Isolation and Characterization of Fluorescent Pseudomonads from Apparently Healthy Peach Trees

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South Carolina Agricultural Experiment Station Technical Contribution No. 1153. Published with the approval of the Director.

We gratefully acknowledge the technical assistance of Ms. Jane Moose and Ms. Deborah Hohla.

Accepted for publication 13 September 1974.

ABSTRACT

Pathogenic and nonpathogenic fluorescent pseudomonads were readily isolated from apparently healthy peach twig and trunk tissue samples collected monthly in Georgia and South Carolina. No pathogenic bacteria were isolated during the summer months. Morphological and biochemical tests showed that the pathogenic isolates were closely related to *Pseudomonas syringae,* but about 50% of the fluorescent isolates were nonpathogenic. Inoculation of mature trees in the field with these isolates during early fall pruning resulted in death of trees by the following March. Heterogeneous populations of pseudomonads exist in apparently healthy peach orchards in the southeastern United States.

Phytopathology 65:233-236

Additional key words: Pseudomonas syringae, pruning, bacterial canker, peach tree short life.

Bacterial canker of stone fruits, caused by *Pseudomonas syringae* van Hall and related bacteria, has become a serious problem in many parts of the world (2). Although bacterial canker has often been implicated in the problem known as "peach-tree short life" in the southeastern United States (11, 19), the precise interaction of *P. syringae,* the induction of bacterial canker, and tree death has been difficult to understand. During the spring of 1972, more than 300,000 peach trees died in South Carolina, Georgia, and North Carolina. Despite several hundred attempted isolations, *P. syringae* or related causal organisms of bacterial canker were seldom found. However, in the spring of 1960 (17) and 1973, when many more trees died in the same area, *P. syringae* was easily isolated from diseased trees. Also cankers were obvious in many trees. It appears that (i) bacterial canker may not be involved in tree death during certain seasons, or (ii) the damage caused by bacterial canker may be subtle at times and difficult to detect.

We report the isolation of virulent pseudomonads from apparently healthy peach orchards in the Southeast and discuss their possible relationship to the problem of peach tree short life.

MATERIALS AND METHODS.—During the fall of 1971, a study was begun to determine the presence of *P. syringae* and related pseudomonads in peach orchards and their importance to the problem of peach-tree short life. Four commercial peach orchards (designated I, II, III, and IV), 2-4 years old, in the Byron-Ft. Valley, Georgia area, were surveyed at monthly intervals. Samples consisted of four terminal twigs, 25-cm long per tree, and six drill cores, 7 mm × 35 mm, taken from the main trunk and scaffold limbs. Four different trees in each orchard were sampled each month. By similar procedures, half the samples were processed in the laboratory at Byron, Georgia, and the other half at Clemson, South Carolina. Samples were transported in plastic bags to maintain cleanliness and prevent contamination. An ice chest was used to prevent extremes in temperatures. At irregular intervals during dormancy, similar samples were obtained from four orchards in South Carolina.

Leaves were removed from twigs, and the twigs were cut into 15-cm pieces. The pieces were blended 1 minute in 100 ml sterile distilled water in a Waring Blendor, and the homogenate was used for dilution plates on Pseudomonas F (Difco) agar. Drill core samples similarly were blended and diluted. The Waring Blendor was sterilized between samples by being rinsed with 0.525% sodium hypochlorite and then sterile, distilled water.

Dilution plates were incubated at 25 C and observed daily for 5 days. Fluorescent colonies were identified by our observing the plates under ultraviolet light at a wave length of 2.537×10^{-7} m (2537 Å), and representative colonies were isolated and purified for further tests. Nonfluorescent, translucent, bacterial colonies were isolated and examined morphologically and biochemically to determine possible nonfluorescent pseudomonads and their relationship to *P. syringae.* Biochemical tests described by Jones (13) were used to compare isolates with known cultures of *P. syringae* (Table 1). To determine hypersensitive reaction (HR), bacterial suspensions containing 10^8 cells/ml were injected into the mesophyll (14) of fully expanded leaves of *Nicotiana tabacum* L. Toxin production was measured by determining the inhibition of *Geotrichum candidum* by each isolate using methods described by DeVay et al. (9). Sensitivity of isolates to ten bacteriophages was determined using methods of Baigent et al. (1). Six phage isolates were obtained from soil in South Carolina using techniques described by Crosse and Hingorani (7), and four (designated 9TD, P19C, P103, and P543) were obtained from N. Baigent, University of California, Davis. Serological tests of selected isolates were conducted by J. Otta, South Dakota State University, Brookings, using previously described techniques (16).

