84-44

Canker Complex in Almond

Annual Report - 1984

PROJECT LEADER: Dr. Richard M. Bostock
Department of Plant Pathology
University of California
Davis, CA 95616

OTHER PERSONNEL: Mark A. Doster, Gail E. Middleton

OBJECTIVES:

1) To conduct studies on infection of pruning wounds and other aerial parts of almond trees by Phytophthora syringae with emphasis on the environmental factors necessary for disease development.
2) To obtain information on disease distribution within trees and in orchards and to assess the importance of P. syringae as a principal causal agent of pruning wound cankers.
3) To evaluate different cultivars for their susceptibility to P. syringae.
4) To continue laboratory studies of canker development using excised branch segments inoculated with P. syringae and Ceratocystis fimbriata.
5) To continue studies on the development of wound resistance to C. fimbriata with emphasis on formation of the lignified and suberized periderm layer.

Interpretive Summary

Cankers with profuse gumming associated with pruning wounds in almond trees were frequently observed in California orchards during the winter and spring of 1984. These cankers frequently girdled and killed small limbs less than 5 cm in diameter and resulted in the loss of some fruiting wood. Phytophthora syringae was isolated with high frequency from these cankers in April but not in June. In an initial survey of a commercial orchard, more than 99% of the cankers were associated with pruning wounds or injuries created during pruning in late fall and winter months. However, no other known pathogens of almond, including Ceratocystis fimbriata which is associated with another canker disease in California almond orchards, were isolated from pruning wound cankers. Although at this time the involvement of other organisms in addition to P. syringae cannot be excluded, the absence of other known pathogens in diseased tissue, and the seasonal occurrence of this disease strongly suggest that P. syringae is a principal causal agent of pruning wound cankers in almond trees.

In an almond variety trial in Butte County naturally infected pruning wound cankers were found in all twenty varieties. In two almond orchards, the percentage of pruning wounds with cankers were 11 and 23% indicating that disease incidence can be very high. Cankers were observed at heights from 0.5 m to greater than 5 m and disease incidence increased as the diameter of the pruning wound increased.

Because other Phytophthora species can cause disease in the aerial parts of almond trees, we propose that the disease resulting from infection of pruning wounds by P. syringae be referred to as "Phytophthora pruning wound canker" instead of the nondescript "Aerial Phytophthora canker."

Bark wounds in almond become resistant to infection by Phytophthora syringae and Ceratocystis fimbriata through temperature-dependent processes which we are studying microscopically and biochemically. We can now rapidly monitor the relative susceptibility of wounds to better implement control strategies based on wound treatments, time of pruning and cultivar resistance.

Experimental Procedures.

Experimental methods for inoculating almond bark with Phytophthora syringae and Ceratocystis fimbriata were essentially the same as those described in our previous annual report. One exception was that wounds inoculated with spore suspensions of C. fimbriata were wrapped with paraffin film for only 24 hr after inoculation. A detailed description of the experimental procedures used for studies on Phytophthora pruning wound canker follows.

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

Disease incidence in almond orchards. In April, 1984, two orchards in Colusa County which 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', '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 ~~other~~ 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 corn meal agar 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., or on acidified potato dextrose agar (APDA). Plates were incubated at 18-20 C and examined daily for 7 to 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, F-97) on corn meal agar.

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 in trees of cultivars 'Nonpareil' and 'Ne Plus Ultra' and were immediately inoculated by transferring a mycelial plug (4 mm dia) 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 similarly treated but not inoculated with mycelium.

Branch segments approximately 15 cm in length 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 then wounded in the center using 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 two weeks to determine canker expansion rates.

Canker development in trees of different cultivars at California State University, Chico and Delta Community College was determined by inoculating pruning cuts with P. syringae. Pruning wounds were inoculated in January, 1984 and canker length was measured in April, 1984.

