Relationship of Wound Periderm Formation to Resistance to *Ceratocystis fimbriata* in Almond Bark

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ABSTRACT

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Bark wounds in branches on almond trees of the cultivar Nonpareil became resistant to infection by *Ceratocystis fimbriata* within 10–14 days after injury in summer. Frequency and size of cankers declined significantly as the time interval between wounding and inoculation increased on Nonpareil and also on the cultivar Carmel. The development of complete resistance in wounds on Nonpareil that had aged for 14 days was related by histochemical analyses to extensive deposition of lignin and suberin in a well-developed wound periderm. The difference in canker size at bark injury sites inoculated after 6, 10, and 14 days was not significant and was related to browning and death of cells at the wound surface followed by deposition of lignin and suberin near a wound meristem. On potted trees, wounds that were reinjured after 14 days of aging became susceptible to infection, similar to fresh bark wounds. These results suggest that treatments applied to fresh bark wounds should protect for 14 days after wounding to be effective and should not impair the development of natural resistance in the host tissue.

Additional key words: Prunus dulcis, wound closure.

Stone fruit trees in California are affected by a number of canker diseases incited by wound-infecting fungal pathogens. One of these diseases, Ceratocystis canker caused by *Ceratocystis fimbriata* (Ellis & Halst.), is most prevalent on almonds (*Prunus dulcis* (Mill) Webb) and French prunes (*P. domestica* L.) (11), although other stone fruits are susceptible. Nitidulid beetles (*Carpophilus* spp.) and other insects carry the fungus from soil or infected trees to fresh bark wounds made by harvesting equipment (11,19). Cankers that develop from these infections appear externally as depressed, darkened areas in the bark with profuse amber gum at the canker margins and entry wounds. The pathogen is primarily restricted to the bark but may also invade young xylem (11). With the improvements made in harvesting equipment and increased grower awareness, Ceratocystis canker has become less widespread in California orchards in recent years (8). Nevertheless, the disease occurs in some orchards and is of concern to growers. No chemical controls are currently available for use against *C. fimbriata* in almonds or prunes, although benomyl has been reported to control the pathogen on *Platanus occidentalis* L. (10).

Because of the difficulty in controlling many canker diseases of trees, precise information on factors that contribute to resistance of bark tissues to canker development may reveal characters for selection in cultivar improvement programs (3–7,21). A focus of recent investigations has been the dynamics of formation of wound (necrophylactic) periderm and associated anatomical barriers. Although the wound periderm has often been considered a passive defense mechanism, as is the outer (exophylactic) periderm, its formation is an active process, and elements of periderm formation (e.g., lignification) are also associated with induced resistance mechanisms in plants (15,21,28). A number of wound-induced reactions in plants are also enhanced during resistance expression to incompatible isolates of pathogens and nonpathogens and by microbial elicitors (9), suggesting that processes occurring during wound closure could be augmented by biological or chemical agents. The rate of wound closure is also an important consideration in relation to the duration of protection required of prophylactic treatments.

The procession of changes in bark tissues adjacent to wounds includes autolysis and death of cells, dedifferentiation of extant parenchyma cells to form a lignosuberized layer, and development of wound periderm and callus from newly formed meristematic cells (6,21,26). Biochemical changes observed in or adjacent to wounded plant tissues in model systems such as the tuber of *Solanum tuberosum* L. include evolution of ethylene and ethane; increased activity of peroxidases, polyphenol oxidase, and phenylalanine ammonia-lyase; accumulation of phenols and oxidized phenols; dramatic changes in isoprenoid metabolism; and deposition of lignin, suberin, and hydroxyproline-rich glycoproteins in cell walls (9,15,21,23,28). Many of the products of these reactions have antimicrobial activity or function as barriers to pathogen ingress by conferring impervious qualities to host tissue.

Although earlier studies indicated that bark wounds on almond trees develop resistance to invasion by *C. fimbriata* (11,20), little information was given about the nature and developmental time course of this resistance. In this study, we examine the relationship between resistance development and formation of wound periderm in almond bark. The primary objective was to determine the time course of wound resistance development in relation to lignification, suberization, and other histological changes during aging of almond bark wounds. A preliminary report of this work has been published (17).

