Interaction of Pseudomonas syringae and Freezing in Bacterial Canker on Excised Peach Twigs

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ABSTRACT

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A test tube incubation technique was used to study the effect of freezing on the development of bacterial canker symptoms in excised peach twigs. Bark cankers with a characteristic water-soaked appearance and "sour-sap" odor developed only on twigs that were frozen at −10 C after inoculation with *Pseudomonas syringae* and then incubated at 15 C; neither freezing nor inoculation alone produced cankers. A brown discoloration noted in the xylem of twigs after inoculation was not correlated with development of bark cankers. Twigs of eight peach cultivars were compared for susceptibility to bacterial canker, and significant differences were found among the cultivars. Longer cankers developed on twigs collected when trees were dormant rather than on twigs collected when the chilling requirement had been satisfied. These results confirm field observations that bacterial canker on peach twigs results from an interaction between infection by *P. syringae* and freeze injury.

Additional key words: Peach tree short life, *Prunus persica*.

The bacterial canker disease caused by *Pseudomonas syringae* van Hall in peach (*Prunus persica* L. Batsch) is associated with the death of trees in the central valley of California (5) and the southeastern United States (7, 15). The disease occurs on the aboveground parts of the trees, and may result in localized cankers or death of limbs or entire trees (9, 15).

Occasionally bacterial canker develops on peach twigs, causing light-tan bark cankers with a water-soaked appearance and a "sour-sap" odor (15, 20). I have observed that twig cankers appeared in naturally infected (20) and artificially inoculated (19) trees within a few days after a freeze that was preceded by mild temperatures for several days.

Klement et al. (11) demonstrated that both *P. syringae* and freezing temperatures were required for the development of bacterial canker of apricots both on trees in the field and on excised limbs in the laboratory. Vigouroux (18) reported a similar interaction between freezing and *P. mors-prunorum* f. sp. *persicae* in the development of bacterial canker in excised peach twigs. This paper presents evidence that the bacterial canker disease of peach twigs likewise results from an interaction between *P. syringae* and freezing.

MATERIALS AND METHODS

Terminal twigs 45 cm long were collected from the periphery of dormant trees of peach cultivars Babygold 5 and Coronet in commercial orchards in central Georgia. A total of 160 twigs from each cultivar was collected from eight dormant trees on 6 December 1976, and again on 4 January 1977, after the trees had been sufficiently chilled to break dormancy. Twigs also were collected from trees of eight cultivars (generally used as rootstocks) budded as scions on Lovell rootstock planted in a single block at the Southeastern Fruit and Tree Nut Laboratory, Byron, Georgia. A total of sixteen twigs was collected from four trees of each cultivar on 9 December 1976, again on 5 January 1977, and again on 17 February 1977. All twigs were placed in polyethylene bags and brought to the laboratory within 1 hr after being removed from the trees.

A technique described by Vigouroux (18) was used to surface sterilize the twigs and incubate them in glass tubes. Each twig was dipped in a 0.01% solution of sodium hypochlorite, rinsed in sterile water, dipped in 95% ethanol, and again rinsed in sterile water. The basal end was then coated by inserting it to a depth of 5 cm in melted paraffin. The twigs were placed on water-saturated cotton in the bottom of glass test tubes 25 cm long and 2.5 cm in diameter. The tubes had been sterilized in an autoclave for 1 hr at 121 C and 1 kg-force/cm^2 pressure. The apical end of the twig was cut off even with the upper edge of the tube and the cut end was inoculated immediately with a drop of *P. syringae* suspension (2.1×10^8 cells/ml) or with sterile water. Inoculum was obtained from a culture of *P. syringae* isolate 026 (8) obtained from peach canker in Georgia, grown on nutrient agar for 24 hr. The tubes were sealed with aluminum foil and placed in an incubator maintained at 15 C. After 7 days, some twigs of both groups (infective and control inoculations) from Babygold 5 and Coronet were incubated at −10 C in an incubator-freezer for 36 hr; other twigs of both groups were left at 15 C. The frozen and nonfrozen twigs then were incubated for 10 days at 15 C and examined for

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injury. The length of each lesion on the twigs was measured and recorded. All twigs of the other eight cultivars, with and without previous inoculation with *P. syringae*, were frozen after incubation at 15 C for 7 days and then incubated and examined as described above.