RESULTS

Disease incidence in almond orchards. The trees in the two orchards surveyed in April, 1984 had cankers around pruning wounds (made three to five months earlier) throughout the trees. Cankers were observed at heights from 0.5 m to greater than 5 m. In the Nickels Estate Orchard, the percentage of pruning cuts associated with cankers increased as the height increased, whereas in the commercial orchard the percentage was approximately equal for all heights (Table 1). The percentage of 1983-84 pruning cuts with cankers was 23.4% in one orchard and 10.5% in the other. More than 99% of the approximately 600 cankers observed were centered around pruning cuts or injuries created during pruning.

At the Nickels Estate Orchard in a block of 11 rows of 28 trees per row and in a commercial orchard, Colusa County, CA, for 485 trees, all the pruning wounds were examined for the association with a canker. The diameter and height of each pruning wound was measured. P. syringae was isolated from pruning wound cankers in these orchards.

In general, a higher percentage of cankers was associated with larger pruning wounds. For the data from the Nickels Estate Orchard (Fig. 1 and Table 2) a regression line, cankers per pruning wound = 0.019 + 0.0034 * diameter of pruning wound (mm), was significant ($P < .0001$, $r^2 = 0.969$). A multiple-infection transformation, which converts mean cankers per pruning

wound to estimated infections per pruning wound, was used. This transformation gave a better fit ($r^2 = 0.971$) of the data to the regression line, infections per pruning wound = 0.015 + 0.0038 * diameter. In Table 2, the infections per unit area and infections per unit circumference were estimated using the assumption that pruning wounds are approximately circular. The number of infections per unit circumference was approximately the same for all diameters of pruning wounds whereas the number of infections per unit area decreased substantially as diameter increased. This could occur if the fungal inoculum can only form cankers by infecting the inner bark around the outside of the pruning wound, but not the area of the exposed wood.

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 3). 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 upon the onset of the warm temperatures of 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 Phytophthora 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 in the literature (Fig. 2). Oospores formed after several weeks at 9 C, had an average diameter of 30 µm and were dark yellow to light brown in color. All antheridia, where apparent, were paragynous. Sporangia had short stalks, were ovoid to obpyriform and semi-papillate with average dimensions of 61 X 31 µm. All isolates had a typical petaloid growth pattern on all media used, and cultures frequently had numerous hyphal swellings (Fig. 2). Linear growth of two isolates was measured over the temperature range 2-27 C (Fig. 3). Growth occurred at 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. 4). These cankers were identical in appearance to those arising from natural infection in commercial orchards (Fig. 4). 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 which 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 4). 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.

Period of Susceptibility of pruning wounds to P. syringae. In preliminary experiments, it was observed that during winter in the orchard some pruning wounds were still susceptible to infection by P. syringae at four weeks after wounding. All pruning wounds observed were completely resistant to infection at six or more weeks after wounding. Sectioning and staining of similar pruning wounds showed that many, but not all, had lignin formed around the wound by four weeks after wounding and all had lignin by seven weeks after wounding, although only a few stained positive for suberin. The formation of lignin and suberin are steps in the formation of a wound periderm which functions similar to the outer bark in excluding pathogens from the tree. In a preliminary experiment, at 7 C no signs of lignin or suberin were detected around wounds in excised almond twigs 10 weeks after wounding, whereas at 20 C lignin and suberin were detected within 4 weeks after wounding. The response to wounding or "healing" of the tree is very temperature dependent and is inhibited at low temperatures.

Susceptibility of almond cultivars to P. syringae. There were significant differences among inoculated cultivars in canker expansion rates (Table 5). However, the expansion rate observed did not always correlate well with disease incidence for the cultivar in naturally infected orchards (Table 6). Nonpareil, the most common cultivar in the state, is very susceptible to P. syringae.

Development of wound resistance to Ceratocystis fimbriata. Bark tissues become resistant in trees of var. Nonpareil to infection by C. fimbriata within 14 days after wounding in late August. Both the percentage of wounds that become infected and the size of cankers that develop are significantly reduced as the wounds age (Figs. 5 & 6).

From the data collected in 1984 the relative risk of infection, a statistically derived numerical estimate of the relative susceptibility of a bark wound, was determined. For example, a wound which has healed 2 days is 19 times more likely to become infected than a wound which has healed 10 days. A wound that has healed 14 days has virtually no chance of becoming infected. Fresh wounds (0 days healing) are all susceptible to infection.

