

Isolation of *Pseudomonas syringae* pv. *syringae* from Cankers and Effect of Free Moisture on Its Epiphytic Populations on Sweet Cherry Trees

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ABSTRACT

Latorre, B. A., González, J. A., Cox, J. E., and Vial, F. 1985. Isolation of *Pseudomonas syringae* pv. *syringae* from cankers and effect of free moisture on its epiphytic populations on sweet cherry trees. *Plant Disease* 69:409-412.

Frequency of isolation of *Pseudomonas syringae* pv. *syringae* (Pss) from bacterial cankers on 3- to 4-yr-old sweet cherry trees (cultivar Napoleon) was highest between late winter (August) and early spring (September). Large, abrupt changes in epiphytic populations of Pss were found on dormant buds of Napoleon cherry shoots under field conditions. These changes closely followed the presence or absence of free moisture resulting from rainfall or dew. Populations of Pss greater than 10^4 colony-forming units per gram of buds were only recorded during wet periods; otherwise, populations remained low or undetectable. Field observations were corroborated by greenhouse experiments on detached, dormant cherry shoots where 10-fold or higher fluctuations in bacterial populations occurred in response to 24 hr of wetness or dryness.

Bacterial canker of stone fruit trees is common in the Central Valley of Chile and primarily affects sweet cherry (*Prunus avium* L.). The disease has been associated with *Pseudomonas syringae* pv. *syringae* (Pss) (16); there is no evidence for the presence of *P. syringae* pv. *morsprunorum*, a major cause of bacterial canker of cherry elsewhere (1,3,14). The most common symptoms of bacterial canker in Chile include dead buds, uneven bud burst, and sunken cankers in bark of twigs, usually associated with a dead bud. Cankers may exude large amounts of light brown gum during late winter or early spring. Dieback of scaffold branches is common in heavily infected orchards. The leaf and fruit phases of the disease are rare or nonexistent under Chilean conditions on cherry. Disease incidence appears to be greatest in new orchards where trees are about 1-5 yr old.

Epiphytic populations (*sensu* Leben, 17) of Pss that survive on symptomless buds or other aerial parts of cherry trees (2,3,7,8,14,16,20) are sources of inoculum. Epiphytic Pss also occurs on nonhosts (3,8,13,15,16,22), but these bacterial populations appear to be lower in virulence (8,16) and their significance in the epidemiology of bacterial canker of stone fruit trees remains to be determined.

Factors affecting population dynamics of Pss on plant surfaces as well as factors promoting infections are not well understood. The population of Pss tends to increase when free moisture is present but not if plant surfaces are dry, regardless of the relative humidity (4,5,8,15). Similarly, Pss appears to be favored by frost injuries on stone and pome fruit trees (6-8,11,21,23).

The objectives of this investigation were to determine the influence of free moisture on population dynamics of epiphytic Pss on sweet cherry shoots during dormancy and to determine the seasonal fluctuation of Pss in cankers.

MATERIALS AND METHODS

Isolation of Pss from cankers. In a heavily infected cherry orchard near Curico, Chile, fifty 3- to 4-yr-old cultivar Napoleon trees showing at least one canker on trunks or scaffold limbs were marked in 1980. Thereafter, 20 canker samples were taken from a group of 10 trees randomly selected each time. Cankers were sampled about every 2 wk. Lesions were swabbed with 90% ethanol, and small pieces (0.5-1.5 cm long) were removed aseptically from margins of cortical lesions just below the periderm. Samples were transferred immediately to 20-ml screw-cap tubes containing 3 ml of sterile distilled water (SDW) and transported to the laboratory. Each

