Project Number: 85-U5

Canker Complex in Almonds

Annual Report - 1985

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Objectives

(1) Continue studies on environmental conditions most favorable for infection of pruning wounds by Phytophthora syringae. (2) Initiate trials on protective chemical treatments for controlling Phytophthora pruning wound cankers. (3) Determine rate of wound resistance development to P. syringae in pruning cuts made at different times of the fall and winter. (4) Evaluate different almond cultivars for their response to P. syringae. (5) Initiate experiments to determine mechanisms of spore dispersal of P. syringae. (6) Finish histopathological studies on invasion of wounds by Ceratocystis fimbriata with emphasis on the relationship between pathogen invasion and wound resistance development. (7) Assess chemicals for their efficacy in protecting fresh wounds from infection by C. fimbriata and in eradicating the pathogen from established ceratocystis cankers.

Interpretive Summary:

Phytophthora pruning wound canker is caused by the fungus Phytophthora syringae. These wounds, although very susceptible when fresh, eventually become highly resistant to infection. During the fall and winter most of the 4 week old wounds in mature almond trees and many of the 2 week old wounds were immune to infection. However, occasionally wounds were still susceptible even 6 weeks after wounding. The tree responds to wounding by producing lignin and suberin which may act as a barrier preventing the fungus from causing cankers. These wound responses were greatly inhibited by the low temperatures of winter. During the fall and winter, the amount of lignin and suberin formed was positively correlated with the cumulative degree days. Lignification in 3 week old wounds was completely inhibited at temperatures less than 13 C (55 F). The amount of lignin and suberin formed increased as the temperature increased. Results from experiments with two almond variety trials for the past two years indicate that there is no substantial difference between the major almond varieties in canker size when fresh wounds were inoculated with P. syringae. Pruning wounds painted with a kocide-linseed oil mixture developed fewer cankers; however, the normal wound healing responses were greatly inhibited and the treatment appeared to be phytotoxic.

EXPERIMENTAL PROCEDURES

The isolation procedure for P. syringae from plant tissue was the same as described in our previous annual report.

Chemical control of Phytophthora pruning wound canker. In December, 1984 twelve blocks of 3 Nonpareil trees were sprayed with a hand gun to runoff with Kocide 101 (4 lbs. + 1/2 gal. supreme oil per 100 gal.). In late April, 1985 the trees were examined for the presence of Phytophthora pruning wound cankers. In another trial, 20 fresh pruning wounds in Nonpareil trees were painted with Kocide in linseed oil (8 lbs. in 3 gal.). One day later half of the wounds were inoculated with a P. syringae zoospore suspension. These were examined for cankers 6 weeks later. Seven weeks after wounding noninoculated wounds were sectioned, stained, and examined for phytotoxicity.

Time course of wound induced lignification. Two similar experiments were performed. The first used Nonpareil trees in an orchard in September and October, 1985. The second experiment used potted Nonpareil trees in a growth chamber at 25 C. The trees were wounded at various times.

The chemical assay for lignin used was the same for both experiments. This assay was adapted from Hammerschmidt (1984, Physiol. Pl. Path. 24:33-42). Wounds were made with a 5 mm diameter cork borer wounding through the inner bark to the wood. After various periods of time the wound areas were excised using a 10 mm diameter cork borer. The outer bark was carefully peeled off. Care was taken to remove the remaining inner bark from the wood. The inner bark tissue was extracted in 4 changes of methanol over 48 hrs. The tissues were then dried in a vacuum desiccator and weighed. The tissue pieces were then each placed in 5 ml of 10:1 mixture of 2 M HCl : thioglycolic acid and then placed in a 95 C oven for four hours. The acid was drained off and the tissue was then rinsed in distilled water. The tissue was then extracted with 4 ml 0.5 M NaOH for 18 hours at room temperature. After extraction the liquid was decanted off, the tissue was washed with 4 ml water, and the NaOH extracts and water washes were combined and placed in 12 ml conical centrifuge tubes. One ml of concentrated HCl was added to each tube and the extract was allowed to precipitate for four hours at 5 C. The precipitate was collected by centrifugation in a clinical centrifuge for 10 min. The supernatant was drained off and the precipitate was dissolved in 0.5 M NaOH. The resuspensions were centrifuged again in a clinical centrifuge for 10 min. The absorbance of the resulting solution was measured at 280 nm.

Effect of chitosan on wound lignification. Ten mg crabshell chitosan (milled #40) was dissolved in 2 ml 0.1 M HCl. The solution was brought up to 5 ml with water and adjusted to pH 6.0 with 0.5 NaOH. Two wounds through the bark to the cambium were made with a 5 mm diameter corkborer in each of seven Nonpareil potted trees. In one wound 0.05 ml of the chitosan solution was placed and in the other distilled water. After three days in a 25 C growth chamber, the outer bark was carefully removed, and the wound area excised with a 10 mm diameter cork borer. Care was taken to separate the inner bark from the wood. The inner bark tissue was assayed for lignin.

Effect of temperature on wound lignification and suberization. Experiments were performed using excised branches and potted trees. For the experiment using excised branches, Nonpareil almond branches of 1 to 2 cm diameter were cut from orchard trees. These were then cut into 15 cm sections and the proximal end placed in warm paraffin wax. Twigs were placed in several incubators at temperatures ranging from 5 to 27 C for various periods up to 6 weeks. The twigs were not kept for long periods at high temperature since they dry out. There were 10 replications. To determine the amount of lignification and suberization the tissue was sectioned (about 0.1 mm thick) on a sliding microtome and then stained with phloroglucinol-HCl and sudan black B, respectively. The sections were examined with the microscope and the percentage of inner bark tissue between the outer bark and cambium that stained with the reagents was measured.

In the other experiment, 1 year old Nonpareil potted trees were wounded with pruning shears by making cross-sectional cuts. After wounding, the trees were kept for 10 or 20 days in growth chambers at various temperatures from 6 to 25 C. The wound areas were then sectioned, stained, and the degree of lignification and suberization determined.

Effect of time of pruning on wound lignification and suberization and resistance to P. syringae. Branches of Nonpareil trees in the Nickels Estate Orchard were pruned every two weeks from October, 1984 through February, 1985 and allowed to heal 0, 2, 4, and 6 weeks. Then 10 branches per time period were inoculated with cornmeal agar plugs containing P. syringae mycelium and another 10 branches were brought back to the lab and sectioned and stained for lignin and suberin as given abouve. Six weeks after inoculation the amount of discoloration indicating canker formation was measured.

Susceptibility of almond varieties to P. syringae. The variety experiment of 1984 was repeated again in 1985. Canker development in trees of various cultivars at California State University, Chico and Delta Community College were determined after inoculating fresh pruning wounds with P. syringae in January and measuring canker length in May.

Conditions favorable for oospore production by P. syringae. P. syringae was allowed to grow in cornmeal agar for three days and then surface sterilized Nonpareil leaf disks (8 mm diameter) were placed on the colony margins. The petri dishes were placed in incubators at various temperatures ranging from 2 to 24 C. Leaf disks placed on noninoculated cornmeal agar plates and inoculated plates without leaf disks served as controls. Four weeks later the plates were examined for oospores.