The development of resistance is correlated in time with the appearance of an organized periderm with cells containing lignified and suberized walls.

Discussion

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 pruning wound cankers 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.

P. syringae seems well adapted for growth and development in almond tissue under the common winter conditions of the central valley of California of mild temperatures and high rainfall. The fungus infected and killed almond

tissues at temperatures as low as 2 C. That we were unable to isolate the pathogen during the late spring and summer is not unexpected since P. syringae will not grow at 27 C (Fig. 3). 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. Other Phytophthora spp. which 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.

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. So far, we have detected P. syringae in pruning wound cankers in at least seven different 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, Fig. 7). We also isolated the pathogen from pruning wound cankers in apricot and French prune.

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 (3rd 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.

We have shown that pruning wounds and bark wounds become resistant to infection by C. fimbriata and P. syringae over time and that this resistance is determined in part by temperature dependent processes. Preliminary experiments have suggested that cool temperatures could be used to study their effects on wound healing and resistance. Suberization, meristem activation, and the formation of lignin-like material occur concomitantly at temperatures typical of those at harvest (late August, early September). However, it appears that these phenomena can be separated by several days at temperatures near 50 F (10 C). This may allow us to determine the contribution that each step in healing makes towards the development of resistance to infection.

In the case of Ceratocystis canker, any protective treatment applied to a wound must be effective for at least 10 days and preferably 14 days after wounding. After this period, it is probably unnecessary to apply protective materials because the trees' own defense mechanisms have taken over.

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 a Phytophthora pruning wound canker problem but confuses this with Ceratocystis canker, then 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 development of control measures.

Conclusions

1. Phytophthora syringae appears to be the principal causal agent of pruning wound cankers in almond and infects wounds throughout the tree.

2. Phytophthora pruning wound canker is widespread in California almond orchards and, in recent years, has been more common than Ceratocystis canker.

3. Phytophthora pruning wound cankers become inactive in late spring but can continue gumming into the following fall.

4. Wounds become resistant to infection by these pathogens by temperature-dependent processes. We may now be able to precisely monitor the status of wounds to better implement potential control strategies for both diseases based on wound treatments, time of pruning and cultivar resistance.

Publications:

Bostock, R. M. and M. A. Doster. 1984. Association of Phytophthora syringae with pruning wound cankers in almond. (Abstr.) Phytopathology 74:840.

Bostock, R. M. and M. A. Doster. 1985. Association of Phytophthora syringae with pruning wound cankers in almond trees. Plant Disease 69: (accepted for publication).

Doster, M. A. and R. M. Bostock. 1984. Development of pruning wound cankers in almond trees caused by Phytophthora syringae. Stone Fruit Decline Workshop, Oct. 30-Nov. 1, 1984. Kearneysville, WV.

Table 1. Percentage of pruning wounds associated with a canker at various heights in two orchards in Colusa County, CA.

	Commercial Orchard		Nickels Estate Orchard	
Height of pruning wound (m)	Diameter of pruning wound (cm) 1.5-3.4	3.5-5.5	Diameter of pruning wound (cm) 1.3-2.7	2.8-4.3
1.0	----[a]	----	4.8	7.7
1.5	20.0	26.4	8.1	12.9
2.0	17.1	25.2	18.9	18.5
2.5	24.3	29.6	---	---
3.0	13.6	31.3	---	---
3.5	----	34.8	---	---
All	19.1	28.4	9.2	13.2

[a]---- means fewer than 20 pruning wounds examined.

Table 2. Disease incidence for pruning wounds of various diameters in Nonpareil almond trees a Nickels Estate Orchard.

Diameter of pruning wound (mm)[b]	Pruning wounds with cankers (%)	Estimated infections[a] per pruning wound	per unit area (m^{-2})	per unit circumference (m^{-}
10	4.8	0.050	631	1.58
15	6.8	0.071	402	1.51
20	10.0	0.105	335	1.68
25	9.7	0.102	207	1.30
30	11.8	0.126	178	1.34
35	13.9	0.150	156	1.36
40	15.3	0.166	132	1.32

[a]Multiple-infection transformation was used to convert cankers per pruning wound to infections per pruning wound.

