Ecology and Epidemiology

Incidence, Distribution, and Development of Pruning Wound Cankers Caused by *Phytophthora syringae* in Almond Orchards in California

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ABSTRACT

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Almond trees in three orchards in California were surveyed for pruning wound cankers caused by *Phytophthora syringae*. In the orchard with the most disease, 23% of all pruning wounds examined had cankers, and some sections of the orchard had more than 50% of the wounds infected. The number of infections increased linearly as the diameter of pruning wounds increased ($r^2 > 0.93$). Pruning wound cankers were frequently observed high in the trees, and, in one orchard, the percentage of pruning wounds with cankers increased with height, while in a second orchard there was no clear trend. Cankers resulting from inoculations with *P. syringae* expanded throughout the fall, winter, and spring but ceased expansion in summer. In the orchards surveyed, there was no sign of canker expansion during or after summer. *P. syringae* was isolated from 18 to 26% of recently fallen almond leaves on the orchard floor. Abundant oospores were formed in the leaves. Pruning wound cankers occurred most frequently in the parts of the orchards pruned during periods of heavy rainfall.

Phytophthora syringae (Kleb.) Kleb. causes crown and collar rot of several species of stone fruit trees in California (13). Recently, *P. syringae* was found to enter through pruning wounds and cause cankers in almond trees *Prunus dulcis* (Mill.) Webb (1). *P. syringae* grows and infects well at low temperatures, but the mycelium dies above 25 C (1). Pruning in almond orchards in California usually takes place from September through February. Pruning wound cankers were observed in winter and spring when the temperatures were relatively low, but seemed to stop expansion in late spring or early summer as the temperature rose (1).

P. syringae has been observed to cause aboveground diseases with several other tree species. In Italy, an aerial fruit rot of peaches was caused by the zoospores of *P. syringae* in the irrigation water being dispersed by sprinklers (2). Apple fruit in England was found to be infected by *P. syringae* while still on the tree, resulting in a fruit rot (16). Brown rot of twigs, leaves, blossoms, and fruit on citrus trees in California is caused by several *Phytophthora* species, including *P. syringae* (5).

Although pruning wound cankers were present in many almond orchards in California, little was known about the disease because it has only been observed recently. The objective of this study was to better understand disease incidence, distribution, and development from the results of surveys conducted in several bearing almond orchards. A preliminary report of this work has been published (4).

MATERIALS AND METHODS

Three bearing almond orchards, from which *P. syringae* was isolated from pruning wound cankers using PVP medium (1), were surveyed (Table 1). Orchards A and B are in Colusa County and orchard C in Yolo County, CA. During the summer, drip irrigation was used in orchard A and sprinkler irrigation at ground level in orchards B and C. Orchards A and B were pruned in December 1983 and orchard C in October 1984 through January 1985, and the orchards were surveyed for cankers the following April. All of the trees were examined in orchards A and C, but only certain rows in orchard B. In orchards A and B, for all trees examined, the diameter and height from the ground for all pruning wounds were measured. In orchard C, only the number of pruning wound cankers was counted for each tree. In orchard C, the progress of the pruners through the orchard was noted throughout the fall. The trees in the surveyed orchards were approximately 5–8 m in height, and the pattern of pruning in the orchards was down the rows. The cultivars in each orchard and the number of trees and pruning wounds surveyed are presented in Table 1. The pattern of disease down the rows in orchards A and B was tested for randomness using runs analysis (12).

Eighty-three leaves on the orchard floor and 13 leaves still attached to the trees were collected throughout orchards A and B in the fall of 1983 and 1984. The leaves were kept in an ice chest and isolations were performed the same day. Leaves were surface sterilized with 95% ethanol. Isolations were made in PVP medium (1) using the petiole and nine 5- × 5-mm pieces from each leaf.

In a fourth orchard in Colusa County, CA, fresh wounds were made and inoculated with *P. syringae* during the period from November 1983 to April 1984. Twenty branches (1–2 cm diameter) were wounded each month by making cross-sectional cuts with pruning shears and inoculated by placing one 9-mm-diameter corn meal agar plug containing mycelium of *P. syringae* (isolate F-79) from the colony margin on each wound. Some wounds were not inoculated as controls. All wounds were wrapped with Parafilm. At various times throughout the winter and spring the length of discoloration in the inner bark from the point of inoculation was measured.