MATERIALS AND METHODS

General. An almond isolate (F-10) of *C. fimbriata* obtained by hyphal tip culture from an active canker and maintained at 22 C on potato-dextrose agar (PDA) slants through ascospore mass transfers was used in all experiments. This isolate consistently produced cankers that expanded at a rate equal to or greater than cankers produced by isolates used in earlier research on Ceratocystis canker (11,19,20). Endoconidial spore suspensions having a germination rate of 90% or better were prepared from 3-wk-old cultures by filtering aqueous washes of the culture through two layers of cheesecloth to remove clumped ascospores and mycelial fragments. Suspensions were diluted to 10^6 spores per milliliter unless otherwise noted. Almond bark wounds were inoculated by applying the spore suspension with a small paintbrush or cotton-tipped applicator. Routinely, the wounds were wrapped with Parafilm for 1 day following inoculation; the Parafilm was then removed, and canker development was assessed

4 wk later.

Almond cultivars used were Nonpareil in the orchard and greenhouse tests and Carmel in the lathhouse. Wounds were made through the bark and cambium with a cork borer (7 mm in diameter unless otherwise specified) and the bark plug was removed. In the orchard and lathhouse experiments, wounded areas of the branch or stem were wrapped with four layers of open-mesh nylon netting (10 strands per centimeter) to exclude insects before inoculation.

An experiment was performed in the greenhouse to determine the effect of inoculum concentration on canker development in fresh wounds. Wounds were inoculated as described above with spore suspensions in concentrations ranging from 10^2 to 10^6 spores per milliliter. Wounds were made along the main stems of six 3-yr-old potted Nonpareil trees. The five inoculum levels were distributed among the wounds along each stem, with an uninoculated wound included on each tree. The treatments were distributed in a Latin square design so that each position along the stem was represented once among the trees. After 4 wk, canker lengths were determined. The outer periderm surrounding each wound was removed to determine the extent of brown, necrotic tissue in the bark cortex, thereby providing a visual assessment of the size of cankers associated with the inoculated wound. The diameter of the wound was subtracted from the measurement reported as the length of the canker. The temperature in the greenhouse was maintained between 22 and 28 C with supplemental lighting during the course of the experiment.

Time course of wound resistance development. Preliminary experiments conducted in the summer and fall of 1982 and 1983 on orchard trees and excised branches indicated that susceptibility of almond bark wounds to *C. fimbriata* generally declined over a 2-wk period. Three experiments were performed to determine more precisely the time course of resistance development. In an experiment conducted in September 1984, 3- to 5-yr-old branches of mature (8-yr-old) Nonpareil almond trees in the orchard were wounded at intervals to provide 0-, 2-, 6-, 10-, and 14-day-old wounds. Branches were selected at random on the north, south, and southwest sides of five trees. One or two complete ranges of aged wounds were included on each branch, and one to four branches per tree were used. In the second and third experiments conducted in June and July 1985, branches and stems of 4-yr-old potted Carmel almond trees in the lathhouse were wounded according to the same schedule as the orchard trees. In the lathhouse experiments, one or two complete ranges of aged wounds were included on each tree. In all experiments, the wounded areas were wrapped with netting immediately. All wounds were unwrapped on the last day of wounding (0 days of aging), inoculated with a suspension of spores of *C. fimbriata* or left uninoculated as controls, and then wrapped with Parafilm for 1 day. Four weeks after inoculation, wounded branches or stems were excised from the trees and the wounds examined. Canker lengths were determined as above. Wounds that did not develop cankers were not included in the calculation of average canker size. In some samples, the presence of *C. fimbriata* in the diseased tissue was verified by taking plugs of bark cortex, each including brown and healthy tissue, from an area where the outer periderm had been removed, then placing the plugs in acidified PDA and incubating the plates at 22 C. Growth of mycelium characteristic of *C. fimbriata* from the canker margin, accompanied by production of endoconidia, was considered to be sufficient to confirm infection by *C. fimbriata*.