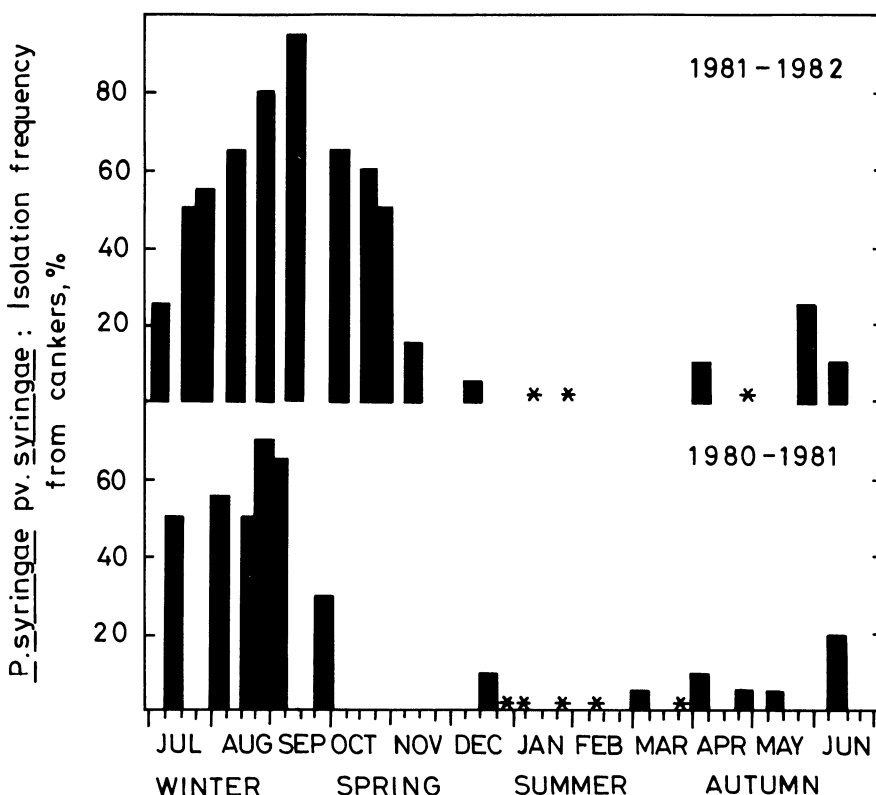


Fig. 1. Seasonal changes in the isolation of *Pseudomonas syringae* pv. *syringae* (Pss) from 20 cankers on 3- to 4-yr-old Napoleon sweet cherry trees. Asterisks mean isolation attempted but Pss not detected.

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sample was triturated with a sterile glass rod, and the material adhering to the glass rods was streaked on medium B (MB) of King et al (9) supplemented with 50 µg/ml of cycloheximide to suppress fungal growth. Two plates were seeded per sample and incubated at room temperature (18–22 C) for 3–4 days. Cankers were considered active if the bark sample yielded at least one bacterial colony that fluoresced under ultraviolet light, showed no cytochrome oxidase activity (12), and induced a hypersensitive reaction in tobacco leaf within 24 hr (10). Ten or more colonies of presumptive pseudomonads from each canker were tested at each sampling time.

Thirteen and 11 representative isolates of oxidase-negative, fluorescent pseudomonads were selected in 1982 and 1983, respectively. These isolates were subjected to GATTa determinative tests (gelatin liquefaction [G], aesculin

hydrolysis [A], tyrosinase activity [T], and tartrate [Ta] utilization as carbon source [14]), levan production on 5% (w/v) sucrose nutrient agar (SNA) (Difco), lactic acid utilization, and growth characteristics in nutrient broth (Difco) supplemented with 5% (w/v) sucrose (SNB). Isolates were maintained on nutrient agar at 5 C.

The pathogenic abilities of 48-hr-old cultures on MB were studied on immature Napoleon cherry fruits, 1-yr-old shoots under field conditions, and immature Packham's Triumph pear fruits. Inoculations were performed as described previously (14,16), using inoculum suspensions adjusted turbidimetrically to about 10^7 colony-forming units (cfu) per milliliter. Isolates were also tested for ice-nucleation activity at -3 to -6 C in glass tubes containing 1 ml of 24-hr-old bacterial suspension prepared from 24- to 36-hr-

old cultures on MB at room temperature. Tubes containing SDW were used as a check. Isolates were recorded as positive if at least one of six suspensions froze before SDW did.

