Association of *Phytophthora syringae* with Pruning Wound Cankers of Almond Trees

R. M. BOSTOCK, Assistant Professor, and M. A. DOSTER, Graduate Research Assistant, Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

Bostock, R. M., and Doster, M. A. 1985. Association of *Phytophthora syringae* with pruning wound cankers of almond trees. Plant Disease 69:568-571.

Cankers on almond trees with profuse gumming associated with pruning wounds were frequently observed in California orchards during the winters and springs of 1982–1984. These cankers frequently girdled and killed limbs less than 5 cm in diameter. *Phytophthora syringae* was isolated with high frequency from these cankers in April but not in June, and its pathogenicity to pruning wounds was proved by artificial inoculations in February. Nearly all cankers caused by *P. syringae* were associated with pruning wounds or injuries created during pruning in late autumn and winter. No other known pathogens of almond were isolated from pruning wound cankers. Excised branch pieces inoculated with *P. syringae* developed cankers at temperatures between 2 and 20 C.

During 1982–1984, we frequently observed cankers with extensive gumming in almond trees (*Prunus dulcis* (Mill.) Webb) in the central valley of California that were unlike those caused by known pathogens. These cankers involved branches throughout the tree and were almost always associated with pruning wounds. The cankers were apparent during winter and spring, and gum exuded well into summer. The cankers had distinct margins with concentric patterns of light and dark tissue and brown discoloration extending into the sapwood beneath the entire canker.

Almonds in California are cultivated on about 172,000 ha, and trees are generally pruned yearly during September to January. Frequently, cankers associated with bark wounds and exuding amber gum have been attributed to infection by *Ceratocystis fimbriata* Ell. & Halst., which causes perennial trunk and branch cankers. *C. fimbriata* is vectored by Nitidulid beetles and other insects that are attracted to bark injuries created by mechanical shakers or other implements during harvesting operations (3,4,12). Our attempts to isolate *C. fimbriata* from cankers surrounding pruning wounds were unsuccessful.

Phytophthora spp. can infect trunks and other aerial parts of almond trees in California (11; S. M. Mircetich, *personal communication*). In the spring of 1983, we isolated *Phytophthora syringae* (Kleb.) Kleb. from cankers surrounding pruning wounds in an orchard in San Joaquin County. We therefore investigated the possibility that the cankers were incited by one or more species of *Phytophthora*. This article details evidence that *P. syringae* causes cankers that develop from infection of pruning cuts and are distinct from those caused by *C. fimbriata*. A preliminary report has been published (1).

Accepted for publication 8 January 1985 (submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Isolates of *P. syringae* (F-78, F-79, and F-97), *P. cactorum* (Lebert & Cohn) Schroeter (F-92), *P. citricola* (Sawada) (F-93), and *P. megasperma* (Drechs.) Waterhouse (F-94) from almond were maintained on lima bean agar (LBA) or cornmeal agar (CMA) at 20 C. An isolate of *C. fimbriata* (F-10) was obtained from a canker in a commercial almond orchard in Solano County and maintained on potato-dextrose agar at room temperature.

Disease incidence in almond orchards. In April 1984, two orchards in Colusa County that had been pruned from late November 1983 through early February 1984 were surveyed for incidence of pruning wound cankers. The almond cultivars were Nonpareil, Ne Plus Ultra, Mission, and Price. The percentage of pruning wounds displaying typical symptoms of Phytophthora canker was determined. Samples were taken from cankers in these and three other orchards in three counties periodically during the spring to determine the presence or absence of *Phytophthora* spp., *C. fimbriata*, or other known fungal pathogens.

Isolation from diseased tissue. Chips consisting of bark and outer sapwood were cut with a sterilized chisel from the upper and lower margins of cankers. These samples were transported in plastic bags on ice to the laboratory, where they were dissected. Tissue pieces (25–50 per canker per medium) were plated on CMA amended with pimaricin (5–10 mg/L), vancomycin (300 mg/L), and pentachloronitrobenzene (25 mg/L) (PVP medium), which is selective for a number of *Phytophthora* spp. (15), or on acidified potato-dextrose agar. Plates were incubated at 18–20 C and examined daily for 7–14 days.

The presence of *P. syringae* was indicated by its typical petaloid colony growth from bark pieces on PVP medium. Mycelium was transferred to LBA or clarified V-8 juice agar amended with β-sitosterol to obtain other characters useful for identification, such as hyphal swellings, sporangia, and oospores. The effect of various temperatures on growth rate as estimated by colony diameter was also determined for two almond isolates (F-79 and F-97) on CMA.