In the orchard experiment, an analysis of variance for the different interactions (dependent variable = canker length) was performed using a general linear models procedure (25). In addition, a stepwise logistic regression (12) was used to model the probability of canker development as a function of wound age. The form of the relationship was $\ln(p/1-p) = u + bt$, where p is the probability of canker development and t is the age. Logistic regression diagnostics indicated that this model fit well.

Effect of wound type on resistance to canker development. Four-year-old potted Carmel trees in the lathhouse were wounded by the cork borer method or by hitting the bark with a rubber mallet until it was crushed and split. The size of the injured area was controlled by holding the small end of a steel bar (1 cm diameter) against the bark and hitting the other end with the mallet. The latter method was designed to simulate the type of bark injury often created by harvesting equipment. Inoculations were made directly on the crushed tissue without removing a bark plug. Two separate experiments were performed, one initiated in June 1985 and the other in July 1985. Wounds of different ages were distributed among the trees as described above. The data were analyzed by a stepwise logistic regression program (12).

Inoculation of aged wounds with *Carpophilus hemipterus* contaminated with *C. fimbriata*. *Carpophilus hemipterus* L. (Coleoptera) individuals were used to determine the relationship between wound age and susceptibility to *C. fimbriata* transmitted via a natural vector of the pathogen. Insects were reared and maintained on figs (*Ficus carica* L.) in mason jars until use (19). Individuals were placed on PDA cultures of *C. fimbriata* for 24 hr. Usually, spore masses of *C. fimbriata* could be seen on the insects. The presence of viable *C. fimbriata* on several insects was confirmed by placing them on acidified PDA or on carrot disks (18). No other fungi pathogenic on almond bark were detected, and cankers did not develop from wounds exposed to individuals not placed on fungal cultures. The contaminated insects were transported to the orchard and anesthetized with CO_2. Then one individual was caged over each cork-borer wound (7 mm diameter) with a plastic Beem (Polysciences, Inc., Warrington, PA) embedding capsule. The capsules were modified with nylon netting to permit gas exchange and were fixed to the bark with modeling clay (19). The insects were encapsulated over the wounds for 10 days and then removed; all were still alive.

Wounds were made as described above in Nonpareil almond branches (1–2 cm diameter) in orchard trees during July 1982. The trees used in this experiment were 6 yr old. For each wound age, 10 wounds distributed over two branches selected at random in two trees were inoculated with the insects and evaluated approximately 4 wk later. Canker development in each wound was rated according to a scale where 0 = no disease and healthy callus development, 1 = small canker with gummosis and necrosis extending up to 5 mm beyond the wound margin, 2 = gummosis and necrosis extending 5–10 mm beyond the wound margin, and 3 = gummosis and necrosis extending more than 10 mm beyond the wound margin. The Mann-Whitney test was used for comparison of mean disease ratings.

Histological studies. One branch from each of 16 Nonpareil almond trees was wounded at three locations at 15-cm intervals on 11 July 1983. Two branches were collected every 2 days for 14 days. The bark margins surrounding the cork-borer wounds were sectioned immediately, using a freezing microtome, then stained and photographed. Transverse sections (from the lateral margins) from at least one-half of each wound were examined. Longitudinal sections (from the distal and proximal margins) were prepared from at least one wound from each sample time. In a second experiment initiated on 5 July 1984, wounds serving as histological checks for the experiment investigating the relationship between wound age and resistance to infection were made at the same time and in the same trees as the wounds to be inoculated, but on separate branches. The branches with wounds for histological examination were collected when the wounds on the other branches were inoculated, and material that could not be examined immediately was stored at 4 C for not more than 3 wk. Wounds for histological examination were either sectioned fresh, using a freezing microtome, or embedded in paraffin (Tissueprep, melting point = 61 C, Fisher) or glycol methacrylate (JB-4 kit, Polysciences, Inc.). Sections obtained with the freezing microtome were approximately 40 μm thick, and sections from embedded tissues were 5–20 μm thick. Bark tissue samples were fixed in a mixture containing Formalin, acetic acid, and ethanol (95:5:5, v/v) and dehydrated in a *t*-butyl alcohol or ethanol series, 50–95%, before embedding (2). Histochemical stains used were phloroglucinol in HCl and Maule's reagent for lignin, Sudan black B for suberin, and toluidine blue for general morphological features (2).

