Pseudomonas Content of Cherry Trees

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ABSTRACT

The systemic distribution and frequency of *Pseudomonas* spp. was determined within diseased and healthy-appearing sweet cherry trees (*P. avium*). Fluctuations in both bacterial distribution and frequency occurred during the seasons, and could be correlated with variations in available moisture and temp. Highest populations were in early spring, with a moderate increase after the first fall rains. Lowest populations were in midsummer and during the coldest weeks of winter.

The systemic existence of *Pseudomonas syringae* Van Hall. helps to explain the lack of effective control from protective bactericides applied to the surface of trees. Phytopathology 60:1343-1346.

Bacterial canker caused by *Pseudomonas syringae* is one of the most serious diseases of stone fruit trees. Control of the canker phase has been erratic and usually unsatisfactory. Most control practices have been based on the application of protective bactericides. Copper compounds, mercurials, and antibiotics have been recommended in various spray schedules. These have been ineffective in about half the cases, and have produced from 10 to 80% control in the other half. Research was initiated to determine the reason for the general ineffectiveness of these materials and to develop more effective control measures.

MATERIALS AND METHODS.—The sampling for *P. syringae* was conducted on 8- to 12-year-old Napoleon sweet cherry trees (*Prunus avium* L.). The trees were photographed in 1966 and the number of each sampling site recorded. Most of the trees had from two to six cankers, but two trees did not have any visible disease symptoms. During the 1st year of the experiment, trees were cut and a 30-cm-long piece was removed at approximately 3-ft intervals. Roots up to a distance of 3 m from the base of the trunk were also sectioned at 3-ft intervals. Sections were surface-sterilized in 20% commercial Clorox (6% sodium hypochlorite) for 4 hr, then washed in running tap water for 4 hr. Tap water did not contain fluorescent bacteria when assayed on King's (Starr's) Medium "B". Sections that were over 2.5 cm in diam were sampled by removing a cross section core with a 5-mm increment borer (Sandvik No. 6, Sweden). The resulting core was cut into 2-3 mm pieces. Samples under 2.5 cm in diam were cut crosswise with a sterile saw, and cross sections taken with a sterile razor blade. Tissue samples were taken 6 inches from the end of each piece to avoid the effect of any Clorox that had penetrated.

During the last 2 years, the procedure was changed to prevent cutting the tree and thereby permitting repeated sampling of the same tree. The trunk and scaffold portions of each tree were sampled at 3-ft intervals by surface-sterilizing the limb with alcohol before inserting the borer. Cross sections were cut from shoots that were too small to be sampled with the increment borer, and adjacent shoots sampled in succeeding intervals.

All cores and cross sections of small stems were plated on medium "B" and scored for occurrence and frequency of *Pseudomonas* spp. (Fig. 1). In subsequent experiments, the bacterial cultures obtained were single-celled by dilution and tested for their oxidase reaction (2, 12) on Bacto Differentiation Disks (Difco Laboratories). Pathogenicity of cultures was determined by inoculating yellow pear tomato (*Lycopersicon esculentum* Mill.), green fruits of prune (*Prunus domestica* L.), sweet cherry, and dormant sweet cherry buds. Inoculated fruits were maintained at 100% humidity for 10 days. Inoculations into cherry buds were made with a dissecting needle in the field during mid-November, and results recorded the following April.

RESULTS.—Previous studies established the occurrence of *Pseudomonas* spp. throughout many of the trees (3, 4). Bacteria were not only isolated from near cankers, but were found as far as 20 ft from obviously diseased tissue (Fig. 2). The highest bacterial counts were obtained from the trunk, roots, and lower scaffold limbs. Smaller limbs were frequently free of bacteria except where a canker was located below the sample site on the same limb.