Inoculation of pruning wounds and

branch segments from almond trees. Pathogenicity of isolates was tested by inoculating trees in the field or excised branches maintained in plastic containers at various temperatures. In February 1983, pruning wounds were made on trees of cultivars Nonpareil and Ne Plus Ultra and were immediately inoculated by transferring a mycelial plug (4 mm in diameter) from a culture of *P. syringae* (F-78) growing on LBA. Mycelial plugs from a culture of *C. fimbriata* (F-10) were also placed on fresh pruning cuts. Inoculated wounds were covered with paraffin film and then flagging tape and observed periodically for 3 mo. Controls were treated similarly but were not inoculated with mycelium.

Branch segments about 15 cm long and 1–2 cm in diameter were inoculated with an almond isolate of *P. syringae* (F-79), *P. cactorum*, *P. citricola*, or *P. megasperma*. The segments were surface-sterilized in 2% sodium hypochlorite for 5 min, rinsed thoroughly in sterile distilled water, and wounded in the center with a no. 1 cork borer. The wound was inoculated with a plug from a growing culture. The cut ends of the segment and the inoculated wound were wrapped with paraffin film and the inoculated branches were then maintained at constant temperatures (2–20 C) in plastic containers with moist paper towels. The length of necrotic tissue from the point of inoculation was measured after 2 wk to determine canker expansion rates.

RESULTS

Disease incidence in almond orchards. Trees in the two orchards surveyed in April 1984 had cankers around pruning wounds (made 3–5 mo earlier) throughout the trees, as high as 6 m above ground in some instances. The percentage of 1983–1984 pruning cuts with cankers was 23.4% in one orchard and 10.5% in the other. More than 99% of about 600 cankers observed were centered around pruning cuts or injuries created during pruning.

Isolation from diseased tissue. *P. syringae* was consistently isolated from pruning wound cankers during April and early May but could not be detected in June (Table 1). Cankers examined during June appeared to have ceased expansion because they had sharply delimited rather than diffuse margins and new callus had formed around the necrotic tissue. The number of tissue pieces from which *P. syringae* grew out on PVP medium declined at the onset of warm temperatures in late spring. *C. fimbriata* was not isolated from any of the pruning wound cankers examined.

No species of *Phytophthora*, other than *P. syringae*, or other fungi known to be pathogenic in almond bark were isolated from cankers, although a number of saprophytic fungi were commonly detected. These included species of *Alternaria*, *Aureobasidium*, *Penicillium*, *Fusarium*, and several unidentified fungi.

Identification of *P. syringae*. Sporangial and oospore characters for an isolate obtained from an aerial canker in an orchard in Colusa County were all consistent with those described for *P. syringae* by Waterhouse (19) (Fig. 1). Oospores formed after several weeks at 9 C, had an average diameter of 30 μm (compared with 31 μm reported by Waterhouse), and were dark yellow to light brown. All antheridia, where apparent, were paragynous. Sporangia had short stalks and were ovoid to obpyriform and semipapillate with average dimensions of 61 × 31 μm (compared with 57 × 36 μm reported by Waterhouse). All isolates had a typical petaloid growth pattern on all media used, and cultures frequently had numerous hyphal swellings (Fig. 1). Linear growth of two isolates was measured over the temperature range 2–27 C (Fig. 2). Growth occurred at

Table 1. Isolation of *Phytophthora syringae* from pruning wound cankers in almond trees in five orchards in California

Sampling date	Location[a]	No. of cankers positive/total sampled	
		P. syringae[b]	*Ceratocystis fimbriata*[c]
2 April	1	7/9	ND[d]
26 April	2	10/13	ND
11 May	3	5/5	0/5
18 May	4	1/6	ND
23 May	2	3/13	0/13
5 June	1	0/18	0/18
	5	0/5	0/5

[a] Five orchards with trees showing typical symptoms were sampled during the spring of 1984. Locations of orchards: 1 = Colusa County, commercial orchard; 2 = Butte County, California State University at Chico, University Farm; 3 = Solano County, University of California, Armstrong Farm; 4 = Yolo County, commercial orchard; and 5 = Colusa County, University of California, Nickel's Estate Orchard.
[b] Twenty-five to 50 tissue pieces from each canker were plated on PVP medium as described in text.
[c] Tissue pieces were plated on acidified potato-dextrose agar medium as described in text.
[d] ND = not determined.

