Overwintering and Survival of *Pseudomonas syringae* pv. *syringae* and Symptom Development in Peach Trees

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ABSTRACT

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Peach trees (cultivar Redhaven) were inoculated in October 1981, when leaves were 5–10% abscised, and again in February 1982 with a suspension (10^7 colony-forming units per milliliter) of a rifampin-resistant strain of *P. syringae* pv. *syringae* (rif 2). Inoculum was applied by either brushing the bacterial suspension onto the surfaces of buds and leaf scars (SI) or by injecting it into internodal punctures (PI). Internal populations of rif 2 were monitored biweekly by plating onto a semiselective medium. October SI resulted in rapid initial colonization followed by a decline in rif 2 populations to below detection levels, whereas October PI resulted in stable population levels throughout the following winter and spring. No internal colonization was detected in February SI sites. In contrast, February PI resulted in larger populations than did October PI and were accompanied by the development of cankers at 98.8% of the inoculated sites. February PI infections were also associated with necrosis of fruit and shoot buds and delayed budbreak. Populations of rif 2 remained viable within February PI sites throughout the summer and fall. Rif 2 could not be reisolated as part of the epiphytic twig, shoot, or blossom flora in March.

Additional key words: bacterial canker, peach tree short life, *Prunus persica*

Bacterial canker of stone fruits, caused by *Pseudomonas syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum*, is annually cyclic (6,22,23), alternating between a foliar phase in summer and a canker and/or bud blight phase in the fall, winter, or spring. The canker phase, which is usually most destructive, often causes death of distal tissues by rapidly girdling the colonized limb (21). Canker development then ceases, due either to structural defense reactions in the host or an inhibition of bacterial growth within the tissue by summer temperatures (25). Losses can also result from the failure of infected buds to open in the spring (21).

Bacterial canker of peach is attributed to *P. syringae* pv. *syringae* (1). In the southeastern United States, bacterial canker is associated with the peach tree short life syndrome (PTSL), which is responsible for reduced tree longevity (11,18,19). Peach trees in South Carolina were most susceptible to infection in October and least susceptible in February (9,10). Peach trees generally are most susceptible when dormant (7,23), despite the continuous availability of inoculum (11). Studies using wound inoculations of plum have shown that trees infected early in the fall formed a callus around the infection site and healed rapidly (1), whereas later infections resulted in continuous canker development throughout dormancy (14).

Besides entering through wounds, *P. syringae* pv. *syringae* may also invade peach trees through natural cracks in bud scales and unhealed leaf traces (1). Invasion through leaf scars is known to occur in cherry (4), whereas inoculation of plum buds has resulted in little or no infection unless accompanied by puncturing (24,25). Little information exists concerning the infection of peach trees in the southeastern growing areas.

This study was done to relate the time and method of inoculation to establishment and survival of the pathogen and development of disease in peach.

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MATERIALS AND METHODS

A rifampin-resistant strain of *Pseudomonas syringae* pv. *syringae*, strain B-15+ rif 2 (rif 2), was used for inoculations. This strain was previously shown to be equivalent in biochemical charactersitcs and virulence to the original wild type (8). Bacteria were grown for 36 hr on nutrient agar, harvested, and diluted to 10^7 colony-forming units (cfu) per milliliter in sterile phosphate buffer (0.02 M, pH 7.0).

Terminals of current year's growth of 6-yr-old trees (*Prunus persica* 'Redhaven') on Lovell rootstock were inoculated in the fall, when 5–10% of the leaves had abscised (6 October 1981), or in late winter (23 February 1982). One set of 240 twigs was surface-inoculated (SI) by detaching the remaining leaves and applying the cell suspension to leaf scars and associated buds with a size 2 camel's-hair brush. A corresponding set of 240 twigs was puncture-inoculated (PI) by inserting a 23-gauge syringe needle internodally and injecting the suspension to saturation. Sterile buffer was used for the control. Treatments were replicated on eight trees, with 10 inoculation sites per twig and eight twigs per treatment for each sampling.

Internal populations of rif 2 were monitored biweekly. Eight twigs from each treatment were surface-disinfested for 3 min in 0.52% sodium hypochlorite and rinsed for 5 min in distilled water; sections of the twig at individual inoculation sites were then split longitudinally and soaked in 0.50 ml sterile buffer for 3 hr to extract bacteria. Twenty-five microliters of the buffer was plated onto King's medium B (15) amended with 50 μg/ml each of rifampin and cycloheximide, and incubated 4 days at 24 C. A rating scale of 0–3, based on colony counts, was used to estimate bacterial growth on the reisolation medium. Ratings were selected instead of colony counts because they could be converted to cfu/cm^3 on the basis of average twig-section volumes. Ratings were as follows: 0 = no colonies; 1 = one to five colonies, equivalent to about 10^3 cfu/cm^3 tissue; 2 = six to 25 colonies, equivalent to about 10^4 cfu/cm^3 tissue; and 3 = 25+ colonies, equivalent to $>10^4$ cfu/cm^3 tissue. The identity of selected colonies was verified by oxidase reaction (20) and virulence tests using seedling bioassays (12).