By the end of 1966, six trees had been sectioned and sampled for *Pseudomonas* spp. Considerable variation in bacterial numbers was encountered, apparently due to individual variation among trees or to influences associated with seasonal changes (Table 1). Because the sampling procedure involved destroying the tree, it was impossible to determine if there was a seasonal fluctuation in a single tree. The sampling method was changed to provide four sets of samples from the same tree. The 1967-68 results, which consisted of duplicate samples from four trees, show that there is a seasonal change in the bacterial population within a tree (Table 2). The results also show fewer bacteria in the upper portion of the tree than in either the middle or lower portion. The number of sites in the tree where bacteria were found increased rapidly during the spring (February-April) and then declined through summer (May-August). There was a small increase in fall and early winter followed by a low incidence in the coldest part of the season (January). Although the method of collecting the 1966 data allowed a tree to be sampled only once, results in Table 1 and 2 are similar, as the slight variation from year to year corresponds with seasonal differences in cli-

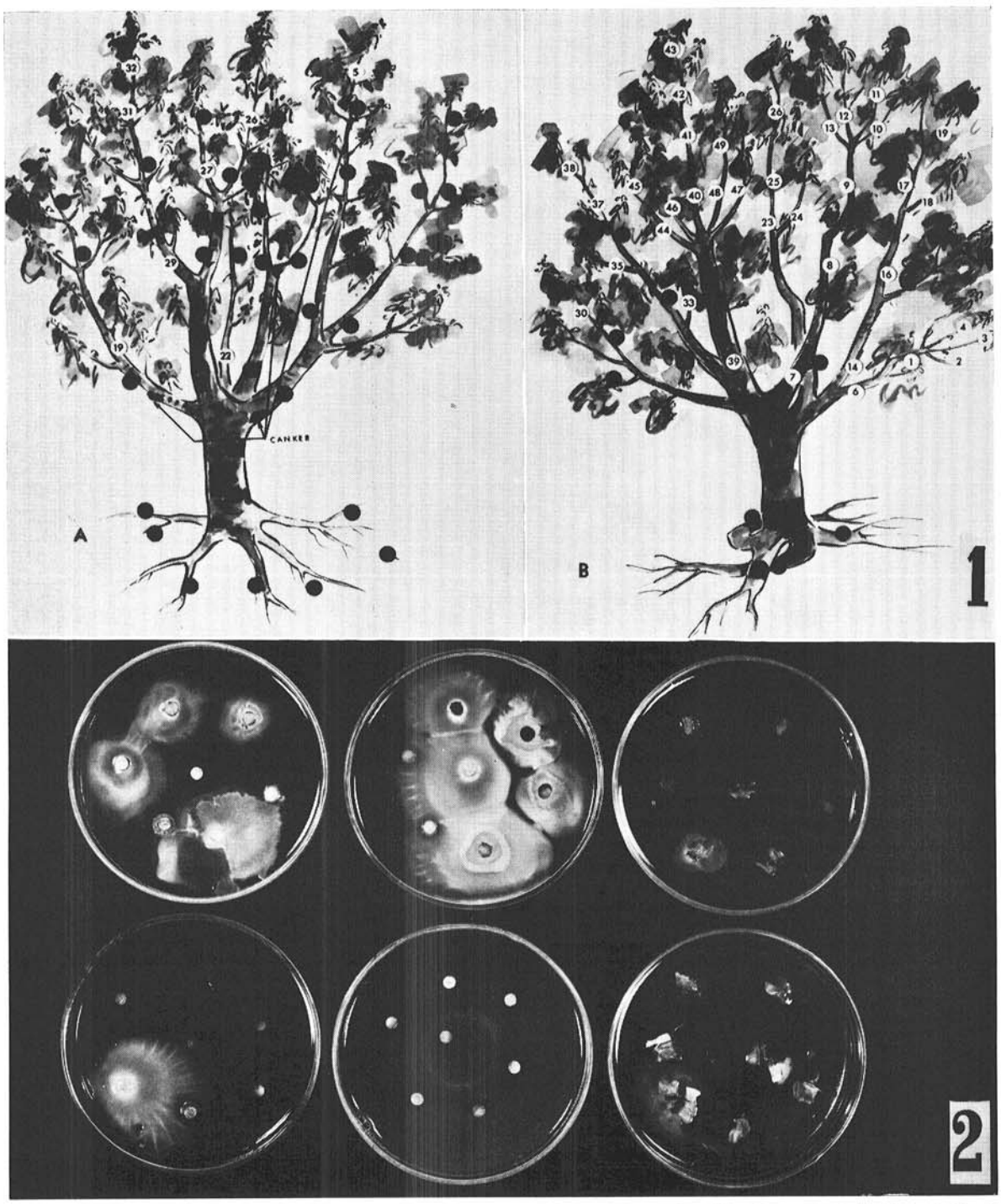

Fig. 1-2. **1)** Diagramatic drawing to show distribution of *Pseudomonas* bacteria within the tree. Black circles indicate sites where *P.* are present. White numbers indicate *P.*-absent. **A)** Tree with four cankers. **B)** Tree with no disease symptoms. **2)** Cultures of *Pseudomonas* spp. on King's Medium "B" photographed under fluorescent light.

matic conditions. The low bacterial population corresponding with the coldest month in 1966 (January 5.4 degrees above normal and February 1.1 degrees below normal) and the coldest month in 1968 (January 1.8 degrees above normal and February 4.9 degrees above normal) (Table 2). The frequency of bacteria from each sample also increased in a similar manner in both Table 1 and Table 3, allowing for yearly climatic changes. Again there is a rapid increase in bacteria from January to March, a gradual decline through