TABLE 1. The number of trees and pruning wounds examined and the incidence of pruning wound cankers caused by *Phytophthora syringae* in the almond orchards A, B, and C[a]

		Number observed		Pruning wounds	
Orchard	Cultivar	Trees	Pruning wounds	with cankers (%)	Cankers per tree
A	Nonpareil	224	785	12.4	0.43
	Mission	56	119	4.2	0.09
	NePlus Ultra	28	105	3.8	0.14
B	Nonpareil	313	1,186	23.2	0.88
	NePlus Ultra	86	408	25.5	1.21
	Price	86	541	22.4	1.41
C	Nonpareil	634	...[b]	...[b]	0.38
	Butte	319	...	...	0.30
	Price	301	...	...	0.23

[a]Orchards A and B were pruned in December 1983 and orchard C in October 1984 through January 1985. The orchards were surveyed the following springs.

[b]In orchard C trees were examined for number of cankers only and individual pruning wounds were not measured or counted.

RESULTS

All of the pruning wound cankers observed in all orchards showed the symptoms typical of cankers caused by *P. syringae*. Of all three orchards, orchard B had the highest disease incidence with about 23% of the pruning wounds surveyed having cankers (Table 1), and from some parts of the orchard the disease incidence was more than 60%. For many of the trees in orchard B, all or most of the pruning wounds developed cankers. In orchard A, the cultivar Nonpareil had a substantially higher percentage of cankers than NePlus Ultra, whereas in orchard B both cultivars had about the same disease levels (Table 1). Although in orchard A the percentage of pruning wounds with cankers in Nonpareil ranged from 0 to 40% for groups of 10 trees (Fig. 1A), the runs analysis on the distribution of cankers did not indicate nonrandomness (Table 2). However, for orchard B the runs analysis indicated that cankers

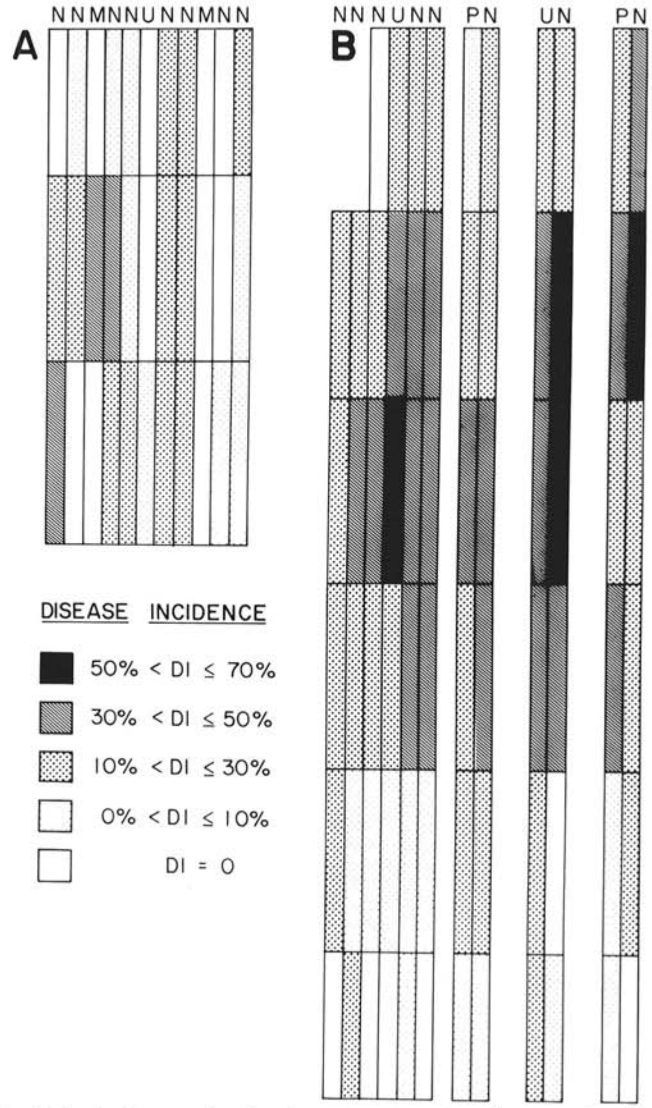

Fig. 1. Orchard maps showing the percentage of pruning wounds with cankers for groups of 10 trees (chosen for presentation purposes) in the same row in orchards A and B. Rows run from the top of the page (north) to the bottom (south). Only data for pruning wounds of diameter between 2 and 6 cm, inclusive, are presented. The cultivar for the row is presented at the top of the row by a capital letter. N represents Nonpareil, M (Mission), U (NePlus Ultra), and P (Price).

were distributed nonrandomly or clustered for all three diameters (2, 3, and 4 cm) for the whole orchard. In orchard B there was less disease in the southernmost third of the orchard (Fig. 1B), and when the orchard was divided into thirds, the runs tests no longer indicated a strongly nonrandom distribution (Table 2).