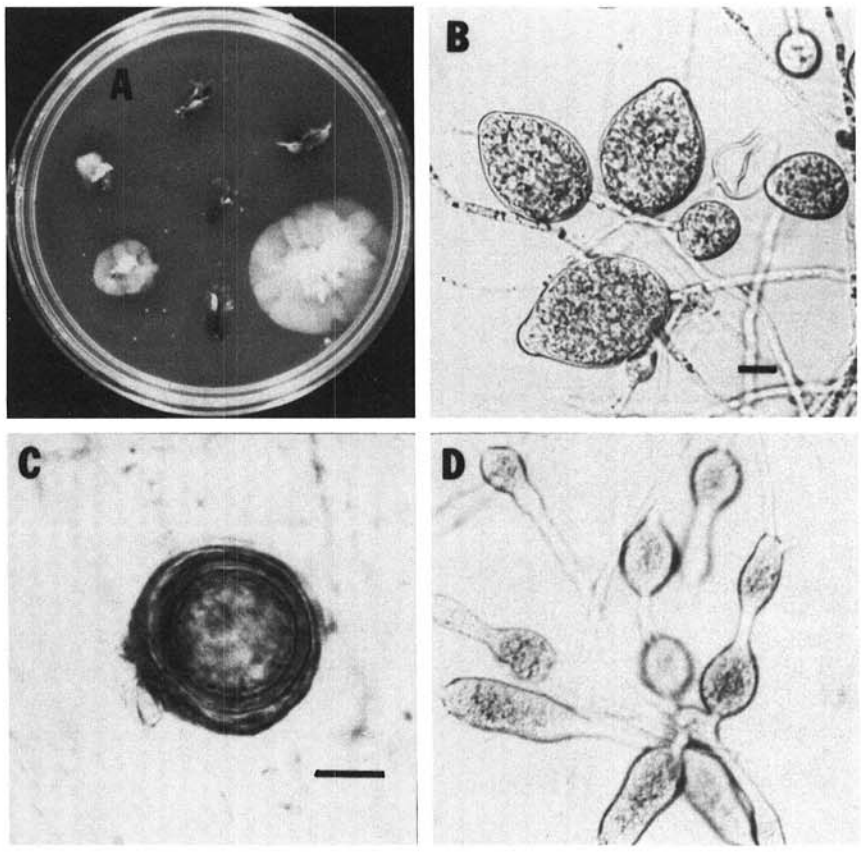

Fig. 1. (A) Growth of *Phytophthora syringae* from almond bark pieces on PVP medium (16). **(B)** Sporangia of *P. syringae* growing on amended lima bean agar. Bar = 10 μm. **(C)** Oospore produced in culture after 8 wk at 9 C. Bar = 10 μm. **(D)** Hyphal swellings produced by an almond isolate of *P. syringae* on cornmeal agar.

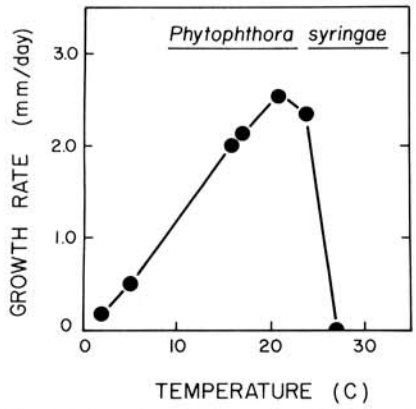

Fig. 2. Effect of temperature on radial growth of two almond isolates of *Phytophthora syringae* on cornmeal agar. Each point represents the averaged data from 10 plates.

Fig. 3. (Top) Gummosis at margins of a canker resulting from natural infection of a pruning wound in an almond tree by *Phytophthora syringae*. **(Bottom)** Canker 3 mo after inoculation of a pruning wound with an almond isolate of *P. syringae*.

temperatures as low as 2 C and was most rapid near 21 C. No growth was apparent at 27 C.

Inoculation of trees and branch segments. Inoculation of pruning wounds with *P. syringae* in February resulted in gum-producing cankers that extended 15 cm or more from the site of inoculation when evaluated in May (Fig. 3). These cankers appeared identical to those arising from natural infection in commercial orchards (Fig. 3). *P. syringae* was reisolated from these cankers. Cankers did not develop at pruning cuts inoculated with a pathogenic isolate of *C. fimbriata*. Necrotic tissue extended only a few millimeters beyond the wound surfaces.