[b]Only those diameter classes for which more than 50 pruning wounds were examined are listed.

Table 3. Isolation of *Phytophthora syringae* from pruning wound cankers in almond trees in five orchards in California.

	Sampling Date						
	April 2	April 26	May 11	May 18	May 23	June 5	June 5
Location[a]	1	2	3	4	2	1	5
Number of cankers positive for *P. syringae*/total sampled[b]	7/9	10/13	5/5	1/6	3/13	0/18	0/5
Number of cankers positive for *Ceratocystis fimbriata*/ total sampled[c]	N.D.[d]	N.D.	0/5	N.D.	0/13	0/18	0/5

[a]Five orchards with trees showing typical symptoms were sampled during the spring of 1984. The location of these orchards were: 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]25-50 tissue pieces from each canker were plated on PVP medium as described in text.

[c]Tissue pieces were plated on APDA medium as described in text.

[d]N.D. = Not determined.

Table 4. Canker development at four temperatures in almond branch segments (cv. 'Drake') infected with various Phytophthora species pathogenic on almond.

	Canker expansion rate (mm/day)			
	Temperature (C)			
Species	2	7	12	20
P. syringae	1.1 a[1]	1.3 a	2.9 a	3.1[2]
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

[1]Numbers within a column followed by the same letter are not significantly different (P = .05) according to Duncan's multiple range test.

[2]No significant differences (P = 0.05).

Table 5. Canker expansion rates in 11 almond cultivars artificially inoculated with *Phytophthora syringae*

Cultivar	Expansion rate (mm/day)
Jordanolo	1.12 a
Carmel	1.09 ab
Nonpareil	1.05 abc
Butte	0.91 abc
Thompson	0.89 abc
Fritz	0.88 abc
Price	0.86 bc
Merced	0.85 bc
Mission	0.85 bc
Ne Plus Ultra	0.84 c
Ripon	0.59 d

Table 6. Natural incidence of Phytophthora pruning wound cankers in three orchards.

Orchard	Year	Cultivar	Mean cankers per 100 trees	Percentage pruning wound with cankers
CSUC Variety trial	1982	All 20	41	----------
Butte Co.	1983	All 20	49	----------
	1984	All 20	43	----------
Nickels Estate	1984	Nonpareil	43	12.4
Orchard		Mission	9	4.2
Colusa Co.		Ne Plus	14	3.8
		All 3	34	10.5
Commercial Orchard	1984	Nonpareil	88	23.2
Colusa Co.		Ne Plus	121	25.5
		Price	141	22.4
		All 3	103	23.4

Figure Legends

Fig. 1. Relationship between pruning wound diameter and % pruning wounds with cankers.

Fig. 2. A. Growth of *Phytophthora syringae* from almond bark pieces on PVP medium.

B. Sporangia of *P. syringae* growing on amended lima bean agar. Bar indicates 10 µm.

C. Oospore produced in culture after 8 wk at 9 C. Bar indicates 10 µm.

D. Hyphal swellings produced by an almond isolate of *P. syringae* on corn meal agar.

Fig. 3. Effect of temperature on radial growth of two almond isolates of *Phytophthora syringae* on corn meal agar. Each point represents the averaged data from ten plates.

Fig. 4. Upper. Gummosis at margins of a canker resulting from natural infection of a pruning wound in an almond tree by *Phytophthora syringae*.

Lower. Canker 3 mo after inoculation of a pruning wound with an almond isolate of *Phytophthora syringae*.

Fig. 5. Effect of wound age on canker development in almond bark (var. Nonpareil) inoculated with *Ceratocystis fimbriata*.

Fig. 6. Effect of wound age on percentage of wounds developing cankers after inoculation with *Ceratocystis fimbriata*.

Fig. 7. Locations (O) where *P. syringae* was positively identified in pruning wound cankers.