As the diameter of pruning wounds increased, the percentage with cankers increased also. The number of infections was estimated using Gregory's multiple infection transformation (7). The estimated number of infections increased linearly as the diameter increased in both orchard A and B (Fig. 2). Only the data from the northern third of orchard B are presented in Figure 2, because of the clustering of cankers in this portion of the orchard.

In orchard A, the percentage of wounds with cankers increased as height increased (Fig. 3). However, in orchard B there was no consistent trend. For example, the percentage of cankers for wounds of 4-cm diameter increased substantially with height, but decreased substantially for wounds of 5-cm diameter (Fig. 3). In orchard B, cankers were observed at heights greater than 5 m.

The year after the surveys in orchards A and B no new pruning wound cankers were observed, even though pruning was performed and there were no signs, such as abundant gumming, of the old cankers being active. In orchard A, the mean distance from pruning wound to the canker margin for nine cankers was measured as 109 mm in the fall 1984 and was still 109 mm in late spring 1985, indicating that the cankers had ceased expansion.

P. syringae was commonly isolated from almond leaves on the orchard floor. *P. syringae* was isolated from 26% of the leaves gathered from orchard A in the fall of 1983 and 1984, and from 18% of the leaves from orchard B in the fall of 1984. The leaves when gathered were in various conditions of decay. *P. syringae* was isolated from 17% of the leaves, which were completely green, as though they had just fallen off the tree, from 10% of the leaves showing some yellowing, and from 50% of the leaves showing some browning. *Pythium* spp. were frequently isolated from leaves with some yellowing or browning. *P. syringae* was never isolated from leaves still attached to the tree. Abundant oospores resembling those of *P. syringae* (1) were observed in 78% of the leaves from which *P. syringae* was isolated but in only 4% of the other leaves.

The disease incidence in orchard C corresponded to the amount of rainfall during the period of pruning (Fig. 4). The part of the orchard with the highest disease incidence (0.62 cankers per tree) was pruned during November, the period of the highest mean daily rainfall (9.6 mm/day). The part of the orchard that was pruned during a period of little rain (0.9 mm/day) had very few pruning wound cankers (0.04 cankers per tree).

TABLE 2. Results of runs tests for random distribution of cankers caused by *Phytophthora syringae* for pruning wounds of the same diameter in whole and parts of orchards A and B[a]

Orchard	Part of orchard	Wound diameter (cm)	Canker (%)	Runs test probability[b]
A	Whole	2	10.0	0.708
		3	11.9	0.319
		4	15.3	0.806
B	Whole	2	19.4	0.000***
		3	19.3	0.049*
		4	27.9	0.000***
	Northern third	2	16.2	0.058
		3	27.2	0.374
		4	30.6	0.015*
	Central third	2	33.3	0.033*
		3	22.1	0.177
		4	38.8	0.136
	Southern third	2	3.7	0.924
		3	4.7	0.872
		4	5.7	0.478

[a] Runs tests were performed on pruning wounds of a specific diameter in Nonpareil trees down one row and then up the next row.

[b] Significance level for one-tailed runs test for random distribution. * indicates significantly ($P<0.05$) nonrandom or clustered. *** indicates highly significantly ($P<0.001$) clustered.

Cankers resulting from wound inoculations with *P. syringae* expanded well throughout the fall, winter, and spring, with the mean canker expansion rate ranging from 1.0 to 1.9 mm/day (Table 3). No cankers were observed in the uninoculated controls. The slowest canker expansion rate was during late spring, and no canker expansion was observed between June 15 and October 24.