Branch segments inoculated with *P. syringae* and then incubated at 12 C developed cankers that extended the length of the segment (15 cm) within 3 wk. *P. syringae* was virulent in branch segments over a range of temperatures from 2 to 20 C and at low temperatures caused larger cankers than *P. cactorum*, *P. citricola*, and *P. megasperma* (Table 2). Isolates of the latter three species did not cause disease in almond branches at 2 C. These species commonly attack the roots and crowns of almond and other stone fruit trees (9–11).

DISCUSSION

Our data clearly show a causal role of *P. syringae* in pruning wound cankers in almond. This disease is distinguished from that incited by *C. fimbriata* by its association with pruning wounds throughout the tree and its occurrence during cool weather. *C. fimbriata* primarily infects trunk and scaffold wounds created during harvest operations in late summer and is most active at warm temperatures (4). Cankers caused by both organisms, however, exude copious amounts of amber gum. All characteristics of our pathogen in culture were consistent with those of *P. syringae* (19). We satisfied Koch's postulates and demonstrated that our isolates of *P. syringae*, unlike those of *C. fimbriata*, were highly virulent on pruning wounds. Pruning cuts appear to be a primary avenue for infection since more than 99% of the cankers we observed were associated with such wounds and we were unsuccessful in infecting uninjured bark. We observed natural infections in more than 20 almond cultivars. No species of *Phytophthora*, other than *P. syringae*, or other known pathogens of almond were detected in the cankers, although excised branch segments were readily infected by the other species of *Phytophthora* tested.

C. fimbriata made only limited ingress into pruning wounds in the field, even though our isolate readily invades other types of bark injuries. It seems unlikely then that *C. fimbriata* is responsible for the aerial cankers reported in this study since most trees are pruned at a time when vectors are not active and since we failed repeatedly to isolate this organism at a time when *C. fimbriata*, if present, would have been readily detected. Furthermore, wounds with crushed bark tissue are a more favorable infection court for *C. fimbriata* than wounds such as pruning cuts with little crushed tissue (3,4,12).

P. syringae seems well adapted for growth and development in almond tissue under the common winter conditions of the central valley of California—mild temperatures and high rainfall (13,18). The fungus infected and killed almond tissues at temperatures as low as 2 C. Our inablilty to isolate the pathogen during late spring and summer was not surprising since *P. syringae* will not grow at 27 C (8,19) (Fig. 2). This temperature is frequently exceeded during May and June in the central valley of California.

Dried gum was observed around inactive cankers in August and September, which may account in part for the confusion between this disease and warm-weather canker diseases such as those caused by *C. fimbriata* and *Botryosphaeria dothidea* (6). Other *Phytophthora* spp. that infect the roots and crowns of almond trees can readily invade the trunk and scaffolds to produce the extensive gummosis also observed with Ceratocystis canker (9–11).

P. syringae occurs in orchard soils throughout the central valley in California and can cause root and crown rots in all the stone fruit trees (9–11). So far, we have detected *P. syringae* in pruning wound cankers in at least seven locations in the central and northern counties of the central valley, and the fungus has been isolated from branch cankers as far south as Kern County (B. Teviotdale, *personal communication*). We also isolated the

Table 2. Canker development at four temperatures in almond branch segments (cultivar Drake) infected with various pathogenic *Phytophthora* species

Species	Canker expansion rate (mm/day) (temperature [C])			
	2	7	12	20
P. syringae	1.1 a[y]	1.3 a	2.9 a	3.1[z]
P. cactorum	0.0 b	0.0 c	3.6 a	4.8
P. citricola	0.0 b	0.5 b	3.3 a	6.8
P. megasperma	0.0 b	0.4 b	0.3 b	0.8

[y] Numbers within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] No significant differences ($P = 0.05$).

pathogen from pruning wound cankers in apricot and French prune.

Although research on *P. syringae* has focused primarily on its role as a crown and root pathogen of fruit trees, its ability to attack aerial parts of trees is well recognized (2,5,7,17,20). Nevertheless, the only other published report of it causing pruning wound cankers is that of Smith (15), who found it to cause numerous stem and branch cankers in young peach and apricot trees in New Zealand. Although the cankers reported in our study became inactive with the onset of warm summer temperatures and did not girdle large limbs, we frequently observed dieback of smaller branches (<5 cm), resulting in loss of some fruiting wood. In a number of trees in one young orchard (third year after planting), numerous cankers killed or weakened main scaffolds. The disease may also compromise the compartmentation of wounds to render branches more susceptible to attack by wood-rotting organisms (14).