Pathogenicity of isolates was tested on peach seedlings in the greenhouse and on mature trees growing in the

TABLE 1. Cultural characteristics of a known isolate (B-3) of *Pseudomonas syringae* and *P. syringae*-like bacteria

Test	Isolate				
	B-3	Group A	Group B	Group C	Non-pathogenic
Fluorescence	+[a]	+	+	+	+
Oxidase	−	−	−	−	−
Aesculin hydrolysis	+	+	+	−	−
Arbutin hydrolysis	+	+	+	−	−
Use of lactate	+	+	−	+	+
Use of tartrate	−	−	+	−	−
Hypersensitive reaction (in tobacco)	+	+	+	V[b]	−
Toxin production on PDA	+	+	+	V	−

[a]+ = positive response; − = negative response.
[b]V = variable response.

TABLE 2. Isolation of *Pseudomonas syringae*-like bacteria from apparently healthy peach trees in commercial orchards in 1971-72

Month	Orchard							
	I		II		III		IV	
	Twigs[a]	Drill	Twigs	Drill	Twigs	Drill	Twigs	Drill
October	−[b]	−	−	+	+	+	−	+
November	+	−	+	−	+	−	−	+
December	+	+	−	−	−	−	+	+
January	−	−	−	−	+	−	+	+
February	+	+	−	−	+	+	+	−
March	+	−	−	−	+	+	−	+
April	+	−	+	−	+	−	+	−
May	+	−	+	−	+	+	+	−
June	−	−	−	−	−	−	−	−
July	−	−	−	−	−	−	−	−
August	−	−	−	−	−	−	−	−
September	−	−	−	−	−	−	−	−
Total (+'s)	6	2	3	1	7	4	5	5

[a]Sampling procedures are described in the text.
[b]− Indicates absence of pathogen; + indicates presence.

TABLE 3. Virulence of selected pseudomonad isolates from South Carolina (SC) and Georgia (GA) compared with that of isolate B-3 of *Pseudomonas syringae* from California (CA)

Isolate No.	Origin	Group[a]	Virulence[b]	
			Seedlings	Mature trees
B-3	CA	A	10.0	10.0
215	SC	A	10.0	7.7
310	GA	A	9.5	1.7
455	GA	A	9.0	1.7
459	GA	A	10.0	4.8
026	GA	B	9.0	10.0
B158	GA	B	5.0	3.3
233	SC	B	8.5	6.7
312	GA	B	9.0	10.0
634	SC	B	6.0	10.0
635	SC	B	4.0	10.0
802	SC	−	0	0.0

[a]See Table 1 for description of Groups A and B.
[b]Virulence rating is based on visual observations for which 10 = a dead seedling tip or a dead tree, and 0 = no damage. Results are the average of ratings from six seedlings and three mature trees.

field. Peach seedlings were inoculated by injecting 0.05 to 0.1 ml of an aqueous suspension containing approximately 5×10^7 cells/ml 1-2 cm below the growing tip. Two-year-old 'Dixired' trees growing in clay soil in the Piedmont area of South Carolina, an area not severely affected by the peach-tree short life problem, were pruned in early fall when about 90% of the leaves had dropped. The entire tree was sprayed with an aqueous suspension of the desired bacteria containing $4\text{-}6 \times 10^7$ cells/ml. Previous results (11) had shown that this method of inoculation with pathogenic bacteria is effective. Bacteria were reisolated from treated trees using the same procedures described for obtaining the original isolates.

RESULTS.—*Pseudomonas syringae*-like bacteria were isolated from apparently healthy trees every month except June, July, August, and September (Table 2). The presence of these bacteria was associated with tree damage caused by bacterial canker in only one orchard. In one area of orchard I, about 20 trees died during the spring, and fluorescent, oxidase-negative bacteria were isolated from some of these trees before their death.