TABLE 1. Frequency of isolation of *Pseudomonas* from fruit trees during 1966

Tree sampled	Date	% Sites containing *Pseudomonas* bacteria	Frequency of *Pseudomonas* in % of isolations/tree
Sweet Cherry	27 Jan.	80	35[a]
Sweet Cherry	16 Feb.	19	20
Sweet Cherry	16 March	86	35
Sweet Cherry	16 May	15	9
Sweet Cherry	19 Dec.	20	32
Pear	26 Nov.	39	27

[a] Calculated as the total number of *Pseudomonas* isolates obtained divided by the total number of isolations attempted.

summer (April-August), and an increase in fall (August-November).

While evidence of the systemic nature of *Pseudomonas* spp. in the host is helpful in explaining the difficulties encountered with protective control measures, it is necessary to determine the percentage of the *P.* isolates that are *P. syringae*. Nine and eight-tenths % of the 9826 isolates were oxidase-negative. All but two oxidase-negative isolates were pathogenic when inoculated into cherry trees, but two isolates that were scored as oxidase-positive were also pathogenic on cherry. Numerous isolates were pathogenic on green fruit of yellow pear tomato, but they were not always the same isolates that were pathogenic on cherry. Pathogenic isolates were not confined to those sites with or adjacent to visible infections.

DISCUSSION.—During the past few years, Cameron (5), Dowler (9), and Keil & van der Zwet (11) have reported isolating pathogenic bacteria from healthy-appearing tissue. The bacteria were grown by single cell culture methods and were proven to be pathogenic on the original host species. There can be little doubt that pathogenic bacteria exist in healthy-appearing tissues. The question then arises, since the organisms are present in the absence of disease, as to what conditions of either bacterial or host metabolism may be necessary for development of disease symptoms? Probably numerous factors interact, and any one of several may be the limiting factor at any given time. The maturity of the host tissues has been shown to have an effect on bacterial infection (7). In most cases, young succulent tissues have been the most easily infected, but van der Zwet (*personal communication*) has found older wood much more susceptible in the case of Magness pear.