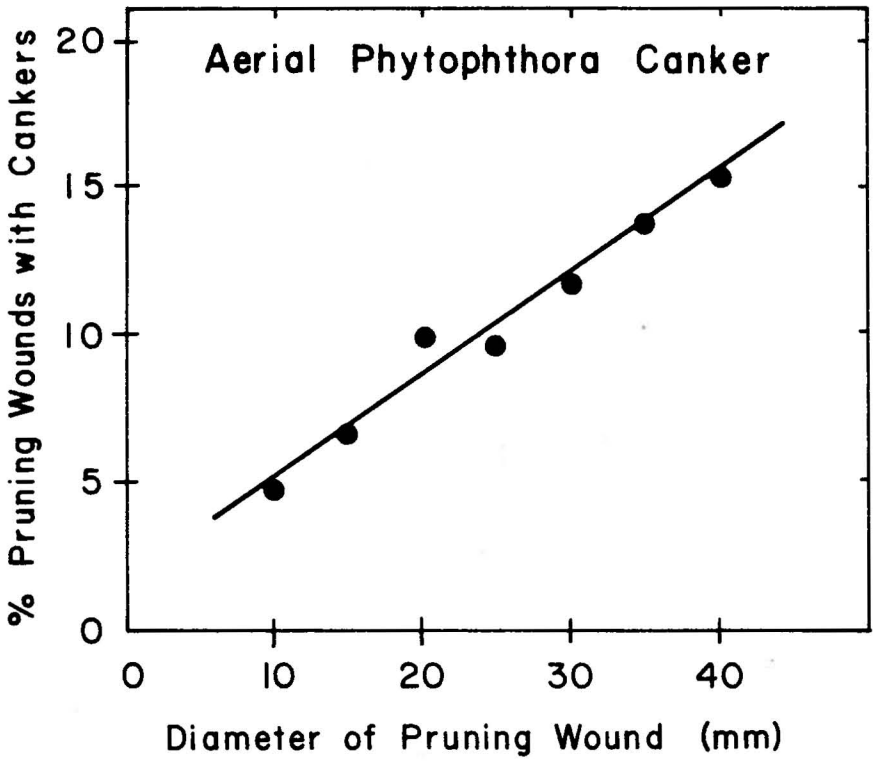
Aerial Phytophthora Canker
% Pruning Wounds with Cankers
0
5
10
15
20
0
10
20
30
40
Diameter of Pruning Wound (mm)

