Susceptibility of Pear Cultivars to Blossom Blast Caused by *Pseudomonas syringae*

Steven K. Whitesides¹ and Robert A. Spotts²

Oregon State University Mid-Columbia Agricultural Research and Extension Center, Hood River, OR 97031

Additional index words. *Pyrus*, disease resistance

Abstract. Conditions were established for inducing pear (*Pyrus communis* L.) blossom blast caused by *Pseudomonas syringae* (Ps) on detached shoots. Highest incidence of infection followed occurrence of a major exotherm in the presence of Ps suspended in water drops on blossom tissue. Eight pear cultivars were evaluated for susceptibility to blossom blast, with the red-fruited 'Beurré d'Anjou' sports 'Gebhart' and 'Columbia' least susceptible and 'Doyenné du Cornice', 'Beurré d'Anjou', and 'Beurré Bosc' most susceptible.

Blossom blast of pear is caused by *Pseudomonas syringae* van Hall (Ps), an epiphytic phytopathogenic bacterium (Barker and Grove, 1914; Crosse, 1959). Under favorable climatic conditions, the disease has caused

serious but sporadic yield reductions in pear-growing regions throughout the world (Cancino et al., 1974; Dye, 1956; Mansvelt and Hattingh, 1986; McKeen, 1955; Panagopoulos and Crosse, 1964; Sands and Kollas, 1974). The most severe and frequent expression of disease symptoms usually follows cool, wet weather (Mansvelt and Hattingh, 1987; Sands and Kollas, 1974; Waissbluth and Latorre, 1978), which favors increases in populations of Ps (Gross et al., 1983).

Ps is one of the most common ice nucleation-active bacteria (Maki et al., 1974) and has been shown to increase frost injury to sensitive plants (Anderson et al., 1982; Arny et al., 1976; Klement, 1974; Klement et al., 1984; Weaver, 1978). High populations of Ps increase the probability of ice nucleation activity (Gross et al., 1984; Lindow et al., 1982) during pear bloom. Although frost damage is often associated with outbreaks of pear blossom blast, the disease can appear

without a frost during bloom periods that are cool and wet (Sands and Kollas, 1974; Waissbluth and Latorre, 1978).

Pear blossoms at the prebloom through postbloom developmental stages are the most sensitive to frost injury (Proebsting and Mills, 1978; Whitesides and Spotts, 1991). There have been reports of differences in cultivar susceptibility to blossom blast (Cancino et al., 1974; Panagopoulos and Crosse, 1964; Waissbluth and Latorre, 1978), but the susceptibility of many cultivars has not been studied. The objective of this study was to compare the blossom blast susceptibility of eight pear cultivars commonly grown in the Pacific Northwest, using a standard procedure under controlled conditions.

Pseudomonas syringae pv. *syringae* strain AP1, isolated in the Hood River Valley, Ore., from an Asian pear (*Pyrus pyrifolia* Burm. Nak.), was used in all experiments. In a preliminary experiment with 25 isolates of *P. syringae* pv. *syringae*, AP1 caused 47% blossom blast of 'Beurré d'Anjou', compared with a mean of 27% for the group of 25 isolates. Although AP1 was more virulent than the group mean, it was well within the group range of 1%–67% blossom blast. To prepare inoculum, strain AP1 was cultured on nutrient agar and incubated for 48 h at 21°C. The bacterial cells were suspended in sterile distilled water (SDW) and adjusted to 0.3 ± 0.02 absorbance at 600 nm on a Spectronic 20 calorimeter (Bausch and Lomb, Rochester, N. Y.) to achieve $\approx 3 \times 10^8$ cfu/ml. This concentration was confirmed by standard dilution plate assay. Inoculum concentration was adjusted to 10^6 by diluting the assayed suspension in SDW.

Pear shoots were collected in the Hood River Valley during bloom periods of 1989 and 1990. Shoots with terminal blossom clusters containing from five to 20 individual flowers (blossoms) were detached from trees

Received for publication 13 Sept. 1990. Oregon Agricultural Experiment Station technical paper 9300. This project was supported in part by the Winter Pear Control Committee, Hood River Grower-Shipper Assn., and Medford Fruit Growers League. Use of trade names in this article does not imply endorsement by Oregon State Univ. of the products named or criticism of similar products not mentioned. We thank Joseph Postman, National Clonal Germplasm Repository, Corvallis, Ore., for providing cultivar rankings. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Postdoctoral research associate.

²Professor.

Table 1. Blossom blast in detached shoots of eight cultivars of pear.