The effect of temperature on oospore production by five isolates of P. syringae in a new medium, Kerwin's medium (Kerwin and Washino, 1984, Exp. Mycol. 8:215-224) was investigated. Kerwin's medium is 1.25 g peptone, 1.25 g yeast extract, 1.25 g glucose, 1.0 g linseed oil, 2.0 g corn oil, 0.075 g CaC12, 20 g agar, brought up to 1 liter with distilled water. The plates were inoculated with the fungus and then placed in incubators ranging in temperature from 6 to 21 C. V-8 agar was also inoculated for comparison. After 4 weeks the plates were examined for oospore production.

Fungicidal control of Ceratocystis canker. Branches of prune and almond orchard trees were wounded with a #3 cork borer in September, 1985. A conidial suspension of C. fimbriata (10^6 conidia / ml) was prepared from 10 day old cultures and applied to the wound with a paint brush. Wounds were either painted using a paint brush with one of the test compounds immediately and wrapped with parafilm for 24 hr or wrapped with parafilm for 24 hr and then after another 24 hr painted with the test compound. The experiment was initiated on September 10, 1985 and evaluated on October 2, 1985. Controls included nontreated, nontreated and inoculated, treated with sodium alginate or xanthan gum (no fungicide) and inoculated, and fungicide treated and inoculated. The experimental fungicides used, PP969 (50% SP) and Imazalil sulfate (Fungaflor, 75% WSP), were dissolved in water and then xanthan gum (12.5 g/1) or sodium alginate (12.5 g/1) were added with mixing until a homogeneous gel formed. Fungicides were tested at 1000 and 2000 ppm.

RESULTS

Continued study of orchards surveyed in 1984 for Phytophthora pruning wound canker. In 1984 two orchards in Colusa Co. were thoroughly surveyed for pruning wound cankers as reported in the 1984 Annual Report. Complementary studies were performed in these orchards in 1985.

In both of these orchards in the fall P. syringae was consistently isolated from recently fallen almond leaves. In the Nickels Estate Orchard P. syringae was isolated from 21.4% of 56 fallen leaves but none of 16 leaves still attached to the tree. In the commercial orchard P. syringae was isolated from 18.2% of 33 fallen leaves.

Nine one-year-old cankers in Nickels Estate Orchard were measured in November, 1984 and then again in May, 1985. The mean canker length from the canker margin to the pruning wound was 10.9 cm for both November and May readings. In both orchards there was no sign (i. e. fresh gumming) of any of the old cankers still being active after the summer.

Chemical control of Phytophthora pruning wound canker. In the spray trial the trees sprayed with Kocide had only 0.03 cankers per tree while the nonsprayed controls had 0.22 cankers per tree. None of the Kocide-linseed oil painted wounds but 40 % of the controls developed cankers; however, the kocide-linseed oil treatment was also phytotoxic.

Time course of wound induced lignification. The results of the orchard experiment are shown in Fig. 1. Significantly more lignin was detected as early as 2 days after wounding. Lignin accumulation was significantly linear (r^2 = .948) over time. The regression line was: relative lignification = 0.471 + 0.216 x days after wounding.

For the wounded inner bark tissue of potted trees kept at 25 C, lignin accumulation was also significantly linear (r^2 = .984) (Fig. 2). The regression line was: relative lignification = 0.263 + 0.141 x days after wounding. As early as 24 hr there was both a significant and substantial increase in the amount of lignin detected.

Effect of chitosan on wound lignification. Chitosan elicited a substantial and significant increase in lignification of the wounded bark tissue (Table 1). Substantially more lignin accumulated in the water treated 3 day old wounds when compared with fresh wounds.

Effect of temperature on wound lignification and suberization. Lignification after wounding the excised twigs was greatly inhibited at low temperatures (Fig. 3). Even after 5 weeks almost no lignin was observed at 5 C. There was about the same degree of lignification at 12 C after 5 weeks as at 27 C after 1 week. Suberization after wounding was also greatly inhibited at low temperatures or less than 12 C. Lignin was detected prior to suberin.

Both lignification and suberization after wounding the potted trees were greatly inhibited at low temperatures (Fig. 5). Lignin again was detected prior to suberin. The amount of lignin observed 20 days after wounding the potted trees was similar to that observed 3 weeks after wounding excised twigs.

Effect of time of pruning on wound lignification and suberization and on resistance to P. syringae. Both wound lignification (Fig. 6) and suberization (Fig. 7) were greatly inhibited during December and January. Both lignin and suberin formation showed the trend of high amounts in October and November, only a little in December and January, and then large amounts again in February. This pattern followed the trend for average temperature with December and January having the lowest temperatures. Lignin was detected prior to suberin.

None of the fresh wounds, some of the 2 week old, most of the 4 week old, and almost all of the 6 week old wounds were immune to infection by P. syringae (Fig. 8). There was no clear pattern for canker development in 2 and 4 week old wounds throughout the fall and winter. There was no indication of a positive correlation between temperature and disease resistance. The discoloration length of the resulting canker (Fig. 9) did not show any pattern related to temperature during the period of healing but did show some relationship to temperature after inoculation. When fresh wounds were inoculated in late March and April, there was a definite inhibition of canker expansion associated with the high temperatures. From October through February, there was little difference in canker length from the inoculated fresh wounds, even though there were substantial differences in temperature during this period.

Susceptibility of almond varieties to P. syringae. A summary of results for 1984 and 1985 for the California State University, Chico variety trial is shown in Table 2 and for the Delta College trial in Table 3. Table 4 gives the average canker expansion rate of those varieties which were at both trials. There was little difference in canker expansion between the varieties except Ripon which had consistently smaller cankers. Both variety trials only had one row of trees for most varieties, so the most reliable indication would be Table 4 which combines the data for the two trials for both years.

Conditions favorable for oospore production by P. syringae. Oospores were readily produced in the leaf disks only at temperatures of 12 C and lower (Table 5) but not in the inoculated cornmeal agar controls at any temperature. In Kerwin's medium four of the isolates produced abundant oospores only at low temperatures. Only isolate #5 produced oospores at all temperatures tested. Enormous numbers of oospores were produced on Kerwin's medium; however, the viability of these oospores was not determined. Only rarely were oospores observed in V-8 medium at any temperature and then only very few.

Fungicidal control of Ceratocystis canker. Under the experimental conditions, the tested fungicides provided no significant protection of fresh or 2 day old inoculated wounds (Table 6). All of the inoculated controls developed cankers but none of the noninoculated wounds did. In some cases, the canker appeared delayed in development by the fungicides.

DISCUSSION

Almond leaf litter may play an important role in the disease cycle of Phytophthora pruning wound canker. P. syringae readily colonizes fallen leaves in orchards in the autumn. Abundant oospores are produced in the leaves which unlike the mycelium could survive the heat of summer and provide the primary inoculum in the following autumn. It seems definite that cankers are not active during or after summer.

Copper compounds applied as a spray or a paint are promising for the control of Phytophthora pruning wound canker. However, the optimal rates have not yet been determined. Further studies are needed.

Increase of lignin in the inner bark after wounding was detected as early as 24 hr after wounding (Fig. 2) with a constant increase over time (Fig. 1,2). A lignified zone around wounds could function as a barrier to fungal ingress. Wound lignin accumulation is positively correlated with the development of resistance to Ceratocystis by wounds (as reported in the previous annual report). Some resistance to C. fimbriata has been detected as early as 2 days after wounding. Although other factors probably contribute to resistance, lignin accumulation seems to be involved in wound resistance to Ceratocystis canker and may be a useful index for the relative resistance of a bark wound.