Fig. 1

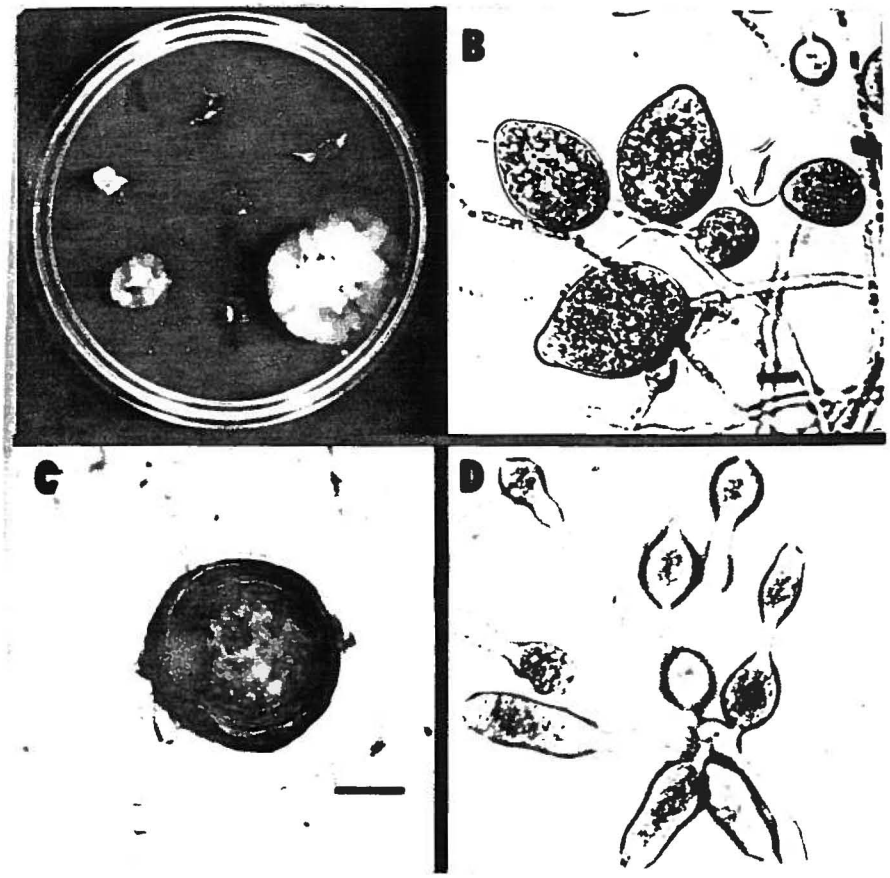

Fig. 2

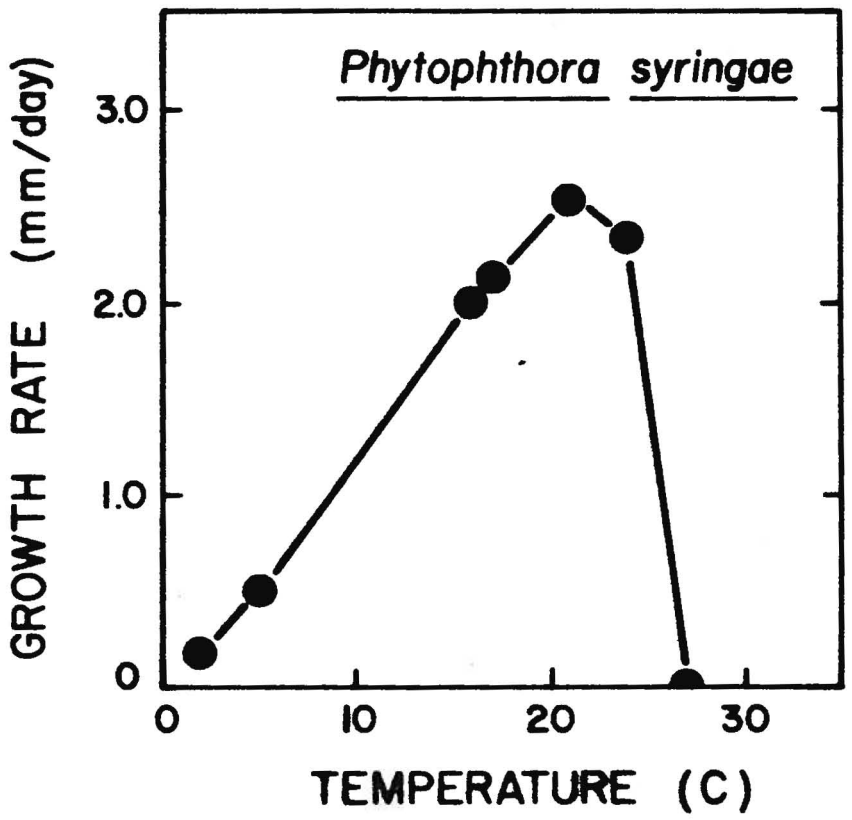
Phytophthora syringae
GROWTH RATE (mm/day)
3.0
2.0
1.0
0
0
10
20
30
TEMPERATURE (C)