Effect of Parafilm on resistance of aged wounds. To determine the effect of Parafilm on the resistance to infection of aged wounds, trees were wounded, then inoculated 14 days later with a spore suspension of *C. fimbriata* (10^6 spores per milliliter). The wounds were either left unwrapped or wrapped with Parafilm for 1–3 days, and canker development was assessed after 4 wk. One experiment used branches on Nonpareil trees in the orchard with 1- and 2-day postinoculation wraps. A second experiment used stems of 3-yr-old potted Nonpareil trees in a greenhouse with 1-, 2-, and 3-day postinoculation wraps. In the greenhouse experiment, four wounds were made at approximately 15-cm intervals along each stem of 10 trees and the four treatments were distributed on each stem. The treatments were distributed so that each position along the stem was represented at least twice among the 10 trees for each wound treatment. In both experiments, fresh wounds inoculated and wrapped for 1 day were also included.

Effect of reinjury on resistance of aged bark wounds. Six wounds were made with a 6-mm-diameter cork borer on each of four potted Carmel almond trees in the lathhouse. All wounds were wrapped to exclude insects and allowed to age for 14 days. Three wounds on each tree were then rewounded with a 7-mm-diameter cork borer to excise developing periderm. All wounds were inoculated with a suspension containing 10^6 spores per milliliter, then wrapped with Parafilm for 1 day.

RESULTS

Time course of wound resistance development. In all experiments, canker size was significantly smaller as the age of the wound before inoculation increased from 0 to 6 days. From 6 to 10 and 14 days, average canker size did not change significantly. This was observed in trees in the orchard (Figs. 1 and 2A) and in potted trees in the lathhouse (Fig. 3A). In Nonpareil almond trees in the orchard, no cankers developed in the wounds that had aged for 14 days before inoculation in experiments using 1-day postinoculation wraps. The number of wounds infected per sampling time also decreased as the age of the wounds at the time of inoculation increased (Figs. 2B and 3B). Uninoculated wounds did not develop cankers.

In the orchard experiment, the analysis of variance of the interactions indicated there was no significant tree-to-tree variation in canker size ($P = 0.05$). The variation in canker development with respect to compass direction of a branch within a tree (side) was slightly significant ($F = 3.38$, $P = 0.037$) but was relatively minor compared with the high significance of the effect of wound age ($F = 58.01$, $P = 0.0001$). The stepwise logistic regression program was used to derive an estimate of relative susceptibility of different aged wounds. Under the conditions of this experiment, for example, a wound that had aged 2 days was 19 times more likely to develop a canker than a wound that had aged 10 days (Table 1).

Average canker length around inoculated fresh wounds in Nonpareil trees in the greenhouse increased as inoculum concentration increased from 10^2 to 10^6 spores per milliliter, with little or no canker development around wounds inoculated with 10^2 spores per milliliter. The relationship between canker length and inoculum density (ID, spores per milliliter) for these

TABLE 1. Relative risk of infection by *Ceratocystis fimbriata* of different aged Nonpareil almond bark wounds[a]

Age of wound before inoculation (days)	Relative risk of infection compared to 10-day-old wound	Relative risk of infection compared to average of all wounds
0	Infinite	Infinite
2	19	53
6	4	12
10	1	3
14	0	0

[a] A stepwise logistic regression program (12) was used to derive relative wound susceptibility, an estimate of the comparative likelihood of infection of a specific age of wound after inoculation with spores of *C. fimbriata*. Values are based on 23 inoculations for each sampling time.