Application of chitosan to wounds caused a substantial increase in lignin deposited in the wounded tissue (Table 1). This may lead to a method of protecting wounds from various canker pathogens such as P. syringae, C. fimbriata, or others by applying compounds which enhance host defense mechanisms.

Responses of the inner bark to wounding such as lignification and suberization were very dependent on temperature and were greatly inhibited at low temperatures (Fig. 3-7). The suberin detected was associated with a wound periderm which would function similar to the outer bark as a barrier to pathogens. The temperatures common in almond orchards in California in the winter are low enough to strongly inhibit both wound lignification and suberization (Fig. 6,7). However, there was no clear indication that wound resistance to Phytophthora pruning wound canker was dependent on temperature (Fig. 8,9). Since most of the 4 week old and almost all of the 6 week old pruning wounds were immune to P. syringae, any protective treatment must protect the wounds for about 5 weeks.

When fresh wounds were inoculated with P. syringae, all of the major almond varieties were very susceptible (Tables 2-4). Ripon seems to be the only variety which is consistently and definitely more resistant (Table 4). However, it seems that canker expansion rate should not be the sole criterion for evaluating cultivar resistance and that other criteria, such as rate of wound healing, should be considered.

Kerwin's medium is promising for use in producing P. syringae oospores (Table 5). This would facilitate identification of isolates, study further the conditions favorable for oospore production, and study the germination and survivability of oospores. Oospores are probably the fungal structure which survives the summer in California. It is not known why oospores tend to be produced only at low temperatures (Table 5).

No fungicide has yet been found which will adequately protect bark wounds from infectin by C. fimbriata (Table 6). Perhaps too much inoculum overwhelmed the effect of the fungicides in this study, but no significant protection was observed. Although the application of a wound protectant is a desirable strategy, our research thus far has not been promising in this regard. Alternative approaches such as the use of biological agents, different formulations which can facilitate penetration of the fungicide into the suberized bark tissue or novel chemical agents (e.g. elicitors of bark defense reactions) are possibilities. However, extensive preliminary studies will need to be performed before embarking upon an investigation into any one or all of these approaches.

PUBLICATIONS:

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

Doster, M. A. and R. M. Bostock. 1985. Effect of temperature on lignification and suberization of almond bark wounds and on wound resistance to Phytophthora syringae. (Abstr.) Phytopathology 75:1313.

Doster, M. A. and R. M. Bostock. 1985. Survey of pruning wound cankers caused by Phytophthora syringae in almond orchards. (Abstr.) Phytopathology 75:1369.

Middleton, G. E. and R. M. Bostock. 1985. Histopathology of wounded almond bark in relation to infection by Ceratocystis fimbriata. (Abstr.) Phytopathology 75:1374.

Table 1.

Effect of chitosan on bark lignification after wounding.

Treatment	Relative lignification[y]
chitosan treated 3 day old wound	0.032 a[z]
water treated 3 day old wound	0.024 b
fresh wound	0.015 c

[y]Lignification expressed as absorbance (280 nm) of lignothioglycolic acids per milligram dry weight of inner bark cell walls.

[z]Numbers followed by the same letter are not significantly different (P = 0.05) DMRT (LSD = 0.005).

Table 2.

Expansion rate of Phytophthora syringae cankers in various almond varieties at the California State University, Chico variety trial, Butte Co. for the winters of 1984 and 1985.

Canker expansion rate (mm/day)

	Year		Mean
	1984	1985	
Norman	1.47	1.08	1.28 a[z]
Milow	1.17	1.34	1.26 ab
Carrion	1.11	1.38	1.25 ab
Padre	1.24	1.16	1.20 abc
Sonora	0.80	1.41	1.10 abcd
Jordanolo	1.23	0.94	1.08 abcd
Butte	0.97	1.08	1.02 bcde
Vesta	0.94	1.08	1.01 cde
Solano	1.00	0.98	0.99 cdef
Merced	0.83	1.08	0.96 def
Robson	1.16	0.74	0.96 def
NePlus Ultra	0.85	1.02	0.94 defg
Mission	0.88	0.95	0.92 defg
Thompson	0.98	0.93	0.91 defg
Fritz	0.83	0.98	0.91 defg
Carmel	0.97	0.84	0.91 defg
Nonpareil	0.94	0.84	0.89 defg
Harvey	0.81	0.88	0.84 efg
Granada	0.78	0.74	0.76 fgh
Price	0.79	0.63	0.71 gh
Ripon	0.61	0.55	0.58 h
Mean	0.97	0.98	

[z]Numbers followed by different letters differ significantly at P = 0.01 by FLSD. $LSD_{.01} = 0.235$.

Table 3.

Expansion rate of Phytophthora syringae cankers in various almond varieties at Delta College almond variety trial, San Joaquin Co. for the winter of 1984 and 1985.

Canker expansion rate (mm/day)

	Year		Mean
	1984	1985	
Livingston	1.51	1.25	1.38 a[z]
Grace	1.30	1.16	1.23 ab
Mono	1.41	1.04	1.22 ab
Sauret #1	1.50	0.82	1.16 bc
Monterey	1.27	0.98	1.12 bcd
Ruby	0.72	1.22	0.97 cde
Merced	0.87	1.04	0.96 de
Mission	0.81	1.07	0.94 def
Fritz	0.92	0.96	0.94 def
Nonpareil	1.16	0.71	0.93 def
Tokyo	0.94	0.87	0.90 efg
Carmel	1.21	0.59	0.90 efg
Butte	0.85	0.96	0.90 efg
LeGrand	0.75	0.98	0.86 efgh
Jordanolo	1.00	0.69	0.85 efgh
Sauret #2	0.68	0.85	0.76 fghi
Yosemite	0.78	0.73	0.76 fghi
Price	0.92	0.60	0.76 fghi
Thompson	0.89	0.61	0.75 fghi
NePlus Ultra	0.83	0.62	0.73 ghi
Monarch	0.69	0.73	0.71 hi
Planada	0.64	0.77	0.70 hi
Peerless	0.65	0.52	0.59 ij
Ripon	0.57	0.40	0.48 j
Mean	0.95	0.84	

[z]Numbers followed by different letters differ significantly at P = 0.01 by FLSD. $LSD_{.01}$ = 0.1955.

Table 4.

Expansion rate of *Phytophthora syringae* cankers in mature almond trees at the Delta College variety trial, San Joaquin Co. and California State University, Chico variety trial, Butte Co. during the winters of 1984 and 1985.

Variety	Canker expansion rate (mm/day)[y]
Jordanolo	0.97 a[z]
Butte	0.96 a
Merced	0.96 a
Mission	0.93 a
Fritz	0.92 a
Nonpariel	0.91 a
Carmel	0.90 a
NePlus Ultra	0.83 a
Thompson	0.83 a
Price	0.73 ab
Ripon	0.53 b

[y]Canker expansion rate averaged for both sites and both years.

[z]Numbers followed by different letters differ significantly at P = 0.01 by FLSD. $LSD_{.01}$ = 0.260.