Attempts were made to recover *P. syringae* from diseased or apparently healthy tissue in twigs whether or not they were inoculated with the bacterium. A sterilized scalpel and forceps were used to aseptically remove pieces of bark or wood, about 2 mm^2, from the twigs. Each piece was put into a separate test tube containing 5 ml of sterile nutrient broth and then was incubated at 28 C for 48 hr. A loopful of broth then was spread on an agar medium selective for *Pseudomonas* spp. (16) and incubated at 28 C for 48 hr. Bacterial colonies were observed for fluorescence under long-wave ultraviolet light, and representative fluorescent colonies were tested for oxidase reaction (12). Colonies that were fluorescent and oxidase-negative were considered to be *P. syringae*-like bacteria based on previous studies of numerous isolations of bacteria from peach twigs in Georgia and South Carolina (8). Selected isolates also were tested for toxin production (6) to confirm their identity.

RESULTS

After 18.5 days, noninoculated twigs appeared fresh and healthy whether they had been frozen or not. No internal discoloration of bark or xylem was observed. All buds were killed on twigs that were frozen whether or not the twigs were inoculated with *P. sryingae*; buds on nonfrozen twigs remained healthy. We did not determine if ice formed in the twigs after the low temperature treatments.

Light-tan bark cankers with a water-soaked appearance and "sour-sap" odor developed on twigs that were frozen after inoculation with *P. syringae* (Table 1). The symptoms were identical with those in twig cankers observed on peach trees in the field. Bark cankers became visible 3 to 4 days after twigs were transferred from the freezer to the incubator and were distinguished easily from healthy bark by the differences in color (Fig. 1). Cankers were significantly larger on twigs of Babygold 5 peach collected in December than on twigs of Coronet collected in December or January and Babygold 5 collected in January (Table 1). However, bark cankers in both cultivars were about three times longer on twigs collected and inoculated with *P. syringae* in December when the trees were dormant rather than on those collected in January when the chilling requirement had been satisfied.

A brown discoloration appeared in the cambium and outer xylem of all twigs, frozen and nonfrozen, that were inoculated with *P. syringae*. The browning was most intense near the inoculated end and lessened as it extended downward. One or more brown streaks usually extended several millimeters below the generally browned area.

In twigs inoculated with *P. syringae* but not frozen, the browned areas in the cambium and xylem were significantly longer in Coronet peach collected in December and

TABLE 1. Effect of inoculation with *Pseudomonas syringae* and subsequent freezing on development of bark cankers and xylem browning in excised twigs of two peach cultivars[a]

		Average length of injury[b] in twigs inoculated with P. syringae and:			
		Frozen		Not frozen	
Cultivar	Sample date (1976-77)	Bark canker (mm)	Brown cambium and xylem (mm)	Bark canker (mm)	Brown cambium and xylem (mm)
Coronet	6 Dec	7.7	41.9	0	44.1
	4 Jan	2.2	45.5	0	41.6
Babygold 5	6 Dec	15.9	36.7	0	31.2
	4 Jan	4.4	36.9	0	35.5
LSD ($P = 0.05$)		7.9	5.0		9.4
r[c]			0.12 NS		

[a]Twigs were placed in sterile test tubes, their apical ends were removed, and the twigs were inoculated with *P. syringae* or sterile water and incubated at 15 C for 7 days. Twigs from each group were then frozen at −10 C for 36 hr or left at 15 C. All twigs were then incubated at 15 C for 10 days and examined.

[b]Each value is the average of 40 samples; length of injured portion of twig was measured beginning at the inoculated end.

[c]Coefficient of correlation between length of bark canker and xylem browning. The abbreviation NS indicates no significance.