Fig. 3

Fig. 4

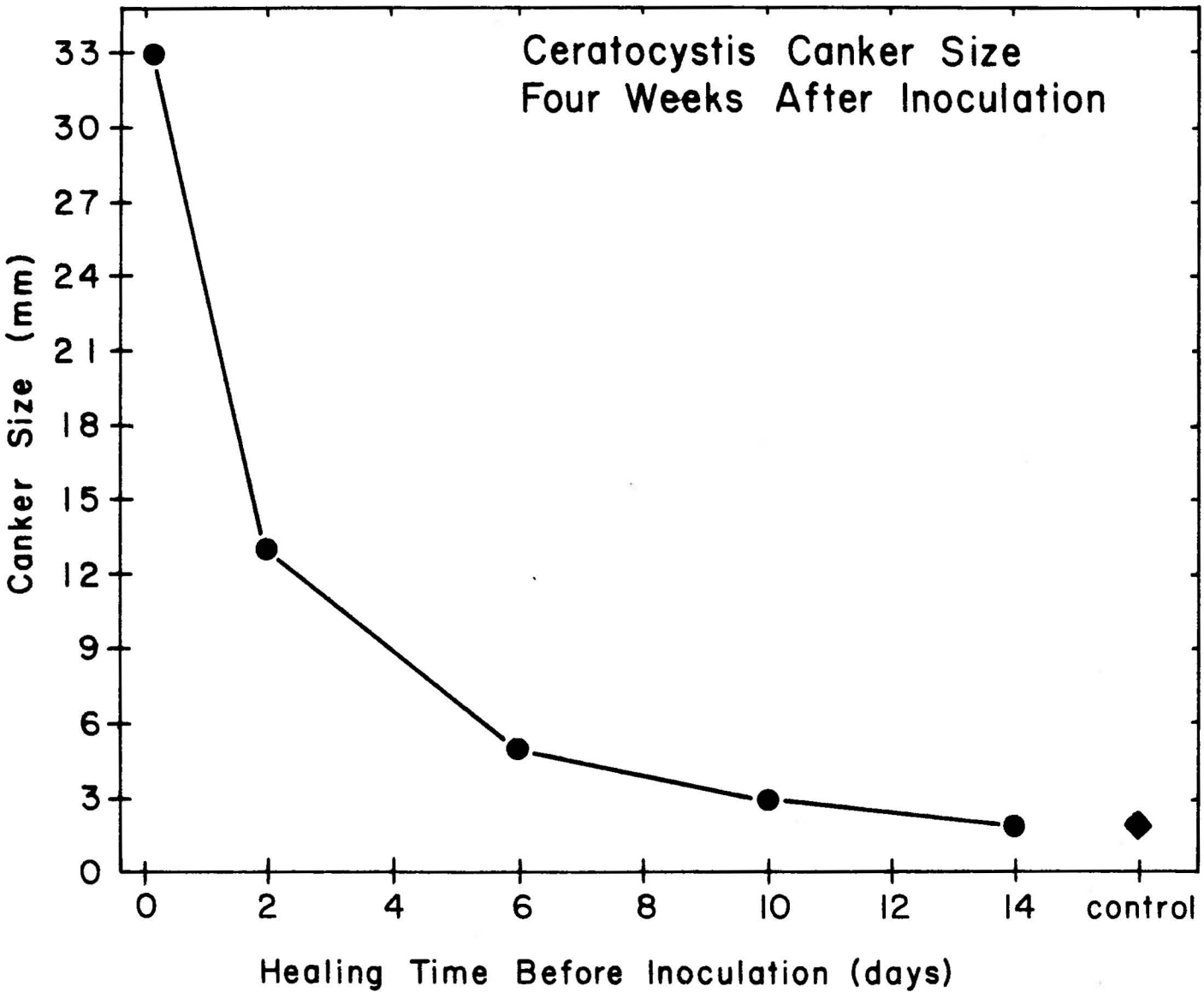
Ceratocystis Canker Size
Four Weeks After Inoculation
Canker Size (mm)
33
30
27
24
21
18
15
12
9
6
3
0
0
2
4
6
8
10
12
14
control
Healing Time Before Inoculation (days)