Monitoring bacterial populations under field conditions. To study the population dynamics of Pss on cherry, spontaneous mutants resistant to rifampicin (Rif^r) were selected by screening on MB amended with 50 µg/ml of rifampicin. Populations of Pss strains C-113 and F-215 isolated from a canker and a blasted blossom of cherry, respectively, were used. Two Rif^r isolates that showed no differences from wild-type strains in colony morphology, oxidase reaction, or GATTa tests (14) were chosen. Mutant isolates were pathogenic to immature cherry fruits.

Rif^r isolates were increased on MB plus 50 µg/ml of rifampicin (rMB) for 36 hr at 25 C and suspended in SDW to a final concentration of about 10^8 cfu/ml (adjusted to 50% transmittance at 580 nm in a Spectronic 20 Bausch and Lomb spectrophotometer). Two 14-yr-old Napoleon cherry trees were sprayed with about 5 L of inoculum suspension, mixing (1:1) isolates Rif^r 1 and Rif^r 2. Trees were inoculated each year in March or April before leaves started to fall.

Total bacterial populations on bud surfaces were determined every 2 wk for a period of about 4–5 mo. The first count was made 1 hr after trees were inoculated. About 10 shoots (1- to 3-yr-old growth) were randomly collected from each tree. From these, a composite sample of 50 g of buds was shaken vigorously for 5 min in 50 ml of SDW. The resulting suspension was diluted in a 10-fold series, and 0.1 ml of each dilution was seeded in four plates containing rMB with 50 mg/ml of cycloheximide. Plates were incubated at 25 C for 4 days.

Daily data for maximum and minimum air temperatures, relative humidity (RH), precipitation, and dew were obtained from a weather station in Curico, Chile (Dirección Meteorológica de Chile, Fuerza Aerea de Chile), about 5 km from the experimental orchard. Dew conditions were determined by observing the presence of water on the trees just before noon.

Effect of moisture. The effect of alternating 24-hr periods of wetness and dryness on the epiphytic bacterial populations of Pss on cherry buds was studied under greenhouse conditions. About 50 shoots (50–80 cm long) taken from the growth of the last 3 yr were removed during dormancy from Napoleon cherry trees. Shoots were placed vertically in wet sand in a mist chamber and sprayed to runoff with an inoculum suspension prepared from 48-hr-old Rif^r isolates on rMB. In preliminary experiments, populations of total Rif^r bacteria were measured at 12-hr intervals for 2 days; thereafter, populations were