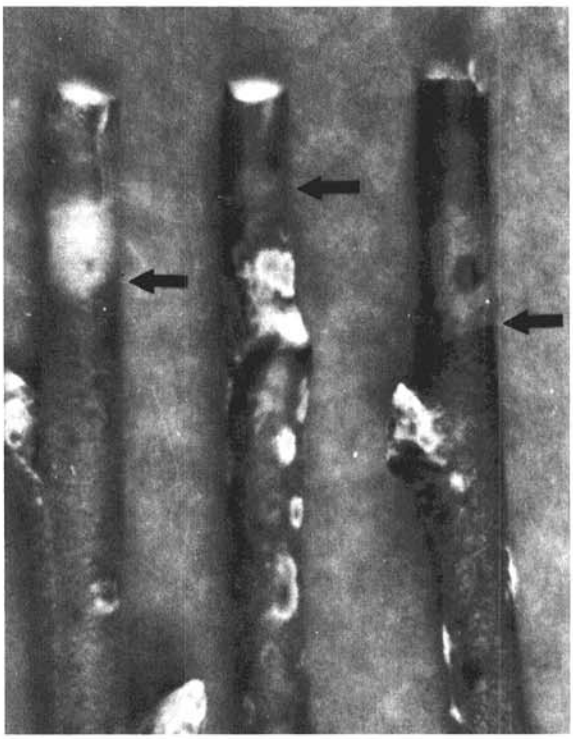

Fig. 1. Bark cankers on excised peach twigs artificially inoculated with *Pseudomonas syringae* and frozen. Note difference in color between diseased bark above canker margins (arrows) and healthy bark below.

January than in Babygold 5 collected in December. In twigs inoculated with *P. syringae* and then frozen, browned areas also were significantly longer in Coronet than in Babygold 5. Differences in the size of browned areas between frozen and nonfrozen *P. syringae*-inoculated twigs of each cultivar were small. The coefficient of correlation between length of bark cankers and browning in the cambium and xylem of twigs that were inoculated with *P. syringae* and frozen was 0.12 (not significant at $P = 0.05$).

When twigs of the other eight cultivars were subjected only to the freezing treatment, with and without previous inoculation with *P. syringae*, no injury was observed on noninoculated twigs. However, both bark cankers (Table 2) and xylem and cambium browning developed in twigs frozen after inoculation with *P. syringae*. The length of browned areas in the xylem and cambium of twigs was extremely variable. Further, the coefficient of correlation between length of bark cankers and browning in the cambium and xylem was 0.23 (not significant at $P = 0.05$). For these reasons data on xylem browning are not included in Table 2.

In all cultivars but NA-8, bark cankers were longest on twigs sampled in December and shortest on twigs sampled in January. The longest cankers developed on Nemaguard and the shortest on Ferris on each sample date, although these cankers did not differ significantly from those on the other cultivars on the same sample date. When data from the three sample dates were averaged, the cankers on Ferris were smaller than those on any other cultivar, and the cankers on Nemaguard were significantly larger than those on Ferris, Tennessee Natural, 152AI-2, and Boone County.

In efforts to culture *P. syringae* from tissues, a minimum of 25 samples of tissue were removed from each of the following: margins of bark cankers, apparently healthy bark (several millimeters below margins of bark cankers), browned xylem, brown streaks in xylem, apparently healthy xylem in all twigs in which injury occurred, and healthy bark and xylem of non-inoculated twigs. *Pseudomonas syringae*-like bacteria were recovered from 95-100% of the samples of bark cankers, browned xylem, and brown-streaked xylem, but never from apparently healthy bark or xylem. No other types of bacteria were recovered.

DISCUSSION

The "sour-sap" phase of bacterial canker was described as early as 1930 by Goldsworthy and Smith (10), but the question of whether *P. syringae* is the sole pathogen still remains. The results of studies designed to determine the effect of temperature on the development of bacterial canker in peach trees inoculated with *P. syringae* have been inconclusive (4, 5). Davis and English (5) found no cankers on peach trees grown in containers of orchard soil held at 15 C, a temperature they considered favorable for disease development, but severe bacterial canker occurred on trees held outdoors in the orchard. They concluded that freezing injury that occurred in the orchard could have promoted infection by the bacterium. Klement et al. (11) reported that severe damage from bacterial canker in apricot trees inoculated with *P. syringae* occurred only in association with orchard temperatures well below freezing.

