirm identity of *P. s. pv. syringae* (Mansvelt and Hattingh, 1986). The two remaining flowers per cluster were left on the tree to allow fruit set to occur. After 8 weeks, the number of fruits per flower that had set was recorded.

Results and Discussion

Regression analyses were performed on the data. No significant differences ($F^* > 0.05$) were found between trends of the same treatment for the two seasons; thus, the results were combined.

The pathogen increased rapidly in the first 24 h, to reach a level of 1.48×10^5 cfu/blossom on inoculated stigmas, after which it stabilized (Fig. 1). Control flowers showed no significant trend ($P = 0.9$) in the viability counts.

Throughout the investigation, fruit set of uninoculated flowers was consistently higher than those inoculated with *P. s. pv. syringae* (Fig. 2). The time of pollination did not influence the numbers of fruit formed—a constant fruit set of 18% was recorded for those inoculated. On uninoculated flowers, the initial percentage set was 18%, which increased to 42% 24 h after inoculation and then declined to 24% after 65 h.

The maximum, minimum, and average temperatures for each season for the 4 days after inoculation were within the normal range for the localities where pear trees are grown (Table

Fig. 1. Viable population levels of *Pseudomonas syringae* pv. *syringae* on 'Packham's Triumph' pear stigmas at selected times after inoculation. Control flowers were treated with sterile distilled water.

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Fig. 2. Percentage fruit set of 'Packham's Triumph' pears after hand-pollination at selected intervals. Flowers were emasculated just before anthesis and then inoculated with *Pseudomonas syringae* pv. *syringae* simultaneously. Controls were treated with sterile distilled water. Fruit set per 50 flowers on 25 clusters was recorded 8 weeks after pollination.

1). No rain fell during the study period, and subzero temperatures resulting in characteristic blast symptoms were absent.

Colonization of pear stigmas by *P. s. pv. syringae* restrained pollination and, therefore, reduced fruit set. Almost all the flowers inoculated with *P. s. pv. syringae* were colonized (data not shown). In both seasons, *P. s. pv. syringae* population levels increased after inoculation. No symptoms developed, although floral structures were exposed upon emasculation. The presence of bacteria on stigmas reduced fruit set by 24%, absolute, and 57%, relative, to the control.

The effective pollination period of flowers can be affected by the length of time that the stigma remains receptive, the suitability of style tissue, or the duration of ovule viability. Whether one or more of these factors limit fruit set depends on genetic, environmental, or cultural factors (Williams et al., 1984). Stigma receptivity and ovule viability are rather short in fruit trees (Egea et al., 1991). For example, in Oregon, the effective pollination period of

'Cornice' is around 5 days (Crisosto et al., 1992). In this study, uninoculated flowers had an adequate fruit set, i.e., >20%, in the 4 days of hand-pollinations.

Scanning electron microscopy (SEM) indicated that the exudates produced by stigma on sweet cherry (*Prunus avium* L.) create a favorable environment for fungal development (Tonutti et al., 1991). The high humidity at stigmatic surfaces for pollen hydration probably favors bacterial multiplication as well. In a previous study, SEM revealed that the papillae of stigma and pollen of 'Packham's Triumph' pears were highly colonized by *P. s. pv. syringae* (Mansvelt and Hattingh, 1987).

No lesions developed on the gynoecia, even though population levels higher than 1.48×10^5 cfu/flower were monitored. Whitesides and Spotts (1991) reported that blossom blast was consistently induced 2 to 3 days after inoculation on flowers in full bloom when appropriate moisture and temperature (21°C) were available. Symptoms on pear stigmas and styles attributable to *P. s. pv. syringae*

have been described (Mansvelt and Hattingh, 1986). However, the nectariferous tissue in the floral cups appears to be the major route for blossom infections (Mansvelt and Hattingh, 1987). In this study, the physiological status of the stigma before anthesis probably inhibited infection, which is why a resident population was established.

Under optimal conditions, a fruit set of 42% was obtained. *Pseudomonas s. pv. syringae* can reduce this figure to 18%, which translates to a crop loss > 50%.

The mechanisms whereby bacteria affect fruit set is uncertain. SEM showed that *P. s. pv. syringae* is capable of colonizing pollen and causing disintegration of the grain (Mansvelt and Hattingh, 1987). The *P. s. pv. syringae* population possibly competes with pollen for stigma exudates, while pollen could be ruptured by the pathogen. Whether colonization of stigma lowers pollen adherence is unknown.

To our knowledge, this is the first report of reduction in fruit set by a bacterial pathogen. These results emphasize the importance of good orchard management during the growing season to keep inoculum levels of *P. s. pv. syringae* low.

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Table 1. Temperature data for Oct. 1989-90 and Oct. 1990-91 (Southern Hemisphere) from 'Packham's Triumph' pear orchards, Cape Province, South Africa, during bloom, when inoculations with *Pseudomonas syringae* pv. *syringae* and emasculations were performed. No rain fell during these investigation periods.

Season	Days after inoculation	Temp (°C)		
		Maximum	Minimum	Avg
1989-90	1	16.6	10.0	13.3
	2	22.0	9.4	15.7
	3	25.0	11.5	18.3
	4	29.8	15.0	22.4
1990-91	1	16.0	12.0	14.0
	2	18.5	10.0	14.3
	3	19.5	11.0	15.3
	4	23.0	12.5	17.8