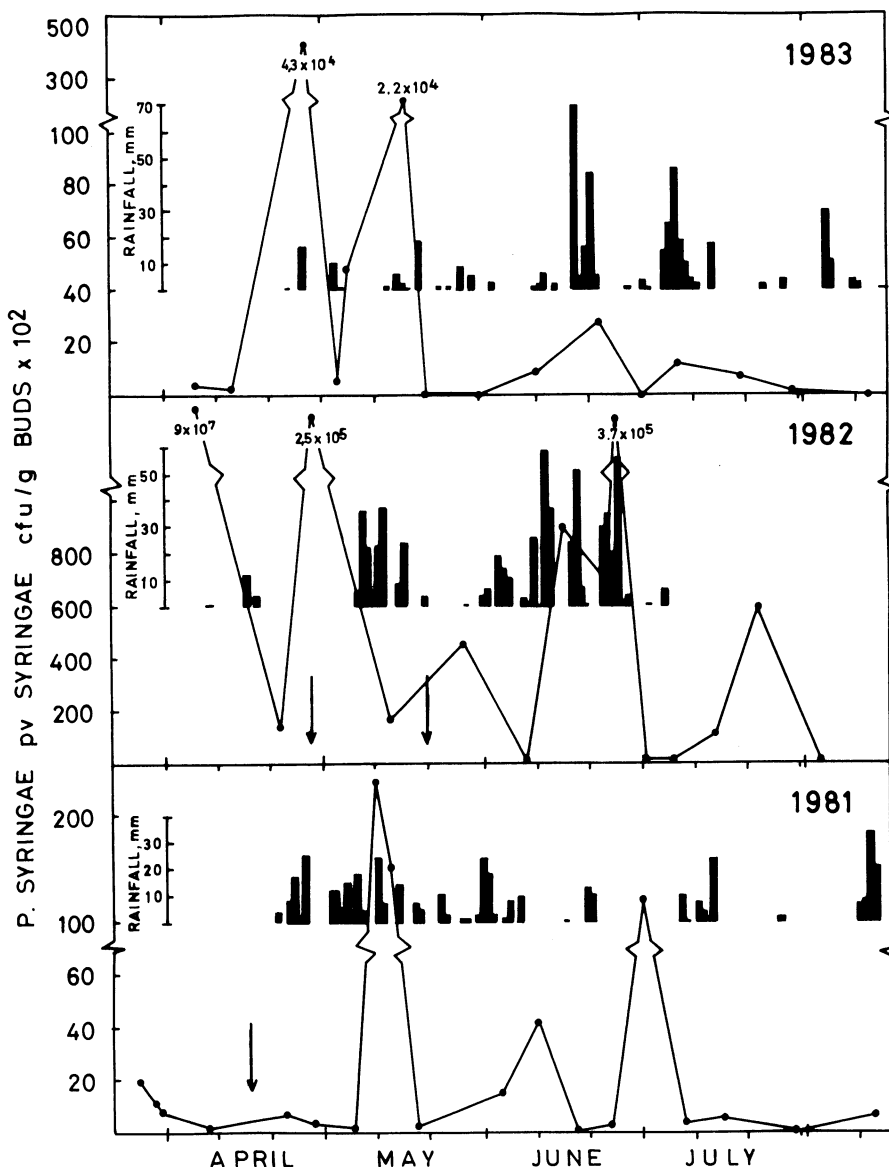


Fig. 2. Population dynamics of *Pseudomonas syringae* pv. *syringae* on Napoleon sweet cherry buds in autumn. Arrows indicate dates sprays (copper oxychloride, 500 g/100 L, not part of this investigation) were applied. Leaf fall was complete by about end of May each year.

measured at 24-hr intervals for 7 days. Shoots receiving a 24-hr period of dryness were removed from the mist chamber and maintained under greenhouse conditions with RH below 60%. No significant differences in temperature or light were observed between the mist chamber and the greenhouse.

Bacterial populations in 10 g of buds from 10–12 randomly collected shoots were assessed. Buds were cut in small pieces and shaken vigorously in 10 ml of SDW in a rotary shaker for 1 hr. The resulting suspension was diluted in a 10-fold series, and four plates with rMB plus 50 µg/ml of cycloheximide were seeded with 0.1 ml from each dilution. Plates were incubated for 5 days at 25 C. Only plates with isolated colonies were counted.

RESULTS

Bacterial characterization. Twenty-four oxidase-negative, fluorescent pseudomonads isolated from cankers were subjected to various tests. Thirteen were GATTa⁺, none were GATTa⁻, and 11 gave variable results (GATTa[±]). Twelve produced levan on SNA, and 16 utilized lactic acid as carbon source. All isolates produced yellow, translucent materials in SNB, and 21 were active in ice-nucleation.

Twenty of 24 isolates were pathogenic to cherry fruits, and 21 were also pathogenic to immature pears. Pathogenic isolates produced dark brown, sunken lesions around the inoculation site on both cherry and pear fruits. Seventeen isolates induced water-soaked lesions that became dark brown and necrotic on green shoots 7–10 days after inoculation.