Cultivar	Blossom blast (%) ^z		
	1989	1990	Mean ^y
Gebhart Red d'Anjou	10 a	18 a	14 a
Columbia Red d'Anjou	30 bc	10 a	20 ab
Forelle	14 ab	37 b	25 ab
Max Red Bartlett	42 c	14 a	28 ab
Bartlett	48 c	19 a	33 b
Doyenné du Comice	46 c	56 bc	51 c
Beurré d'Anjou	75 d	44 b	60 c
Beurré Bosc	97 e	66 c	82 d

^yMean separation within columns by Duncan's new multiple range test ($\alpha = 0.05$). Values corrected for background *Pseudomonas syringae* population.

^zTwo-way ANOVA.

of eight pear cultivars, cut to ≈ 30 cm in length, then placed into 250-ml beakers filled with 100 ml tap water. Cultivars included: 'Beurré Bosc', 'Forelle', 'Doyenné du Comice', 'Bartlett', 'Max Red Bartlett', 'Beurré d'Anjou', 'Gebhart Red d'Anjou', and 'Columbia Red d'Anjou'. The total number of blossoms per cultivar in each year was similar. In the field, these cultivars were not synchronous in bloom stage. Typically, 'Beurré d'Anjou' and 'Forelle' were the earliest to bloom with 'Beurré Bosc' or 'Doyenné du Comice' blooming latest. About 5 days separated early and late-blooming cultivars. To synchronize the bloom stage of all eight cultivars, shoots with blossoms were collected from late-blooming cultivars located at sites with warm microclimate, whereas the early blooming cultivars were collected at sites with cool microclimates. All samples were collected on the same day. Following collection, cultivars were placed at 5, 10, 15, or 20°C, depending on their bloom stage. The more mature cultivars were kept at lower temperatures and the less mature cultivars at higher temperatures. Within 2 days, all cultivars were synchronous in bloom stage development. There were three replicates of five shoots per replicate beaker of each cultivar.

Inoculum suspensions and a SDW control were sprayed to runoff over blossoms and leaves with a hand-pump sprayer. All inoculations of plant materials were done at 18 to 21°C. After inoculation, a 3.8-liter plastic bag was used to cover each beaker and enclose the blossom clusters to ensure high relative humidity. The beakers were placed in cardboard fruit boxes (five to six per box) with lids to ensure more uniform temperature of the plant material during low temperature treatment. The boxes were placed in a $3 \times 3 \times 3$ m storage room at 6 to 8°C for up to 2 h before temperature reduction. All cultivars were included in a single experiment, and cultivars were randomized among boxes.

Tissue temperature was reduced at a rate of 1.0 ± 0.2 °C per 15 min until an exotherm was detected (George et al., 1974; Quamme, 1974; Quamme et al., 1972), indicating ice formation within tissues (Modlibowska, 1962). Temperatures were monitored during each treatment using copper-constantan thermocouples placed at the following locations: 1) internal tissue by inserting thermocouples (1-mm diameter) into ovaries, 2)

air temperature within bagged blossom clusters, 3) air temperature within fruit boxes, and 4) air temperature within the cold room. Temperatures at the first two locations were monitored for samples treated with bacterial suspensions, samples treated with SDW, and samples of dry untreated tissue. Thermocouples were inserted into ovaries of one to four cultivars in each experiment. A datalogger (Campbell Scientific, Model CR5, Logan, Utah) was used to record temperatures at 5-min intervals during each experiment. Once the exotherm was detected at locations 1 and 2, the exotherm-inducing temperature was held for 30 min, then temperature was increased at a rate of 2.0 ± 0.2 °C per 15 min until reaching 5°C. Samples were kept at 5.0 ± 0.2 °C for up to 16 h, then the plastic bags were removed and samples were placed at 18 to 23°C for 3 to 5 days to allow for development of symptoms.

Flowers within each blossom cluster were evaluated individually for blossom blast. Each ovary was longitudinally sectioned and evaluated for brown streaking or necrosis of internal tissues. Data were analyzed with one-way analysis of variance (ANOVA) within years with variance among beakers within cultivars as the error term. Two-way (cultivar $\times$ year) ANOVA was used for combined yearly data with cultivar as a fixed effect tested with the interaction error term and time as a random effect tested with the residual error term. The arcsin square root transformation of percent blasted blossoms was used in both analyses. Separation of means was done with Duncan's new multiple range test. Because a linear relationship occurs between the log of the inoculum concentration and the amount of blossom blast (Whitesides, 1989), observed percentages were adjusted to account for background populations of Ps by subtracting the percentage disease in the noninoculated controls from the disease incidence value of the inoculated cuttings. Mean blossom blast of controls was 14% in 1989 and 9% in 1990.