TABLE 2. Monthly variation in percentage of *Pseudomonas*-infected sections from Sweet Cherry

Month	% Sections/portion of tree			Avg for tree
	Lower (0′-6′)	Middle (6′-12′)	Upper (12′-)	
Nov. 1967	45.5	33.3	14.3	30.9
Dec. 1967	62.5	85.7	41.4	63.2
Jan. 1968	44.4	11.1	7.1	20.9
Feb. 1968	50.0	52.9	20.0	41.0
March 1968	100.0	78.6	14.3	64.3
April 1968	85.7	92.8	31.0	69.8
May 1968	100.0	77.8	28.0	68.6
June 1968	70.0	76.5	23.3	56.6
July 1968	81.8	71.4	33.3	62.2
Aug. 1968	37.5	50.0	10.3	32.6
Sept. 1968	100.0	77.8	60.7	79.5
Oct. 1968	50.0	29.4	26.6	35.3
Nov. 1968	90.9	92.9	47.6	77.1

TABLE 3. 1968 rainfall and percentage of samples containing *Pseudomonas*

Month of sample	Rainfall during 30-day period prior to sample[a]	% Total samples/tree containing *Pseudomonas*
Jan.	7.9	7.9
Feb.	13.6	18.0
March	11.6	58.7
April	8.3	19.9
May	2.0	23.4
June	5.8	27.6
July	.6	23.9
Aug.	.6	3.4
Sept.	12.2	22.0
Oct.	4.0	5.4
Nov.	18.6	24.8

[a] Recorded in cm.

Host nutritional levels are thought to affect susceptibility. Beard & Wormald (1) reported low available phosphate reduced the host's susceptibility to *Pseudomonas*. Later studies where N, phosphate, and potassium were applied separately and in all combinations showed no significant differences (15, 16). In Oregon, no differences in the number of cankers were noted with applications as high as 3 lb. of actual N/tree per year or with N plus either boron, zinc, potassium, phosphate, or lime (Cameron, *unpublished data*).

The water content of the host has been reported to have an effect on symptom development only when there was either excessive water, such as water-soaking after freezing (6, 8, 10), or when water content was below permanent wilting points (14). Wilson (13) was the first to suggest a fluctuating cycle of canker development. Large cankers developed during late October and November, smaller cankers through December and January; but optimum canker development was from February to April. Allowing for differences in climate, due to Oregon being at a higher latitude than California, Wilson's conclusions can be explained by the data in Tables 2 and 3. Both the distribution and numbers of bacteria increase during the periods of greatest symptom development. That canker development is not the result of the bacterial increase is shown by the corresponding bacterial increase in symptomless trees. Wilson correlated the changes in canker development with fluctuations of temp, but mentions that ". . . resumption of canker activity cannot be explained as a direct response to temperature". The data in Table 3 suggest that there is a correlation of numbers of bacteria with rainfall, and that the resulting increase in symptom development may be the effect of both temp and moisture on the bacterial population of the host. If this is true, then an increase in moisture during otherwise favorable conditions might be expected to result in an increased bacterial population. This is apparently what happened follow-

ing the very uncommon rains between the August and September sample (Table 3). Because moisture levels are usually replenished more rapidly than they are depleted, the bacterial population tends to increase more rapidly than it decreases. This is most apparent in the May and July readings. Climatological data suggest that if other factors, such as temp, are favorable, between 3 and 12 days are needed after an increase in available moisture before an increase in bacterial population is observed. If there is ample available moisture, then temp may become the limiting factor. This would explain the population drop during the colder portions of winter. Thus, the increase in available water probably not only spreads the bacteria as it moves through the tree, but also may stimulate an increase in the total number of bacteria.

The erratic results from present control measures may be partially explained as follows: In trees where the systemic existence of the pathogen is negligible, a protective spray may be quite effective in reducing the entrance of bacteria from the surface into wounds, bud scales, or other avenues of infection. If the pathogen is already systemic in the host, then the effectiveness of the protectant will decrease as the ratio of internal to external infections increases. This probably accounts for the fact that protective copper and mercury sprays applied to external surfaces have not reduced the incidence of cankers in many orchards. Satisfactory control will, therefore, depend on either modifying host-pathogen metabolism or on the development of systemic bactericides.

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