Isolation of Pss from cankers. Fluorescent, oxidase-negative pseudomonads that induced a hypersensitive reaction on tobacco were recovered more frequently from cankers sampled during winter (June through September) or early spring (September and October) than in spring or summer (December through March). For instance, 70 and 95% of the samples yielded Pss on 28 August 1980 and 9 September 1981, respectively. However, percent recovery progressively decreased to undetectable levels (none of 20 cankers tested) during the summer (Fig. 1).

Abundant light brown gum exuded from cankers during late winter or early spring, coincident with the high canker activity. Most cankers during winter showed necrotic cortical tissues surrounded by diffuse water-soaked margins. This contrasted with the dried lesions with sharp margins most often found during summer from which Pss could not be isolated.

Monitoring the population of Rif⁺ Pss under field conditions. Initial bacterial populations of about 2×10^3 , 9×10^7 , and 4×10^2 cfu/g (fresh weight) of buds were obtained 1 hr after inoculation in 1981, 1982, and 1983, respectively. The large

difference in recovery of the bacteria 1 hr after application in 1982 compared with 1981 and 1983 was attributed to the highly humid conditions prevailing during the postinoculation hours (Fig. 2). Rif⁺ Pss was consistently recovered until August. However, the population level varied considerably. High populations were associated with free moisture (rainfall or heavy dew) at the time trees were sampled. For instance, the rapid decline in the initial bacterial population in 1981 (Fig. 2) from 2×10^3 on 27 March to about 5 cfu/g of buds on 8 April was associated with dry weather (RH below 80%) after inoculation. On the other hand, Rif⁺ Pss population increased rapidly after the onset of a free moisture period, with peak populations of about 10^4 cfu/g of buds in 1981 and 1983 and greater than 10^5 cfu/g of buds in 1982. The low populations measured shortly after dates marked by arrows in Figure 2 may have resulted from copper sprays.

Effect of free moisture on changes in population densities of Pss. The population of Rif⁺ Pss on detached sweet cherry shoots decreased from 4.8×10^6 to 1.6×10^4 cfu/g of buds during the first 12 hr after inoculation. Thereafter, the bacterial population rapidly increased and equaled the initial bacterial density about 36 hr after inoculation. The highest densities occurred 48 hr after inoculation, with bacterial populations exceeding 10^8 cfu/g of buds (Fig. 3A). This experiment was repeated three times with similar results.

When cherry shoots were inoculated and subjected to alternate 24-hr periods of wetness and dryness (Fig. 3B), the bacterial population increased or decreased according to the presence or

absence of free water, respectively. For instance, 2.17×10^4 cfu/g of buds were recorded after 24 hr of wetness, but this bacterial population abruptly dropped to 8.95×10^2 cfu/g of buds when shoots were removed from the mist chamber and placed in a dry environment for 24 hr. The population increased again to 1.82×10^4 cfu/g of buds after a further 24 hr of wetness. Similar changes in bacterial densities were obtained after two other wet and dry cycles. The population of Rif⁺ Pss on shoots maintained continuously under free moisture conditions progressively increased from 1.09×10^4 to 1.11×10^6 cfu/g of buds during 168 hr (Fig. 3B). These experiments were repeated five times with comparable results.

DISCUSSION

Pathogenic Pss was consistently isolated from cankers, and there was no evidence of the presence of *P. syringae* pv. *morsprunorum* (Psm). This report confirms previous work associating bacterial canker of sweet cherry with only Pss in Chile (16). However, isolates intermediate between Pss and Psm were identified on the basis of GATTa tests and found pathogenic. Similar results have been reported (14,16,20).