Table 5.

Effect of temperature on formation of oospores by Phytophthora syringae in leaves and in Kerwin's medium.

Temperature	Leaves Isolates #1	Kerwin's medium #1	#2	#3	#4	#5
-	++z	++	++	++	++	++
6	++	++	++	++	++	++
12	+	++	++	-	-	++
15	-	+	-	-	-	++
18	-	-	-	-	-	+
21	-	-	-	-	-	++

z - no oospores, + only a few, ++ abundant oospores observed.

Table 6.

Fungicidal control of Ceratocystis canker in orchard trees.

French Prune

Treatment	Number cankers/Number wounds	
	Applied at inoculation	Applied 48 hr post-inoculation
Imazalil/2000 ppm/alginate	5/5	5/5
Imazalil/2000 ppm/xanthan gum	5/5	5/5
Imazalil/1000 ppm/alginate	5/5	5/5
Imazalil/1000 ppm/xanthan gum	5/5	5/5
PP969/2000 ppm/alginate	5/5	5/5
PP969/2000 ppm/xanthan gum	3/5	3/5
PP969/1000 ppm/alginate	4/5	4/5
PP969/1000 ppm/xanthan gum	4/5	5/5

Almond

Treatment	Applied at inoculation	Applied 48 hr after inoculation
Imazalil/2000 ppm/alginate	10/10	10/10
Imazalil/2000 ppm/xanthan gum	8/10	9/10
Imazalil/1000 ppm/alginate	10/10	10/10
Imazalil/1000 ppm/xanthan gum	10/10	10/10
PP969/2000 ppm/alginate	9/10	10/10
PP969/2000 ppm/xanthan gum	8/10	9/10
PP969/1000 ppm/alginate	10/10	10/10
PP969/1000 ppm/xanthan gum	8/10	9/10

Figure Legends

Fig. 1. Development of bark lignification after wounding orchard trees. Relative lignification expressed as absorbance (280 nm) of lignothioglycolic acids per wound.

Fig. 2. Development of bark lignification after wounding potted trees kept at 25 C. Relative lignification expressed as absorbance (280 nm) of lignothioglycolic acids per wounds.

Fig. 3. Effect of temperature on lignification of bark wounds in excised almond twigs as measured 1, 3, and 5 weeks after wounding.

Fig. 4. Effect of temperature on suberization of bark wounds in excised almond twigs as measured 1, 3, and 5 weeks after wounding.

Fig. 5. Effect of temperature on lignification and suberizatin of bark wounds in potted trees as measured 10 and 20 days after wounding.

Fig. 6. Development of bark lignification of pruning wounds in orchard trees throughout the fall and winter as measured after 2, 4, and 6 weeks after wounding.

Fig. 7. Development of bark suberization of pruning wounds in orchard trees throughout the fall and winter as measured after 2, 4, and 6 weeks after wounding.

Fig. 8. Canker development of 0, 2, 4, and 6 week old pruning wounds in orchard trees inoculated with Phytophthora syringae throughout fall and winter. Extensive necrosis means bark discoloration extending more than 1 cm from wound margin indicating the presence of a canker.

Fig. 9. Discoloration length of pruning wounds in orchard trees inoculated with Phytophthora syringae 0, 2, 4, and 6 weeks after wounding throughout the fall and winter. If canker is present then discoloration length is the same as canker length.

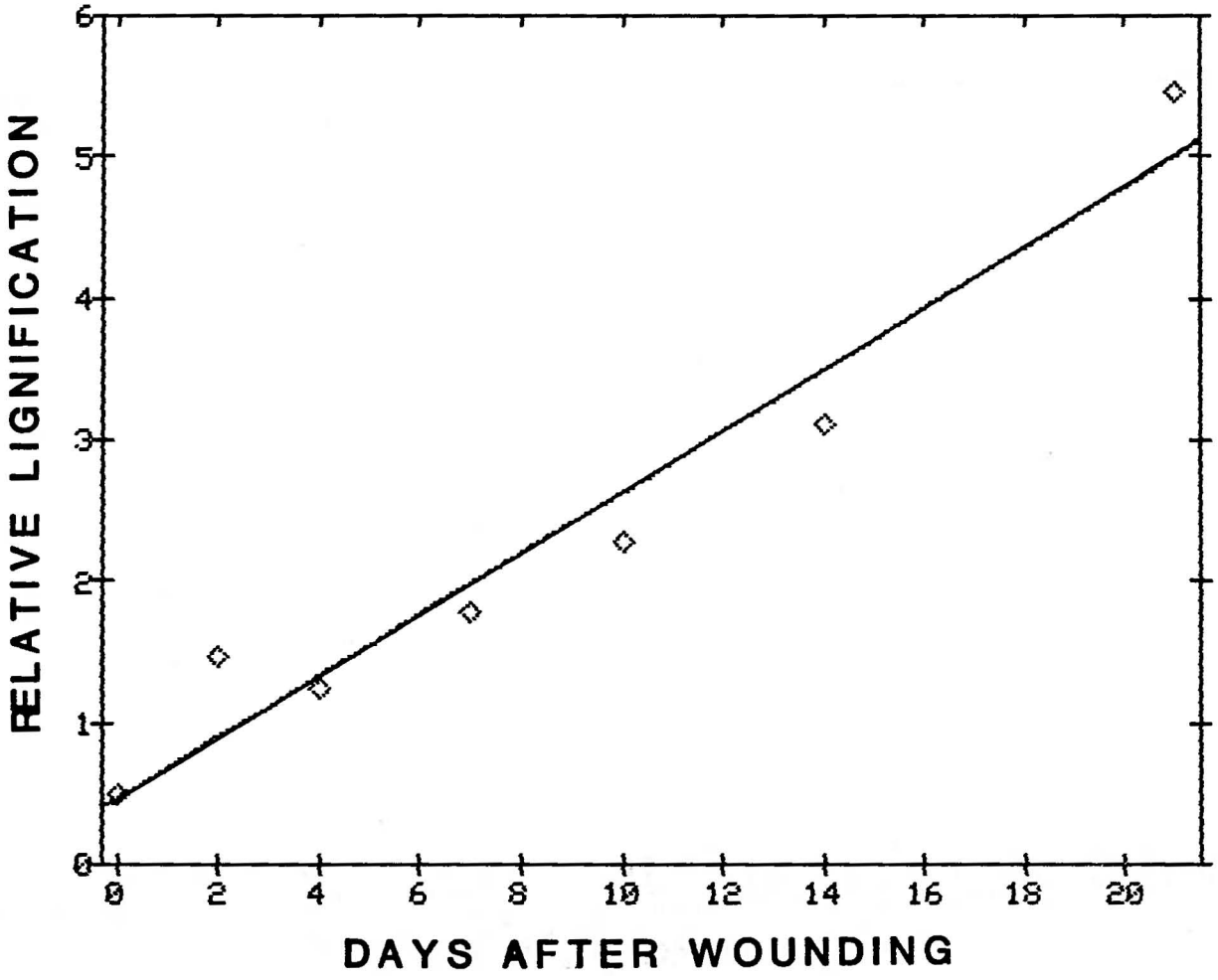
RELATIVE LIGNIFICATION
0
1
2
3
4
5
6
0
2
4
6
8
10
12
14
16
18
20
DAYS AFTER WOUNDING