RESULTS

October inoculations. Internal populations were initially greatest in October PI sites (Fig. 1, solid line). Six weeks after inoculation, however, rif 2 was reisolated infrequently as populations declined below detection levels (Fig. 1). In contrast, rif 2 was reisolated from PI sites throughout the fall, winter, and spring. Mean populations per colonized site (Fig. 1, broken line) remained relatively stable throughout this period. Both SI and PI sites remained asymptomatic and did not

contain detectable rif 2 when sampled the following fall (Table 1).

February inoculations. No internal colonization was detected within February SI sites; however, February PI resulted in the largest internal populations of rif 2 encountered in this study (Table 1). These populations were larger than those resulting from October PI. Mean population ratings per colonized site ($\bar{x}$= 2.98) approached the maximum possible reisolation rating ($\bar{x}$= 3). Furthermore, a low percentage of February PI sites (3.8%) still contained detectable rif 2 the following fall (Table 1).

Symptoms. February PI was the only treatment that resulted in development of external symptoms. Large internal populations occurred 2 wk after inoculation and were followed by the appearance of cankers in 98.8% of inoculated sites 1 wk later (Table 2). Cankers were black, sunken, accompanied by gumming, and often coalesced. Most of the February PI twigs died within 2 mo after inoculation. Of the eight cankered twigs that survived to the fall 1982 sampling, six showed premature leaf discoloration.

February PI were also associated with development of fruit and shoot bud necrosis (Table 2). Fruit bud symptoms varied from browning of the stigma or entire gynoecium to necrosis of the entire bud. Chi-square analysis of fruit bud injury and frequency of reisolation of rif 2 indicated that these two events were highly dependent (χ^2 = 15.3, $P > 0.99$). Likewise, leaf bud injury also varied from slight to moderate internal necrosis to bud death. Leaf buds that were only slightly affected showed no external symptoms other than delayed bud development, similar to the response described for cherry (7). Chi-square analysis in this case indicated that symptom occurrence and frequency of reisolation of rif 2 were independent events (χ^2 = 2.2).

Epiphytic survival. Expanding leaf buds, flowers, and twigs from SI treatments were sampled during full bloom (23 March 1982) to determine the presence of rif 2 on these surfaces. Samples were washed individually by intermittent agitation in 0.50 ml sterile buffer for 3 hr, after which 25 µl of the suspension was plated onto the reisolation medium. Although several fluorescent pseudomonads were recovered from both bud and twig samples, restreaking onto fresh medium showed that none of these isolates were resistant to rifampin and therefore were not the inoculated strain.

Natural infections. Leaf and flower buds from all control treatments were sampled internally at the pink bud growth stage (16 March) for presence of fluorescent bacteria. For these attempts, rifampin was omitted from the reisolation medium. Only two strains of fluorescent bacteria were isolated from symptomless fruit buds; one was subsequently identified as rif 2 by its resistance to 50 µg/ml rifampin, whereas the other was sensitive to rifampin and nonpathogenic in seedling bioassays.

DISCUSSION

Although rif 2 entered twigs through natural openings in October, the bacteria apparently were unable to establish sufficient internal populations for long-term survival. Bark infections may be short-lived; histological observations of plum have suggested that internal survival of *P. syringae* may depend on colonization of the xylem (14). The most probable route of entry in October SI was through leaf scars because SI made in February, when leaf traces were healed (4), resulted in no colonization. Invasion through peach leaf scars was clearly less successful than that reported for cherry (4). This difference may be partially explained by the fact that *P. syringae* pv. *syringae* is considered less capable of invading twigs through leaf scars than is *P. syringae* pv. *morsprunorum* (5). However, the higher frequency of reisolation from wound inoculation than from surface inoculations is consistent with trends observed on cherry (5) and plum (24,25).