The results of the present study provide direct evidence supporting previous observations that the development of bacterial canker in peach twigs is associated with below-freezing temperatures that follow mild periods during the winter. These results confirm other reports that both freezing and inoculation with a pathogen were required for the development of a bacterial canker disease in peach twigs (18) and apricot branches (11).

The potential for involvement of *P. syringae* in the freeze injury of plant cells recently was demonstrated by Arny et al. (1). They showed that *P. syringae*, which promotes ice nucleation (13), increased the frost sensitivity of corn leaves. Thus, leaf tissue inoculated with *P. syringae* was injured at temperatures not low enough to injure noninoculated leaves. This phenomenon may have been at least partly responsible for the bark injury observed in my tests and those of others (11, 18). Based on our results, it is possible that bark tissue inoculated with *P. syringae* was infected with the bacterium during

TABLE 2. Effect of inoculation with *Pseudomonas syringae* and freezing on development of bark cankers in excised twigs of eight peach cultivars[a]

	Length of bark cankers on twigs inoculated:			
Cultivar	9 Dec 1976 (mm)	5 Jan 1977 (mm)	7 Feb 1977 (mm)	Average
Ferris	1.8 A[b]	0.7 A	1.7 A	1.2 A
Tennessee Natural	4.7 AB	1.6 A	3.9 AB	3.4 B
152AI-2	6.5 AB	1.8 A	2.2 A	3.5 B
Boone County	6.7 AB	1.2 A	3.3 AB	3.7 B
NA-8	3.9 A	1.2 A	6.7 B	3.9 BC
Halford	6.1 AB	4.6 BC	4.6 AB	5.1 BC
Rutgers Red Leaf	10.2 BC	4.5 B	5.9 AB	6.7 BC
Nemaguard	14.9 C	5.0 C	7.5 B	9.1 C
Average	6.9	2.6	4.5	

[a]Eight twigs of each cultivar were placed in sterile test tubes and their apical ends were removed. The twigs were inoculated with *P. syringae*, incubated at 15 C for 7 days, frozen at −10 C for 36 hr, and incubated at 15 C for 10 more days before cankers were measured.

[b]In each column, values followed by a letter in common do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

incubation at a temperature favorable for its growth, and that the infected bark then became sensitive to freeze injury. Freeze-injured tissue may have stimulated the bacterium or induced it to produce syringomycin (6, 17) which caused the final symptoms of light-tan discolored bark with a water-soaked appearance and a "sour-sap" odor.

Browning in the cambium and outer xylem of twigs inoculated with *P. syringae* apparently was due to invasion by the pathogen which was readily isolated from discolored xylem, but not from apparently healthy xylem. Discoloration of the cambium and xylem of peach shoots inoculated with *P. syringae* has been reported (5, 7, 11). Histological studies have shown that *P. syringae* primarily invades the inner bark adjacent to the cambium on diseased peach branches (5).

Several studies have shown that peach trees inoculated with *P. syringae* in the field were more susceptible to infection during late fall and winter than at other times (2, 5, 7, 10). Davis and English (5) further concluded that susceptibility varied directly with the degree of dormancy of the tree. My results with excised peach twigs (Tables 1 and 2) support this conclusion; in most cases, longer cankers developed on twigs removed from trees and inoculated in December, when the trees were dormant, than on those removed and inoculated in January or February when the trees had been sufficiently chilled to break dormancy. However, the cultivars varied greatly in the relative length of cankers produced on twigs collected before and after the chilling requirement was satisfied.

Differences in susceptibility of peach cultivars to *P. syringae* have been observed and the rootstock also has been shown to affect the susceptibility of peach trees to bacterial canker and cold injury (3, 21). Greatest tree loss in the southeastern USA consistently has occurred where Nemaguard rootstock was used (21). My results with excised twigs of several cultivars showed that Nemguard was the most susceptible to bacterial canker.

The results of the present study suggest a close interaction between infection by the bacterium *P. syringae* and freeze injury in the formation of typical bacterial canker symptoms in peach twigs. Further evidence for this interaction is provided by the observation that practices such as soil fumigation (3, 5, 9, 14, 21) and pruning at the proper time (2, 3, 7, 14), which reduced the susceptibility of peach trees to bacterial canker, also increased their cold hardiness (14). Studies are underway to elucidate further the role of *P. syringae* in the bacterial canker disease of peach trees in the southeastern USA.