TABLE 2. Canker development in aged wounds on branches of Nonpareil almond trees inoculated by *Carpophilus hemipterus* contaminated with spores of *Ceratocystis fimbriata*

Age of wound at inoculation (days)	Disease rating[a]
2	2.9 x
4	2.9 x
6	2.3 x
8	1.4 y
10	0.5 z
12	0.3 z

[a] Derived from a scale where 0 = no disease and healthy callus development, 1 = small canker with gummosis and necrosis extending up to 5 mm beyond the wound margin, 2 = gummosis and necrosis extending 5–10 mm beyond the wound margin, and 3 = gummosis and necrosis extending more than 10 mm beyond the wound margin. Each value is the mean of 10 determinations. Values followed by the same letter are not significantly different according to the Mann-Whitney test ($P = 0.05$).

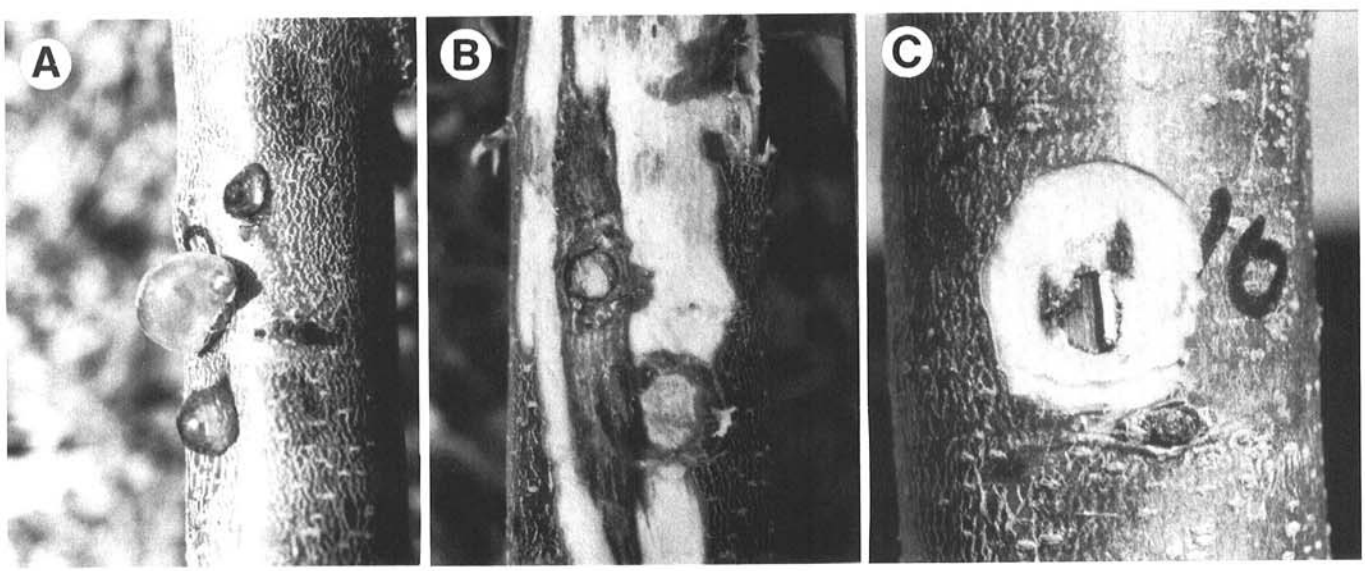

Fig. 1. Canker development in cork borer wounds in Nonpareil almond branches approximately 4 wk after inoculation with *Ceratocystis fimbriata*. **A,** External symptoms of bark canker resulting from inoculation of fresh wound. **B,** Outer periderm excised to show canker development and underlying necrotic tissue. **C,** Appearance of underlying bark tissue after inoculation of 10-day-old wound.

concentrations was described by the regression: canker length (mm) = 8.65 × log (ID) − 17.8 (F = 150.5, P = 0.0001). The analysis of variance indicated the interactions between canker length and stem position and canker length and tree were not significant (P = 0.05).

Inoculation of aged wounds with *Carpophilus hemipterus* contaminated with *C. fimbriata*. The insects efficiently transmitted *C. fimbriata* to bark wounds, with 100% of 2- and 4-day-old wounds developing cankers. The susceptibility of wounds to insect-transmitted inoculum declined during aging until wounds that had aged for 10–12 days before inoculation had little or no canker development (Table 2).