Large seasonal variations occurred in the proportions of cankers from which Pss could be isolated. Pss was isolated most frequently from bud swell (late August) until budbreak (early September), when cool wet weather (minimum air temperature 0–5 C) prevailed. The population of Pss was low or undetectable in cankers during summer (December through March) under warm (daily minimum 8 to 15 C) and dry conditions.

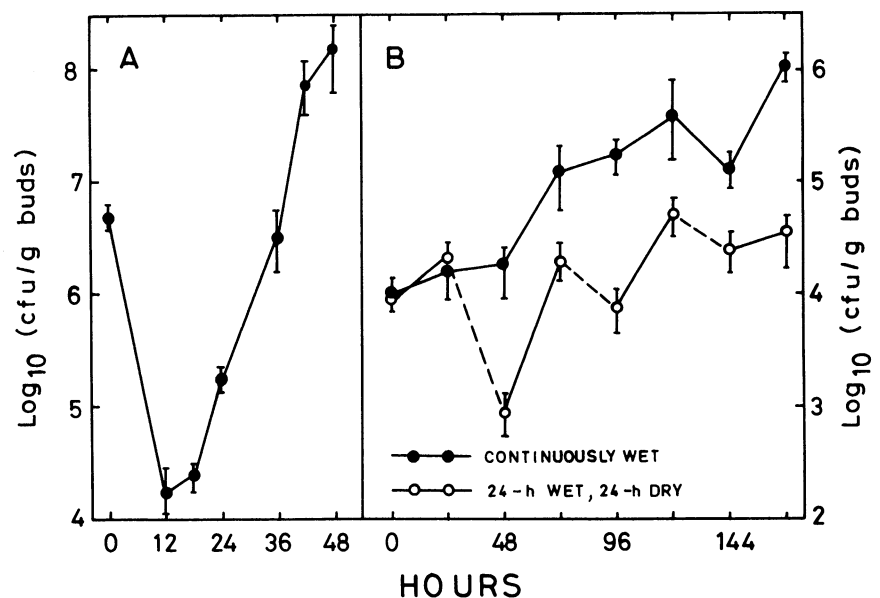


Fig. 3. Effect of free moisture on the epiphytic population of *Pseudomonas syringae* pv. *syringae* on detached, dormant sweet cherry shoots in a mist chamber. (A) Population changes during 48 hr of continuous wetness. (B) Population dynamics observed during a 7-day period. Broken lines denote a 24-hr period of dryness (relative humidity below 60%), and solid lines denote a continuous wet condition. Vertical bars represent standard deviation.

Similar seasonal changes were reported by Wilson (24) on plum and cherry and by Davis and English (4) on peach trees in California. Perhaps, isolates with ice-nucleation activity predisposed tissue to bacterial colonization during winter (7,8,11,18,19,21,23). Our results also agreed with the difficulties encountered in England in isolating Psm from canker lesions during the summer (3) and explain our own failure to isolate Pss consistently from cankered tissue of cherry trees during the warm, dry months of the year (16).

Populations of Pss showed abrupt changes under field conditions, apparently caused by changes in meteorological conditions. Bacterial populations greater than 10^4 to 10^5 cfu/g of buds were recorded only during wet periods in 1981, 1982, and 1983 (Fig. 2), but invariably, populations rapidly declined to very low or undetectable levels during dry periods. The relatively high bacterial populations observed in 1982 may have been related to the highly humid weather prevailing between May and July 1982.

The significant, rapid changes in the epiphytic populations of Pss on detached cherry shoots during dormancy (Fig. 3) reinforce our field observations (Fig. 2) that these bacterial populations multiply or die in direct response to moisture and show that samples for determining populations trends on buds or other plant parts should be taken at intervals as short as possible, as was suggested by Hirano and Upper (8). Our data also demonstrate that Pss may colonize cherry buds and develop high populations ($>10^4$ cfu/g) there during dormancy. Therefore, these epiphytic populations may constitute an important inoculum source for outbreaks

of bacterial canker in areas characterized by wet weather.

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