DISCUSSION

In the orchards surveyed, the incidence of pruning wound cankers caused by *P. syringae* was high (Table 1), especially in some sections of the orchards (Fig. 1). Although these almond orchards were chosen for their high disease incidence, there were many other orchards observed during the course of this study with similar disease incidences. In media, *P. syringae* grew well at low temperatures, but the mycelium was killed above 25 C (1). In the orchard, canker expansion was similarly affected by temperature. Cankers produced from artificial inoculation expanded throughout the fall, winter, and spring but ceased expansion during summer (Table 3). It is difficult to isolate *P. syringae* from pruning wound cankers in early summer (1), and in orchards A and B there were no signs of canker expansion during or after summer. So, even though disease incidence can be high and the cankers expand relatively fast, the direct damage to the tree as a result of these cankers was limited because the cankers were annual. However, the damage to developing scaffolds of young almond trees can be serious.

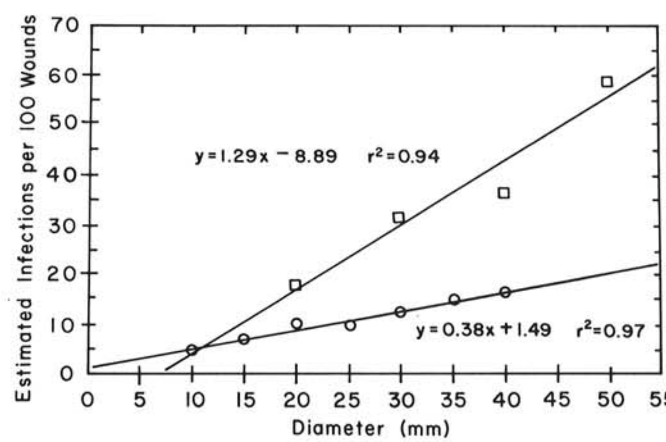

Fig 2. Number of infections by *Phytophthora syringae* per 100 wounds as estimated by the multiple infection transformation for pruning wounds of various diamaters in Nonpareil trees in orchard A and the northern third of orchard B. o = orchard A, □ = orchard B.

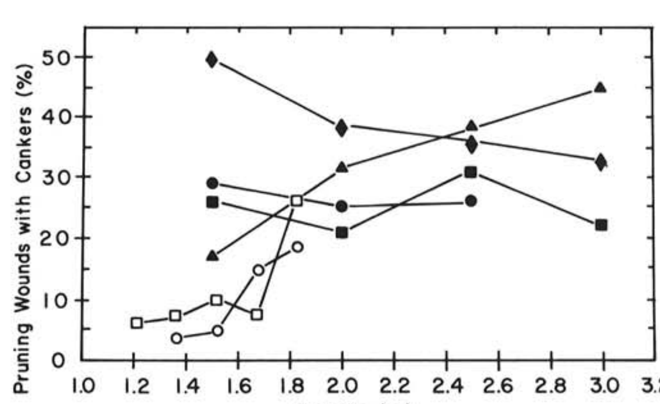

Fig. 3. The percentage of pruning wounds with cankers at various heights from the ground for different pruning wound diameters for Nonpareil trees in orchards A and B. Only those data points representing more than 15 pruning wounds are presented. The data points represent on the average 26 pruning wounds for orchard A and 46 for orchard B. Orchard A: o = 2.5 cm diameter, □ = 3 cm; orchard B: ● = 2 cm, ■ = 3 cm, ▲ = 4 cm, and ◆ 5 cm.

The results of the surveys indicate the difficulty in comparing the susceptibility to *P. syringae* of various almond cultivars by observing disease incidence in the orchard. In orchard B, if we considered the percentage of pruning wounds with cankers, Price had the lowest percentage (Table 1). However, when we examined the number of cankers per tree, Price had substantially more cankers than Nonpareil or NePlus Ultra, because Price had more pruning cuts. In orchard A, there was a substantially lower percentage of pruning wounds with cankers for NePlus Ultra than for Nonpareil, whereas in orchard B, NePlus Ultra had a slightly higher percentage than Nonpareil. Because disease severity is influenced by wound diameter, rainfall, inoculum levels, and amount of pruning, observations of disease levels in orchards could be misleading in discerning relative susceptibility of almond cultivars. In inoculation studies there was little difference in canker development in fresh wounds among the major almond cultivars except in wounds in Nonpareil, which remained susceptible to *P. syringae* longer (3).