Fig. 1

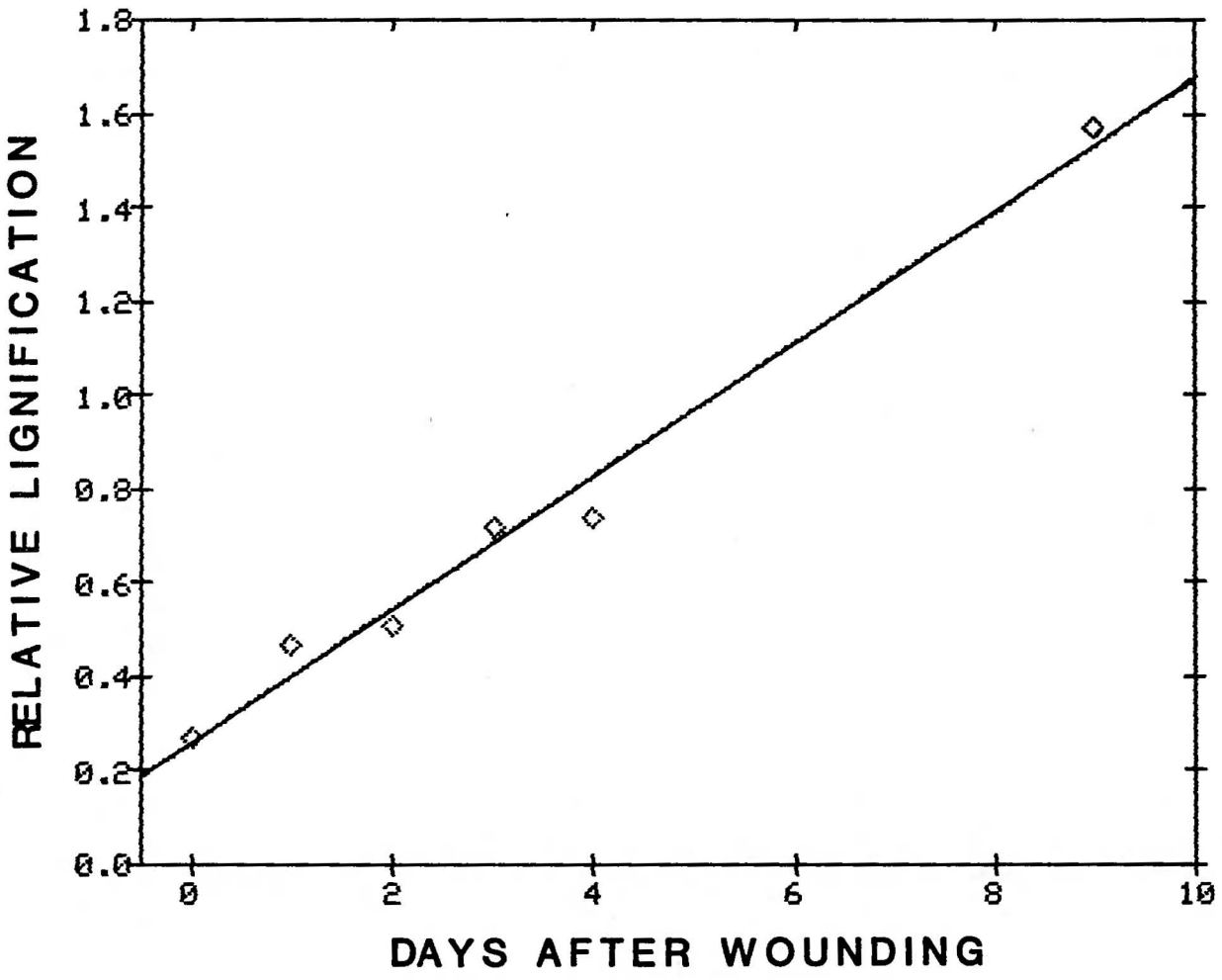
RELATIVE LIGNIFICATION
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
DAYS AFTER WOUNDING

Fig. 2

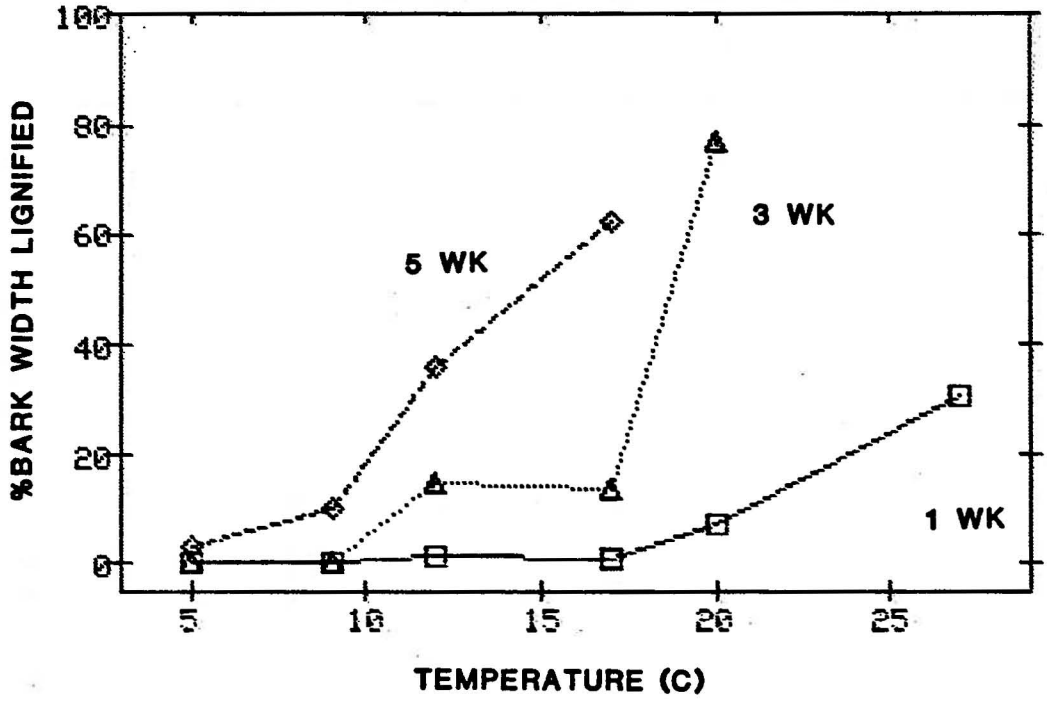
%BARK WIDTH LIGNIFIED
100
80
60
40
20
0
5
10
15
20
25
TEMPERATURE (C)
5 WK
3 WK
1 WK