About 50% of the fluorescent isolates were gram-

negative rods that gave a positive oxidase reaction, a negative HR, and were nonpathogenic as determined by tests in peach seedlings in the greenhouse. Pathogenic isolates caused pronounced wilting of the tips of seedlings within 72 hours, while nonpathogens or water produced no noticeable symptoms. The pathogenic isolates comprised two major groups (Table 1), both of which appear to be closely related to *P. syringae*. A few isolates similar to *P. morsprunorum* were found (Group C). Our biochemical tests showed that Group A and B-3, a known *P. syringae* isolate from California, were identical, but several bacteriophages that lysed B-3 did not cause lysis of cultures in Groups A and B. Serological tests showed close similarities between isolates of the various groups and B-3. Groups A, B, and C did not separate serologically. Similar isolates also were found in the surveys of trees from South Carolina orchards. About 350 isolates were studied. Biochemical tests were conducted for a year, and isolates maintained in stock culture on nutrient agar (Difco) slants at 3-5 C did not change their responses to the tests.

The lack of tree damage in the presence of the bacteria raised the question of what role these bacteria play in the peach-tree short life problem. Although the tests used to characterize the bacteria showed that they were indeed *P. syringae* or closely related, we wondered if they could kill peach trees. This question led to inoculation of trees in the field with selected isolates of Groups A and B. These results (Table 3) show that these isolates did kill trees under field conditions. Of 36 trees, 27 were killed by isolates that had shown virulence in peach seedlings in the greenhouse. The nonvirulent isolates caused no noticeable damage, and trees pruned early without inoculation also survived. Isolates of Groups A and B caused similar damage, but there was little correlation between virulence to seedlings in the greenhouse and virulence under field conditions. Some isolates that had markedly damaged seedlings caused only minor damage to mature trees. Response to inoculation varied greatly. With some isolates, all treated trees were killed, while other isolates killed only one tree or parts of trees.

DISCUSSION AND CONCLUSIONS.—These results reinforce the hypothesis that peach trees must be predisposed before they are seriously damaged by bacterial canker (10). Early fall pruning has previously been associated with bacterial canker (11), and research in Georgia (8, 18) and North Carolina (4) also has shown that early pruning is potentially damaging. More evidence in support of this idea has been presented by Lownsbery et al. (15), associating *P. syringae* and the nematode *Criconemoides xenoplax*. Other factors might act similarly, causing the tree to weaken and to increase in susceptibility to bacterial canker. Therefore, *P. syringae* is probably an important factor in the death of trees under certain conditions, but at other times the observed widespread death of trees may not be associated with bacteria. Weaver et al. (19) have compared symptoms of cold injury and bacterial canker, emphasizing the need to observe trees in early spring. If bacterial canker is involved, twig and trunk cankers may be observed, and there is a characteristic sour-sap odor. At this stage, causal organisms can be isolated readily. Cold injury causes brown discoloration of the cambial area, but the bark is not completely browned. As the season progresses, symptoms of the two disorders become similar, especially the sour-sap odor and the total browning of the bark. At this stage, it may be difficult to isolate a causal organism, making an accurate diagnosis difficult.

Bacteria with the same characteristics as the inoculum were not always recovered from inoculated trees. This suggests the possibility that bacteria were transmitted from tree to tree in the orchard, or natural populations of bacteria were in the orchard. Because no similar bacteria were found in the few isolations from noninoculated trees, tree-to-tree spread appears to be responsible. Besides indiscriminate spread of the bacteria during spray-inoculation of the trees, insects may have transferred bacteria from tree to tree.

The observed variability is not unusual among pseudomonads. The variants among isolates might be considered ecotypes (6) or physiotypes (5), but no differential host effect similar to that reported by Crosse (5) was observed. The difficulty in isolating *P. syringae*-like organisms during the summer has been reported by other researchers (12) and possibly reflects a lower population of bacteria present then. Cameron (3) has reported that *Pseudomonas* spp. are systemic in cherry trees, and that similar variation occurs among his isolates, especially regarding pathogenicity.

Our results suggest that heterogeneous populations of pseudomonads, identical to or closely related to *P. syringae*, exist in apparently healthy peach orchards in the southeastern United States. These pathogens are a potential threat to peach trees, but may be less so without the presence of predisposing factors such as early fall pruning. Recommended preventive measures for bacterial canker are delay of pruning until late winter, disinfesting of pruning shears, and removing diseased trees from the orchard. However, from our results one might question whether disinfesting of pruning shears or removal of diseased trees is of significant value for control of this disease. No effective cure for established infections is known.

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