Fig. 5

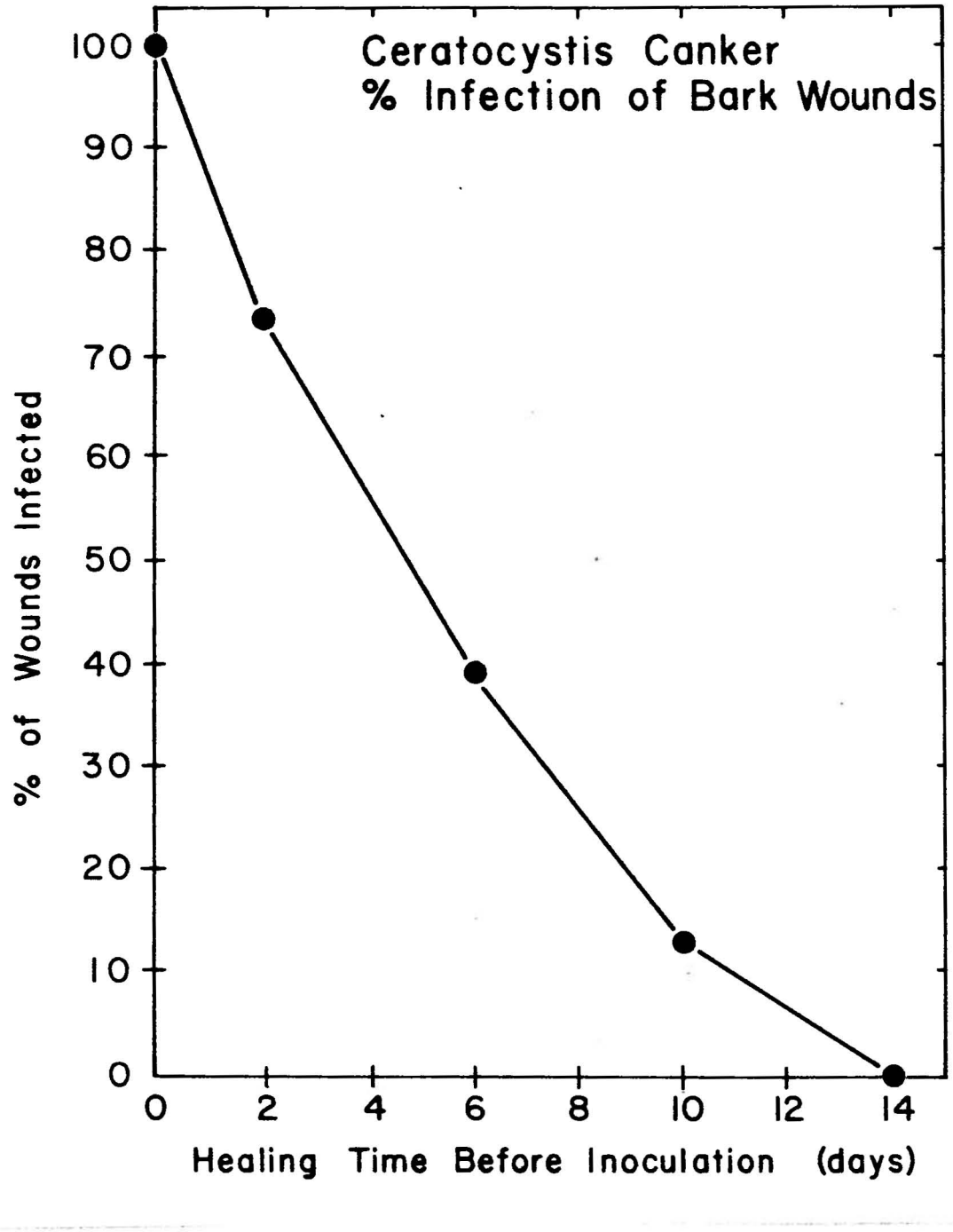
Ceratocystis Canker
% Infection of Bark Wounds
% of Wounds Infected
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Healing Time Before Inoculation (days)

Fig. 6


Del Norte
Siskiyou
Modoc
Humboldt
Trinity
Shasta
Lassen
Tehama
Plumas
Mendocino
Butte
Glenn
Sierra
Colusa
Yuba
Nevada
Lake
Sutter
Placer
Yolo
El Dorado
Sonoma
Napa
Sacramento
Amador
Alpine
Solano
Mono
Marin
San Joaquin
Calaveras
Contra Costa
Tuolumne
S.F.
Alameda
Stanislaus
San Mateo
Mariposa
Inyo
Santa Clara
Merced
Madera
S.Cruz
San Benito
Fresno
Monterey
Tulare
Kings
San Bernardino
San Luis Obispo
Kern
Santa Barbara
Ventura
Los Angeles
Riverside
Orange
San Diego
Imperial

Fig. 7