Fig. 3

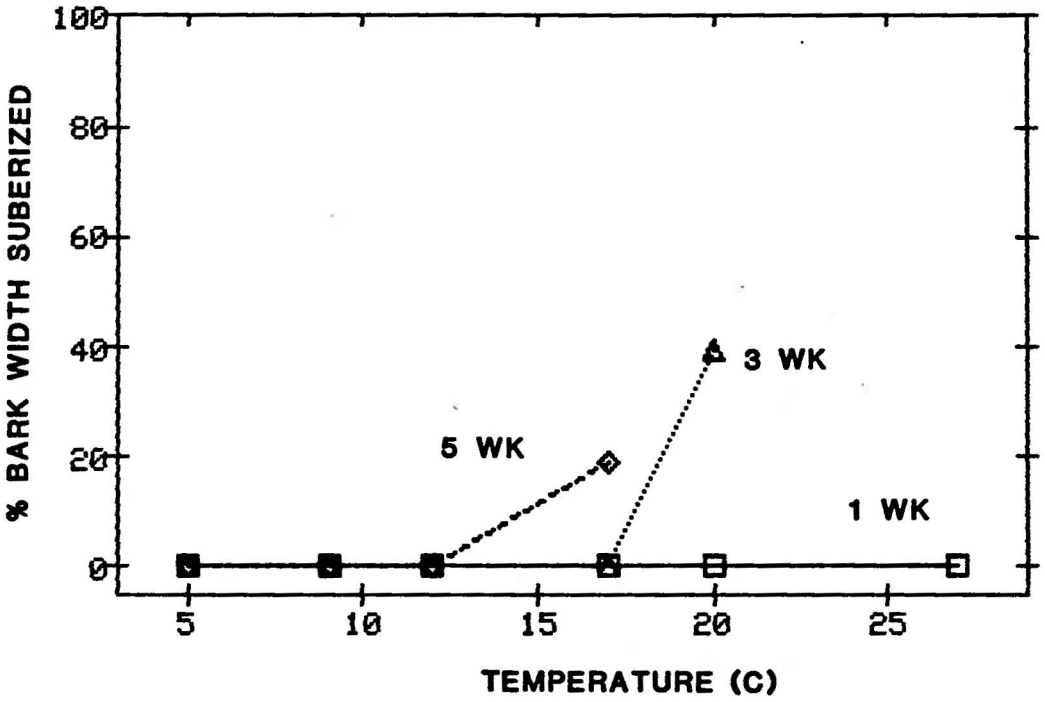
100
80
60
40
20
0
% BARK WIDTH SUBERIZED
5 WK
3 WK
1 WK
5
10
15
20
25
TEMPERATURE (C)

Fig. 4

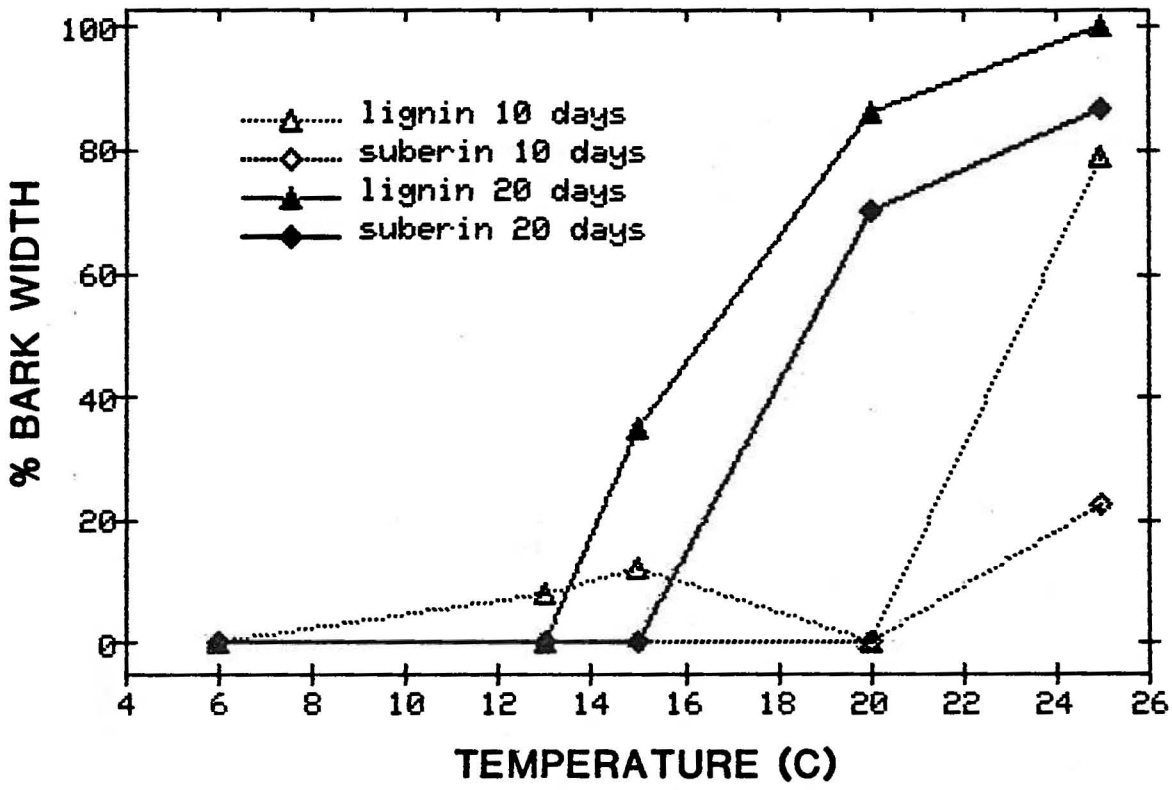
lignin 10 days
suberin 10 days
lignin 20 days
suberin 20 days
% BARK WIDTH
TEMPERATURE (C)
0
20
40
60
80
100
4
6
8
10
12
14
16
18
20
22
24
26

Fig. 5

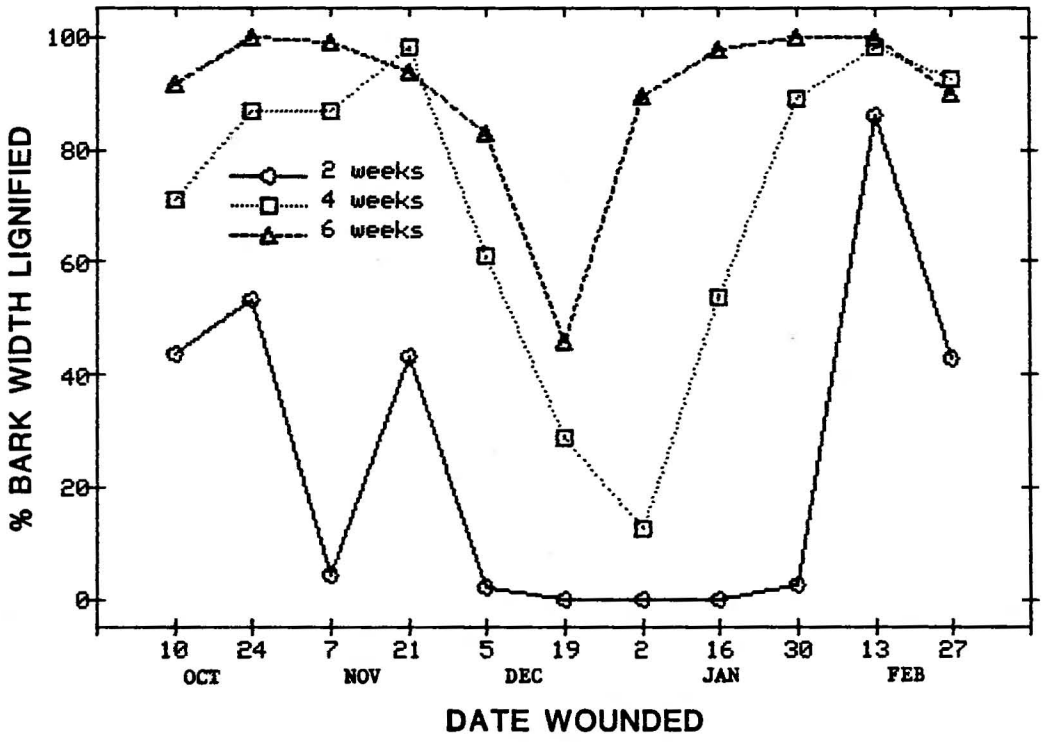
% BARK WIDTH LIGNIFIED
100
80
60
40
20
0
2 weeks
4 weeks
6 weeks
10
24
7
21
5
19
2
16
30
13
27
OCT
NOV
DEC
JAN
FEB
DATE WOUNDED