A current recommendation for controlling Ceratocystis canker is to excise diseased bark during the winter, when insect vectors are not present. However, if a grower has an aerial Phytophthora canker problem but confuses this with Ceratocystis canker, such a practice would only serve to expose more susceptible tissue to infection by *P. syringae*. Our results clarify the differences between these two canker diseases and provide a basis for developing control measures.

ACKNOWLEDGMENTS

We thank S. M. Mircetich for his advice and for providing cultures of *Phytophthora* spp., B. Teviotdale for helpful discussions, and J. Sommer for assistance in portions of this study. Research supported by a grant from the California Almond Board.

LITERATURE CITED

1. Bostock, R. M., and Doster, M. A. 1984. Association of *Phytophthora syringae* with pruning wound cankers in almond. (Abstr.) Phytopathology 74:840.
2. D'Ercole, N., and Flori, P. 1978. A case of peach rot due to *Phytophthora syringae* (Kleb.) Kleb. Inf. Fitopatol. 28:19-21.
3. DeVay, J. E., Lukezic, F. L., English, W. H., Moller, W. J., and Parkinson, B. W. 1965. Ceratocystis canker of stone fruit trees. Calif. Agric. 19:2-4.
4. DeVay, J. E., Lukezic, F., English, H., Trujillo, M., and Moller, W. J. 1968. Ceratocystis canker of deciduous fruit trees. Phytopathology 58:949-954.
5. Edney, K. L. 1978. The infection of apples by *Phytophthora syringae*. Ann. Appl. Biol. 83:1-36.
6. English, H., Davis, J. R., and DeVay, J. E. 1975. Relationship of *Botryosphaeria dothidea* and *Hendersonula toruloidea* to a canker disease of almond. Phytopathology 65:114-122.
7. Feld, S., Mange, J., and Pehrson, J. 1979. Brown rot of citrus: A review of the disease. Citrograph 64:101-106.
8. Kouyeas, H., and Chitzanidis, A. 1968. Notes on Greek species of *Phytophthora*. Ann. Inst. Phytopathol. Benaki 8:175-192.
9. Mircetich, S. M. 1981. Phytophthora root and crown rot of deciduous fruit trees in California. Proc. B.C. Fruit Growers Assoc. Hortic. Forum 13:42-48.
10. Mircetich, S. M. 1982. Phytophthora root and crown rot of apricot trees. Acta Hortic. 121:385-396.
11. Mircetich, S. M., Moller, W. J., and Chaney, D. H. 1974. Phytophthora crown rot and trunk canker of almond trees. (Abstr.) Proc. Am. Phytopathol. Soc. 1:59.
12. Moller, W. J., and DeVay, J. E. 1968. Insect transmission of *Ceratocystis fimbriata* in deciduous fruit orchards. Phytopathology 58:1499-1508.
13. Sewell, G. W. F., Wilson, J. F., and Dakwa, J. T. 1974. Seasonal variations in the activity in soil of *Phytophthora cactorum, P. syringae* and *P. citricola* in relation to collar rot disease of apple. Ann. Appl. Biol. 76:179-1.
14. Shortle, W. C. 1979. Mechanisms of compartmentalization of decay in living trees. Phytopathology 69:1147-1151.
15. Smith, H. C. 1956. Collar-rot of apricot, peaches, and cherries. Orchard N.Z. 29:22-23.
16. Tsao, P. H., and Ocana, G. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature (Lond.) 223:636-638.
17. Upstone, M. E. 1978. *Phytophthora syringae* fruit rot of apples. Plant Pathol. 27:24-30.
18. Upstone, M. E., and Gunn, E. 1978. Rainfall and the occurrence of *Phytophthora syringae* fruit rot of apples in Kent 1973-75. Plant Pathol. 27:30-35.
19. Waterhouse, G. M. 1963. Key to the species of *Phytophthora* de Bary. Mycol. Pap. 92. Commonw. Mycol. Inst., Kew, Surrey, England. 22 pp.
20. Young, R. A., and Milbrath, J. A. 1959. A stem canker disease of fruit tree nursery stock caused by *Phytophthora syringae*. (Abstr.) Phytopathology 49:114-115.