Exotherms were detected at temperatures ranging from -1.8 to -3.5 °C in ovary tissues of blossoms treated with *P. syringae* isolate API. No obvious differences were detected among the cultivars or among boxes in the temperature required to produce an exotherm. The magnitude of exotherm response ranged from increases of 1.5 to 2.6°C. An exotherm of smaller magnitude, from 0.7

to 1.6°C, also was detected in the air temperature among blossoms enclosed by the plastic bags. This exotherm occurred within the same temperature range observed for ovary tissues. Air temperature reached -6.0 and -9.0 °C within the boxes and in the cold room, respectively. Exotherms were not detected at these locations.

Initial symptom development was observed after 2 days of incubation at 21°C. Small brown-to-black lesions appeared in the calyx cup and on sepals. In 3 to 5 days, the lesions enlarged and coalesced, blackening portions of styles and stamens and often the entire floral receptacle and pedicel. Less frequently, the necrosis spread down the peduncle and into the spur. Lesions also were observed on leaves adjacent to the flowers. Brown-to-black streaks and discoloration were observed in sections of necrotic ovary tissues. Infected petals usually turned brown, then abscised, leaving a blasted cluster of petalless floral trusses. Differences among cultivars in the extent of spread of the infection were not observed within the time the shoots were retained for evaluation.

Green-fruited pear cultivars Beurré Bosc and Beurré d'Anjou had a significantly higher incidence of blossom blast than did the red-fruited 'Beurré d'Anjou' sports 'Gebhart' and 'Columbia' or 'Max Red Bartlett' in 1989 and 1990 (Table 1). The remaining cultivars were of intermediate susceptibility and frequently varied in relative resistance between years as indicated by significance of the interaction of cultivar $\times$ year.

Background populations of Ps ranged from 2.9×10^2 cfu/g for 'Bartlett' to 4.3×10^3 cfu/g for 'Doyenné du Comice' in 1989. In 1990, background populations of Ps were below detectability.

The frequency of disease incidence and the development of infection have been reported to differ among pear cultivars. Panagopoulos and Crosse (1964) reported that 'Winter Nelis', 'Josephine de Malines', and 'Beurré Superfin' were susceptible to a rapid spread of infection down the receptacle and into the pedicel. Waissbluth and Latorre (1978) stated that orchard-wide blossom blast resulted in no yields in several orchards of 'Packham's Triumph', 'Winter Nelis', and 'Bartlett'. Cancino et al. (1974) observed that the disease was especially destructive to 'Packham's Triumph', 'Anjou', and 'Bartlett'. Several pear cultivars at the National Clonal Germplasm Repository, Corvallis, were rated in 1988 for blossom blast susceptibility on a scale of 1 (none) to 9 (severe). 'Red Bartlett', 'Bartlett', 'Doyenné du Comice', and 'Beurré d'Anjou' were rated as 2.3, 3.5, 3.5, and 4.5, respectively. Between eight and 24 trees were evaluated for each cultivar (J. Postman, personal communication). This ranking is similar to that obtained in our study. In another study (Whitesides and Spotts, 1991) to evaluate the effect of inoculum dose of strains API and Pss2-3RNH, we found that the detached shoot method for evaluating blossom blast agreed with a method using a portable, controlled-temperature cage in the orchard on attached limbs. These studies in-

dicates that our detached shoot method gives comparable results to those obtained with attached limbs and field rankings.

Our results indicate significant differences under controlled test conditions between the red-fruited sports of 'Beurré d'Anjou' and the green-fruited clone of 'Beurré d'Anjou'. Generally, red-fruited cultivars are less vigorous than green-fruited. Future experimental comparisons between cultivars should include studies to evaluate the correlation between growth rate and blossom blast susceptibility. Also, the high variability of background Ps populations and its apparent influence on blossom blast among cultivars and between years require that comparisons of cultivar susceptibility should be continued for several additional years and compared with disease ratings of cultivars in the field.