Fig. 6

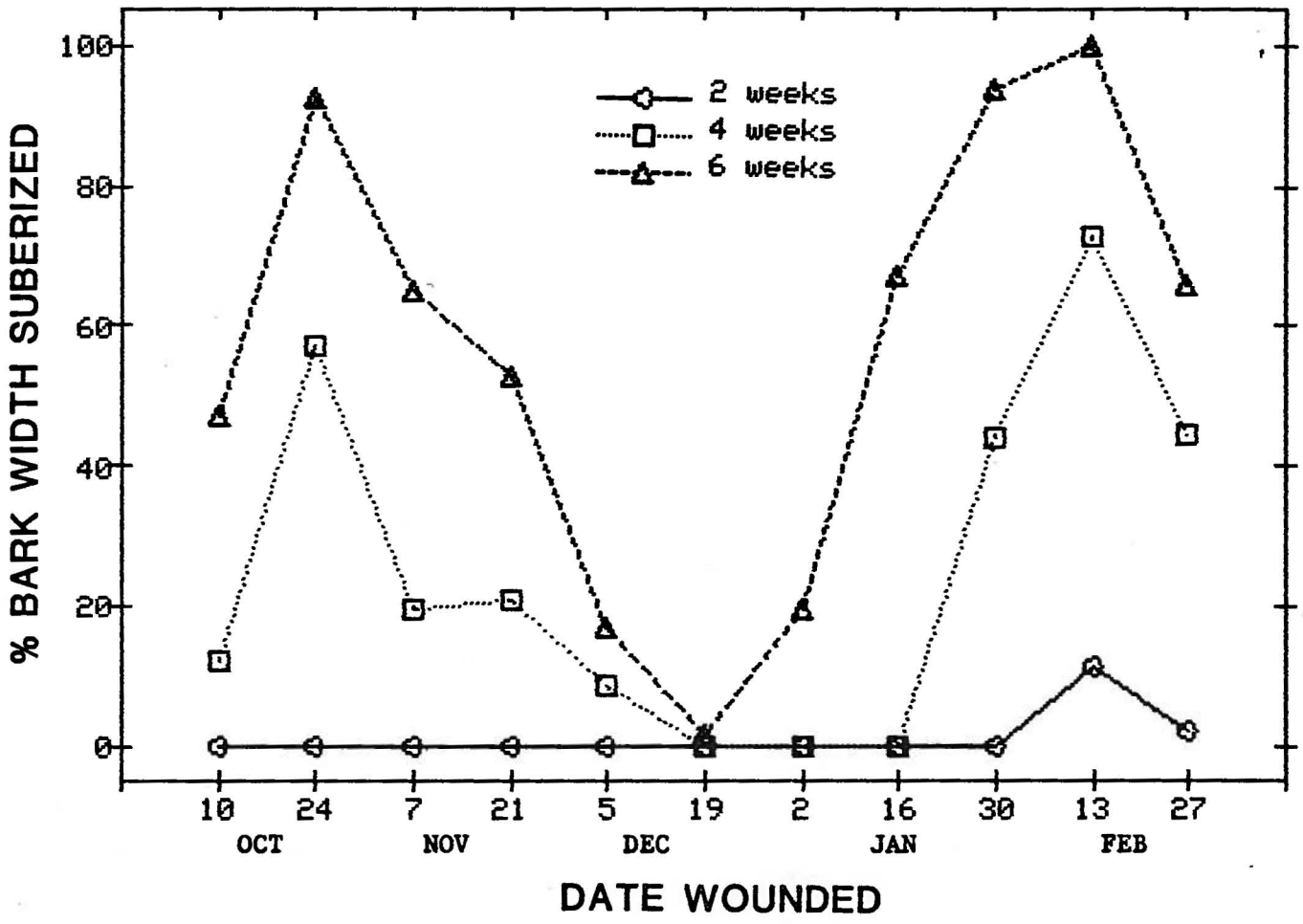
% BARK WIDTH SUBERIZED
100
80
60
40
20
0
2 weeks
4 weeks
6 weeks
10
24
7
21
5
19
2
16
30
13
27
OCT
NOV
DEC
JAN
FEB
DATE WOUNDED

Fig. 7

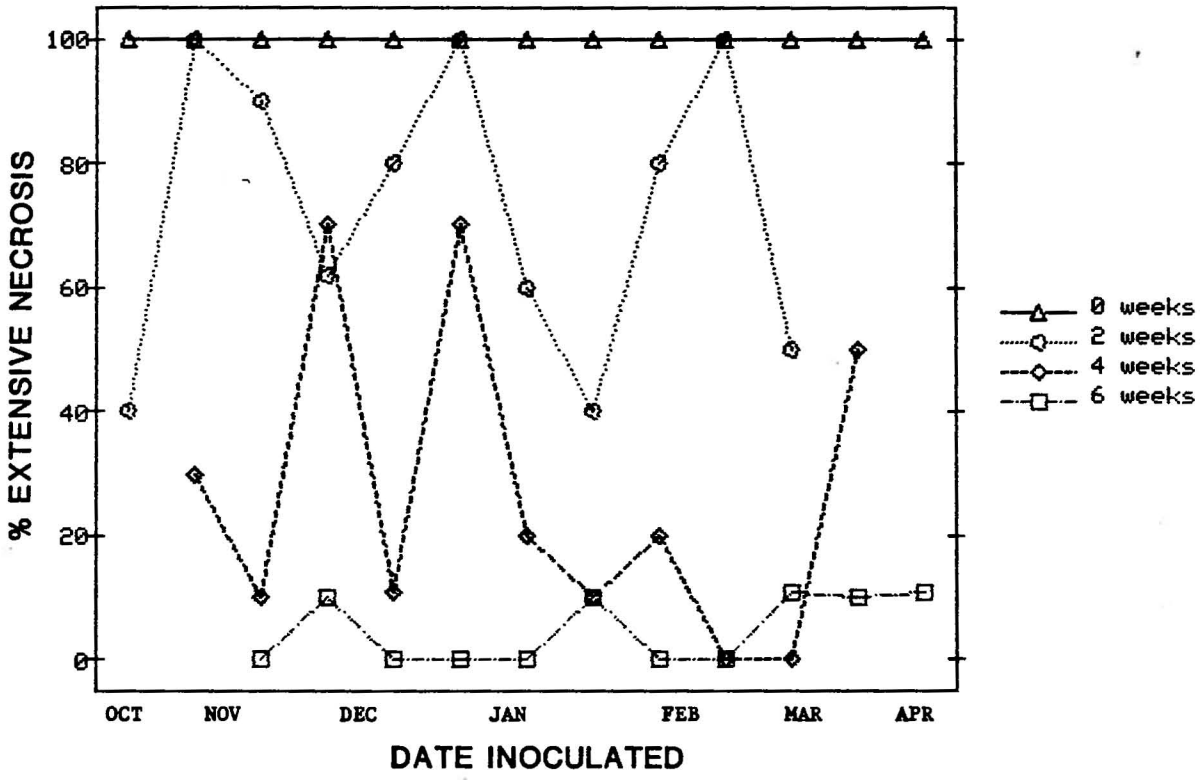
100
80
60
40
20
0
% EXTENSIVE NECROSIS
OCT
NOV
DEC
JAN
FEB
MAR
APR
DATE INOCULATED
0 weeks
2 weeks
4 weeks
6 weeks