P. syringae was frequently isolated from recently abscised almond leaves on the orchard floor and abundant oospores were observed in almond leaves from which *P. syringae* was isolated. Similarly, fallen apple leaves from orchards in England were found to contain oospores of *P. syringae* (8), which were observed to germinate and remained viable for 32 mo in the orchard (9). *P. syringae* rapidly infected freshly abscised apple leaves and substantial populations of *P. syringae* in the apple orchards could be maintained by the development of *P. syringae* in fallen apple leaves (10). In almond orchards, oospores could function as the means of survival for the fungus through the hot summers. The abundance of fallen almond leaves on the orchard floor in autumn during the season of heavy rainfall in California and the high percentage of these leaves containing *P. syringae* (18–26%) indicate that colonization of the leaves by *P. syringae* could be important for inoculum maintenance and increase in almond orchards in California.

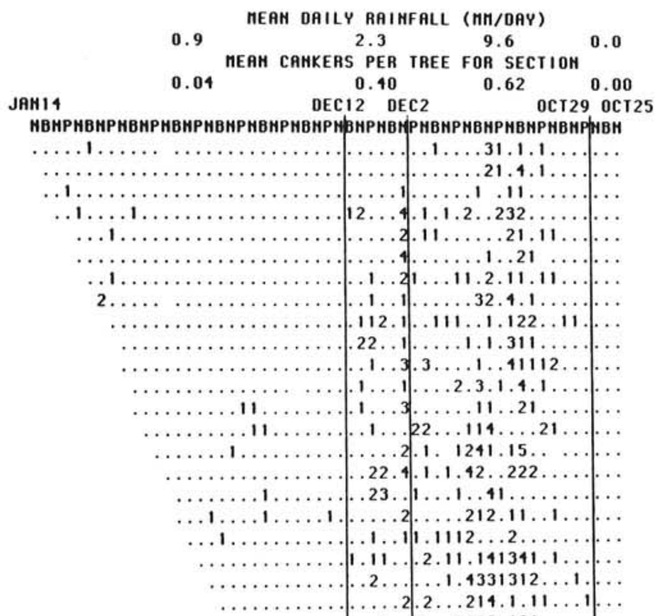

Fig. 4. The number of pruning wound cankers per tree in orchard C. The cultivar for each row is represented by N, B, or P, which stands for Nonpareil, Butte, and Price, respectively. The dots represent trees with no cankers. The numerals represent the number of pruning wound cankers per tree. The mean daily rainfall, mean cankers per tree, and inclusive dates for pruning for each of the four sections of the orchard are presented. Pruning was performed on rows that run from top to bottom starting with the rows at the right and moving left.

The estimated number of infections of pruning wounds by *P. syringae* increased linearly as the diameter of the pruning wound increased in both orchard A and B (Fig. 2). However, the surface area of the pruning wound does not increase linearly but according to the square of the diameter. For pruning wounds in apricot trees attacked by *Eutypa armeniacae* Hansf. & Carte, which invades the xylem of the tree through pruning wounds, it was found that when the wound diameter was doubled, the estimated infection was not doubled but increased 4.8 times or approximately the square of the increase in diameter (15). However, *P. syringae* seemed to be restricted to the inner bark and did not penetrate deeply into the xylem (*unpublished*). If infection only occurred in the exposed inner bark of the pruning wound, then the surface area of the pruning wound would not be important but only the circumference, which increases linearly as the diameter increases. In general, the relationship between disease incidence and diameter of wound depends on which of the exposed wounded tissue can be invaded by the pathogen.

Frequently, pruning wound cankers were high in the tree. In orchard A there was actually a higher percentage of cankers higher in the tree, whereas in orchard B there was no clear trend between disease incidence and the height of pruning wounds (Fig. 3). With apple fruit rot caused by *P. syringae*, infection occurred mainly on fruit within 50 cm of the soil, although infected fruit have been observed at heights over 1 m (16). In citrus orchards in California, brown rot caused by several *Phytophthora* species was usually found on fruit within 1 m of the ground, although occasionally *P. hibernalis* was isolated from diseased fruit up to 3 m in the tree (5,11). In these cases, where the inoculum was from the soil, there was a definite decrease in disease as height increased. Infections in cocoa pods by *P. palmivora* were mainly near the soil early in the year when inoculum came from the soil, but late, high levels of infection occurred high in the trees when inoculum came from previously infected pods (11). Although an aerial fruit rot of peaches caused by *P. syringae* was found to be dispersed by sprinklers (2), irrigation practices seem not to be involved in the development of pruning wound cankers in almond trees. Drip irrigation was used in orchard A, and although sprinkler irrigation was used in orchards B and C, no irrigation was done during the fall and winter when infection occurred. Pruning wound cankers caused by *P. syringae* may be common high in the tree because inoculum was coming from previously infected pruning wounds, because of a means of dispersal at present unknown, or because wounds high in the tree are more readily infected.