Literature Cited

- Anderson, J.A., D.W. Buchanan, R.E. Stall, and C.B. Hall. 1982. Frost injury of tender plants increased by *Pseudomonas syringae* van Hall. J. Amer. Soc. Hort. Sci. 107:123-125.
- Army, D.C., S.E. Lindow, and C.D. Upper. 1976. Frost sensitivity of *Zea mays* increased by application of *Pseudomonas syringae*. Nature (London) 262:282-284.
- Barker, B.T.P. and O. Grove. 1914. A bacterial disease of fruit blossom. Ann. Applied Biol. 1:85-97.
- Cancino, L., B. Latorre, and W. Larach. 1974. Pear blast in Chile. Plant Dis. Rptr. 58:568-570.
- Crosse, J.E. 1959. Bacterial canker of stone-fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Ann. Applied Biol. 47:306-317.
- Dye, D.W. 1956. Blast of pear. Orchardist of N.Z. 29:5-7.
- George, M.F., M.J. Burke, H.M. Pellet, and A.G. Johnson. 1974. Low temperature exotherms and woody plant distribution. HortScience 9:519-522.
- Gross, D.C., Y.S. Cody, E.L. Proebsting, Jr., G.K. Rademaker, and R.A. Spotts. 1983. Distribution, population dynamics, and characteristics of ice nucleation-active bacteria in deciduous fruit tree orchards. Applied Environ. Microbiol. 46:1370-1379.
- Gross, D.C., Y.S. Cody, E.J. Proebsting, Jr., G.K. Rademaker, and R.A. Spotts. 1984. Ecotypes and pathogenicity of ice nucleation-active *Pseudomonas syringae* isolated from deciduous fruit tree orchards. Phytopathology 74:241-248.
- Klement, Z. 1974. Apoplexy of apricots. III. Relationship of winter frost and bacterial canker and die-back of apricots. Acta Phytopathol. Acad. Sci. Hungary 9:35-45.
- Klement, Z., D.S. Rozsnyay, E. Baló, M. Panczel, and G. Prileszky. 1984. The effect of cold on development of bacterial canker in apricot trees infected with *Pseudomonas syringae* pv. *syringae*. Physiol. Plant Pathol. 24:237-246.
- Lindow, S.E., S.S. Hirano, W.R. Barchet, D.C. Army, and C.D. Upper. 1982. Relationship between ice nucleation frequency of bacteria and frost injury. Plant Physiol. 70:1090-1093.
- Maki, L.R., E.L. Galyan, M.M. Chang-chien, and D.R. Caldwell. 1974. Ice nucleation induced by *Pseudomonas syringae*. Applied Microbiol. 28:456-459.
- Mansvelt, E.L. and M.J. Hattingh. 1986. Pear blossom blast in South Africa caused by *Pseudomonas syringae* pv. *syringae*. Plant Pathol. 35:337-343.
- Mansvelt, E.L. and M.J. Hattingh. 1987. *Pseudomonas syringae* pv. *syringae* associated with apple and pear buds in South Africa. Plant Dis. 71:789-792.
- McKeen, W.E. 1955. Pear blast on Vancouver Island. Phytopathology 45:629-632.
- Modlibowska, I. 1962. Some factors affecting supercooling of fruit blossoms. J. Hort. Sci. 37:249-261.
- Panagopoulos, C.G. and J.E. Crosse. 1964. Blossom blight and related symptoms caused by *Pseudomonas syringae* van Hall on pear trees. Ann. Rpt. E. Malling Res. Sta. 1963. p. 119-122.
- Proebsting, E.L., Jr. and H.H. Mills. 1978. Low temperature resistance of developing flower buds of six deciduous fruit species. J. Amer. Soc. Hort. Sci. 103:192-198.
- Quamme, H.A. 1974. An exothermic process involved in the freezing injury to flower buds to several *Prunus* species. J. Amer. Soc. Hort. Sci. 99:315-318.
- Quamme, H.A., C. Stushnoff, and C.J. Weiser. 1972. The relationship of exotherms to cold injury in apple stem tissues. J. Amer. Soc. Hort. Sci. 97:608-613.
- Sands, D.C. and D.A. Kollas. 1974. Pear blast in Connecticut. Plant Dis. Rptr. 58:40-41.
- Waissbluth, M.E. and B.A. Latorre. 1978. Source and seasonal development of inoculum for pear blast in Chile. Plant Dis. Rptr. 62:651-655.
- Weaver, D.J. 1978. Interaction of *Pseudomonas syringae* and freezing in bacterial canker on excised peach twigs. Phytopathology 68:1460-1463.
- Whitesides, S.K. 1989. Epidemiology of disease caused by *Pseudomonas syringae* as an epiphyte and its frequency, distribution, and characteristics as an endophyte of pear. PhD Diss., Oregon State Univ., Corvallis.
- Whitesides, S.K. and R.A. Spotts. 1991. Induction of pear blossom blast caused by *Pseudomonas syringae* pv. *syringae*. Plant Pathol. 40:118-127.