Fig. 8

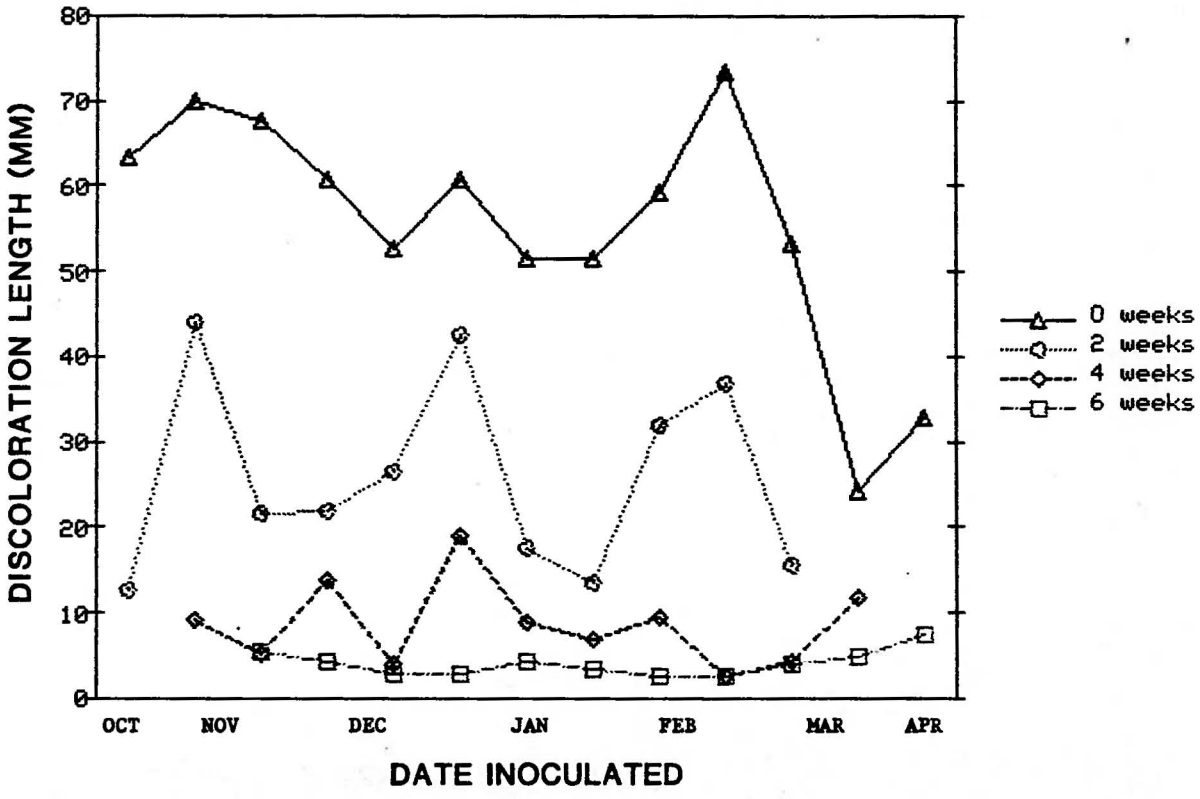
DISCOLORATION LENGTH (MM)
80
70
60
50
40
30
20
10
0
OCT
NOV
DEC
JAN
FEB
MAR
APR
DATE INOCULATED
0 weeks
2 weeks
4 weeks
6 weeks

Fig. 9

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

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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).

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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.

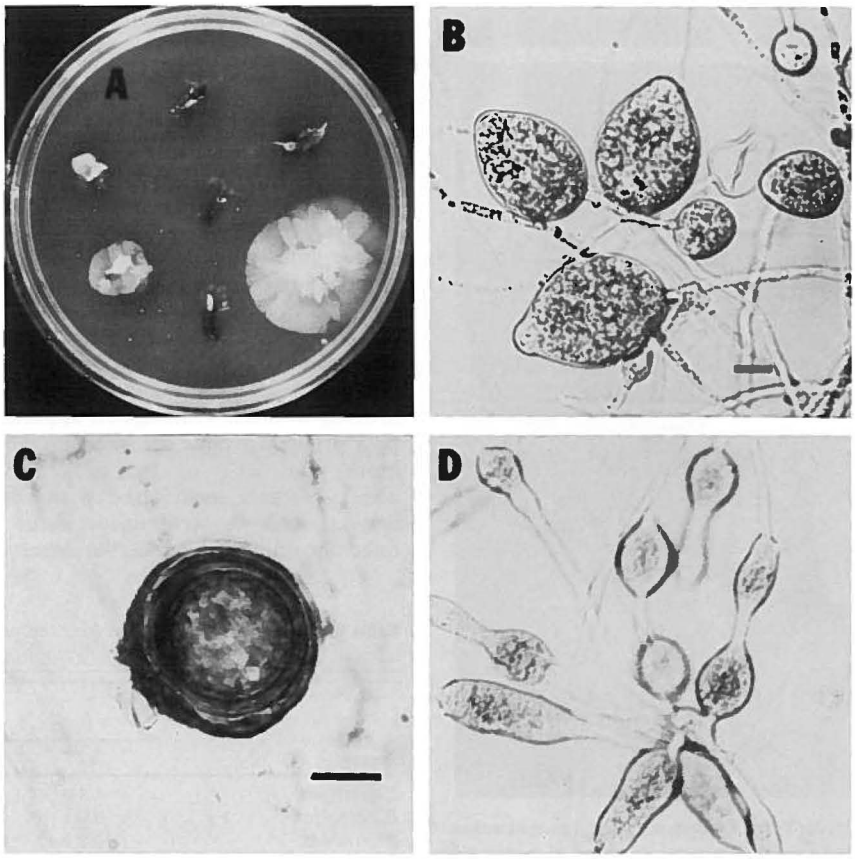

Fig. 1. (A) Growth of *Phytophthora syringae* from almond bark pieces on PVP medium (16). **(B)** Sporangia of *P. syringae* growing on amended lima bean agar. Bar = 10 μm. **(C)** Oospore produced in culture after 8 wk at 9 C. Bar = 10 μm. **(D)** Hyphal swellings produced by an almond isolate of *P.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