LITERATURE CITED

1. ARNY, D. C., S. E. LINDOW, and C. D. UPPER. 1976. Frost sensitivity of Zea mays increased by application of Pseudomonas syringae. Nature 262:282-284.
2. CHANDLER, W. A., and J. W. DANIELL. 1976. Relation of pruning time and inoculation with Pseudomonas syringae van Hall to short life of peach trees growing on old peach land. HortScience 11:103-104.
3. CLAYTON, C. N. 1977. Peach tree survival. Fruit South 1:53-58.
4. DANIELL, J. W., and W. A. CHANDLER. 1974. Effect of temperature on bacterial canker in peach seedlings grown in old and new peach soil. Phytopathology 64:1284-1286.
5. DAVIS, J. R., and H. ENGLISH. 1969. Factors related to the development of bacterial canker in peach. Phytopathology 59:588-595.
6. DE VAY, J. E., F. L. LUKEZIC, S. L. SINDEN, H. ENGLISH, and D. L. COPLIN. 1968. A biocide produced by pathogenic isolates of Pseudomonas syringae and its possible role in the bacterial canker disease of peach trees. Phytopathology 58:95-101.
7. DOWLER, W. M., and D. H. PETERSEN. 1966. Induction of bacterial canker of peach in the field. Phytopathology 56:989-990.
8. DOWLER, W. M., and D. J. WEAVER. 1975. Isolation and characterization of fluorescent pseudomonads from apparently healthy peach trees. Phytopathology 65:233-236.
9. ENGLISH, H., and J. E. DE VAY. 1964. Influence of soil fumigation on growth and canker resistance of young fruit trees in California. Down Earth 20:6-8.
10. GOLDSWORTHY, M. C., and R. E. SMITH. 1930. "Sour-sap" in trees of the genus Prunus. Science 71:506-507.
11. KLEMENT, Z., D. S. ROZSNYAY, and M. ARSENIJEVIĆ. 1974. Apoplexy of apricots. II. Relationship of winter frost and the bacterial canker and die-back of apricots. Acta Phytopathol. Acad. Sci. Hung. 9:35-45.
12. KOVACS, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature 178:703.
13. MAKI, L. R., E. L. GALYAN, M. CHANG-CHIEN, and D. CALDWELL. 1974. Ice nucleation induced by Pseudomonas syringae. Appl. Microbiol. 28:456-459.
14. NESMITH, W. C., and W. M. DOWLER. 1975. Soil fumigation and fall pruning related to peach tree short life. Phytopathology 65:277-280.
15. PETERSEN, D. H., and W. M. DOWLER. 1965. Bacterial canker of stone fruits in the southeastern states. Plant Dis. Rep. 49:701-702.
16. SANDS, D. C., L. HANKIN, and M. ZUCKER. 1972. A selective medium for pectolytic fluorescent pseudomonads. Phytopathology 62:998-1000.
17. SINDEN, S. L., J. E. DE VAY, and P. A. BACKMAN. 1971. Properties of syringomycin, a wide spectrum antibiotic and phytotoxin produced by Pseudomonas syringae, and its role in the bacterial canker disease of peach trees. Physiol. Plant Pathol. 1:199-213.
18. VIGOUROUX, A. 1974. Obtention de symptômes de bacteriose du pêcher (Pseudomonas mors-prunorum f. sp. persicae) sur rameaux de pêcher détachés et conservés en survie. Effet du froid. Ann. Phytopathol. 6:95-98.
19. WEAVER, D. J. and E. J. WEHUNT. 1975. Effect of soil pH on susceptibility of peach to Pseudomonas syringae. Phytopathology 65:985-989.
20. WEAVER, D. J., E. J. WEHUNT, and W. M. DOWLER. 1974. Association of tree site, Pseudomonas syringae, Criconemoides xenoplax, and pruning date with short life of peach trees in Georgia. Plant Dis. Rep. 58:76-79.
21. ZEHR, E. I., R. W. MILLER, and F. H. SMITH. 1976. Soil fumigation and peach rootstocks for protection against peach tree short life. Phytopathology 66:689-694.