TABLE 3. Canker expansion rate in Nonpareil almond trees resulting from inoculations with *Phytophthora syringae* for different inoculation dates in fall 1983 through spring 1984[a]

Date inoculated	Mean canker expansion rate (mm/day) Feb. 2	March 29	June 15	Canker length (mm) March 29
Nov. 8	1.3	1.1[b]	...[c]	161
Dec. 6	1.5	1.2	...	135
Jan. 5	1.5	1.4	...	115
Feb. 2	...	1.5	...	82
March 1	...	1.9	1.2	54
April 12	...	...	1.0[d]	...

[a] The length was determined by measuring the furthest range of discoloration in the inner bark from the inoculated wound. The canker expansion rate was calculated by dividing the canker length by the total number of days from inoculation. The average monthly temperatures from November 1983 to June 1984 were 11, 9, 8, 9, 14, 15, 21, and 23 C (mean of four nearby weather stations, NOAA).

[b] The $LSD_{0.05}$ was 0.3 mm/day for the cankers measured March 29.

[c] ... indicates not determined.

[d] Mean canker size for inoculations made on April 12 was 65 mm when measured on June 15 and 65 mm when measured on October 24, confirming that cankers had ceased expansion in June.

The distribution of cankers in orchard C seemed strongly affected by the amount of rainfall, since only the parts of the orchard pruned during heavy rainfall had many cankers (Fig. 4). In orchards A and B there was almost 200 mm of rainfall during the month the trees surveyed were pruned, which was 2.4 times the normal rainfall (the mean of three nearby weather stations, NOAA). Cankers have been observed in pruning wounds made throughout the fall and winter in various almond orchards (*unpublished*), indicating that the time of year was not as important as the amount of rainfall for disease development. For citrus brown rot, the propagules of *Phytophthora* species are believed to be dispersed to the fruit on the tree by wind-blown rain (5,6). In orchard A, cankers in Nonpareil were distributed randomly (Table 2), although there was a substantial difference between cultivars in percentage of pruning wounds with cankers. In orchard B the cankers in Nonpareil were not distributed randomly (Table 2), but most of the nonrandomness was derived from the southern third of the orchard having very few cankers. No cultural practice or other factor seems to explain this distribution, although it could be caused by the inoculum distribution.

At this time, the mechanism for dispersal of *P. syringae* to pruning wounds is unknown. Field studies on pruning wound cankers are difficult because, although the disease is severe in some orchards, its occurrence is sporadic. The fungus was abundant in the leaf litter of orchards A and B, which would seem to be an inoculum source. However, most aerial diseases with an inoculum source in the soil have the highest disease incidence closest to the soil, whereas this is not so with pruning wound cankers caused by *P. syringae* (Fig. 3). Another possibility is the transmission of the inoculum by pruning equipment. The causal agent of Phytophthora canker of cacao was easily transmitted by pruning equipment, but the disease still occurred when pruning equipment was sterilized (17). However, in our study, although pruning was performed down the rows, the runs analysis showed no clear evidence of clustering down the row (Table 2), which suggests that transmission was not by pruning equipment. In preliminary experiments, pruning shears dipped in a zoospore suspension transmitted *P. syringae*, but the extent that transmission by pruning equipment may be involved in disease development in almond orchards is unknown.

A grower may want to only protect the larger pruning wounds generated before or during favorable infection periods because large wounds were more likely to develop cankers caused by *P. syringae*. Favorable infection periods occurred during the late fall and winter during periods of heavy rainfall. Preliminary work on protecting wounds from infection by *P. syringae* using fungicides has been done (3). Because pruning wound cankers caused by *P. syringae* can be very damaging in young almond trees, causing the loss of developing scaffolds, the grower may wish to avoid the disease by pruning young trees during those months when temperatures are warm enough to inhibit the fungus.

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