Rif 2 was evidently capable of

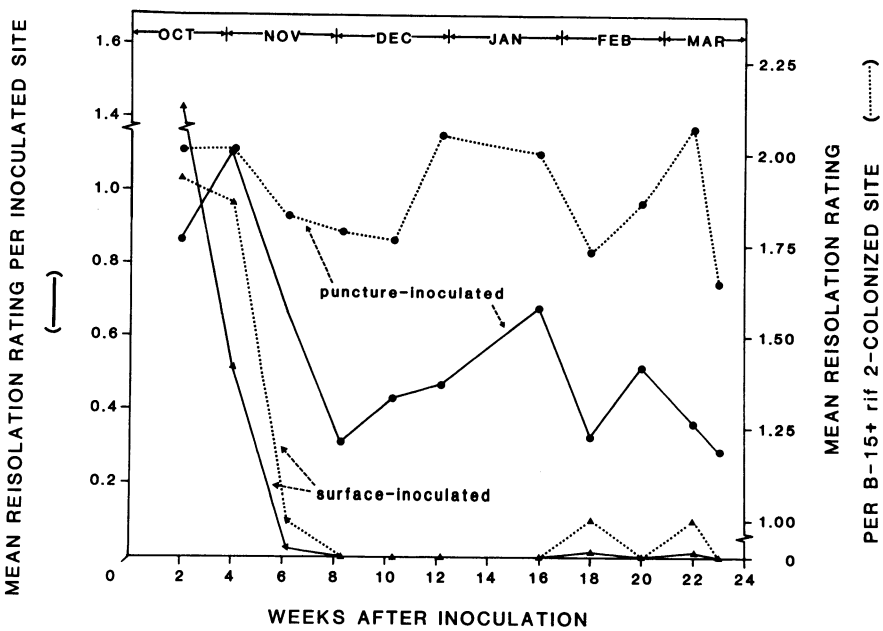

Fig. 1. Population dynamics of *Pseudomonas syringae* pv. syringae B-15+ rif 2 within inoculated twigs (as measured by a reisolation rating scale of 0–3) from fall 1981 to spring 1982. Each point represents samples from 80 inoculated sites.

Table 1. Effect of time and method of inoculation on internal populations of *Pseudomonas syringae* pv. *syringae* rif 2 in peach twigs

Time of inoculation	Method of inoculation	Mean reisolation ratings[a] 2 Wk after inoculation	Pink bud stage (16 Mar. 82)	4 May 82	6 Oct. 82
6 Oct. 1981	Puncture	0.73	0.29	0.26	0.00
	Surface	1.43	0.00	0.00	0.00
23 Feb. 1982	Puncture	2.98	2.75	0.87[b]	0.04[b]
	Surface	0.00	0.00	0.00	0.00

[a] Based on reisolation rating scale of 0–3, where 0 = no colonies, 1 = one to five colonies, 2 = six to 25 colonies, and 3 = 25+ colonies. Means represent samples from 80 inoculation sites.
[b] Distal inoculation sites were completely necrotic and dehydrated at these samplings.

Table 2. Effect of time and method of inoculation on symptom development and reisolation frequency of *Pseudomonas syringae* pv. *syringae* rif 2 from peach tissues

Time of inoculation	Method of inoculation	Sites developing cankers (%)	Fruit buds necrotic[a] (%)	Fruit buds colonized[b] (%)	Leaf buds necrotic (%)	Leaf buds colonized (%)
6 Oct. 1981	Puncture	0.0	2.3	0.0	19.1	0.0
	Surface	0.0	4.9	0.0	13.9	0.0
23 Feb. 1982	Puncture	98.0	38.8	30.6	20.0	15.0
	Surface	0.0	3.8	1.9	10.0	0.0

[a] Symptom data were taken at pink bud stage (16 March 1982).
[b] Based on frequency of reisolation.

overwintering in twig tissue without producing external symptoms. This mode of internal survival has been reported previously in England on cherry (3) and in Chile on pear (20). Internal distribution of bacteria was generally limited to the vicinity of the inoculation site and was not systemic. However, in association with large internal populations within the inoculation site (February PI), rif 2 moved into flower and leaf buds, indicating a passive form of translocation caused by increasing pressure from accumulating bacterial cells. One important observation involving symptom development was that bud infection was the result of internal movement of rif 2 and not of suface inoculation. Direct bud inoculations have previously been unsuccessful unless the inoculum was introduced through wounds (16,17). Thus, despite the apparent resistance of bud scale scars to infection, buds may nevertheless undergo necrosis following internal colonization of adjacent twig tissues. Bud blighting by *P. syringae* pv. *syringae* has been reported in South Carolina (18) and deserves further study in the Southeast.

Because no naturally occurring, virulent strains of *P. syringae* pv. *syringae* were found during the experiment, the source of natural inoculum remains unknown. Oversummering populations within cankers, such as those resulting from February PI, could act as potential sources. When such inocula become available, wounds could be important infection sites on peach.

Although February PI resulted in consistent canker formation and twig dieback, only the portion of the twig distal to the inoculation site was killed. Previous attempts to reproduce large-scale injury or tree death through field inoculations have often failed (1,18,24); where severe symptoms developed, large concentrations of inoculum had been used (7). It is possible that environmental conditions in 1981–1982 were favorable for rapid wound healing or less conducive to extensive development of bacterial canker. Accumulated data from surveys of bacterial canker of cherry in England suggest that environmental conditions favoring extensive canker development rarely occur (2). Our data suggest that a similar situation may exist for bacterial canker of peach in North Carolina. *P. syringae* pv. *syringae* may play a diminutive role in the PTSL complex so that its predominant activity is a localized killing of small areas of tissue rather than the induction of large cankers that result in tree death. This mode of pathogenesis could account for the difficulty in isolating *P. syringae* pv. *syringae* from injured trees and the failure to induce severe tree injury by artificial inoculation (1,13,18,22).

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