Histological studies. Browning of the cell layers adjacent to the cut edge of almond bark was observed within hours after wounding. By the second day after wounding in all cases, the browning had extended 3–20 cell layers inward from the wound margin, with the widest zone of browning in the cortex (Fig. 4A).

In the wounds examined after aging 4 days, the zone of browning was no deeper than at 2 days, and faint traces of material that stained with phloroglucinol/HCl were observed in the healthy cortex of the bark adjacent to the brown zone in some sections. Six days after wounding, signs of wound periderm development were more apparent, although the periderm was incomplete. Some wounds had a thick layer of three to five hypertrophied cells extending the width of the bark but not connecting to the outer periderm (Fig. 4B). In other wounds examined after aging 6 days, the hypertrophied layer was visible only in patches beginning near the phloem fibers and extending toward the cambium. Callus

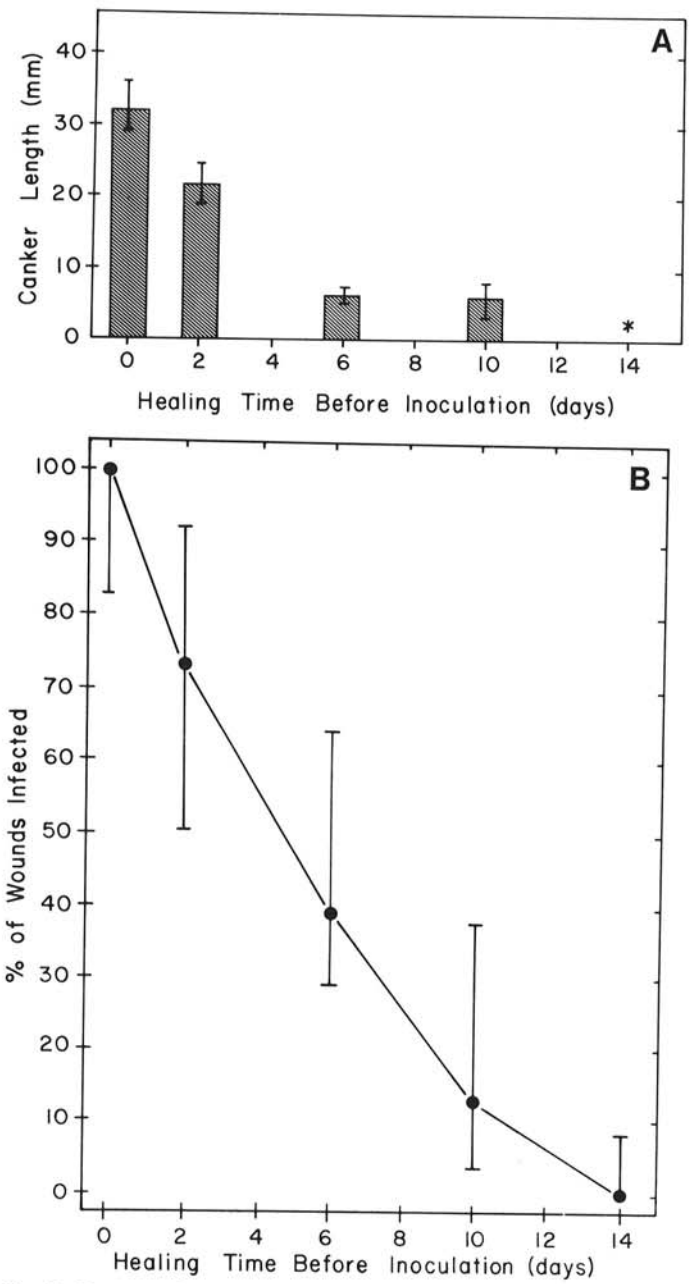

Fig. 2. Ceratocystis canker development in fresh or aged wounds in branches on Nonpareil almond trees in the orchard. **A,** Canker size 4 wk after inoculation. Standard errors of the means are indicated; the asterisk signifies no cankers developed. **B,** Percentage of wounds infected. Confidence intervals for a 0.05 significance level are indicated (n = 23 for each sampling time).

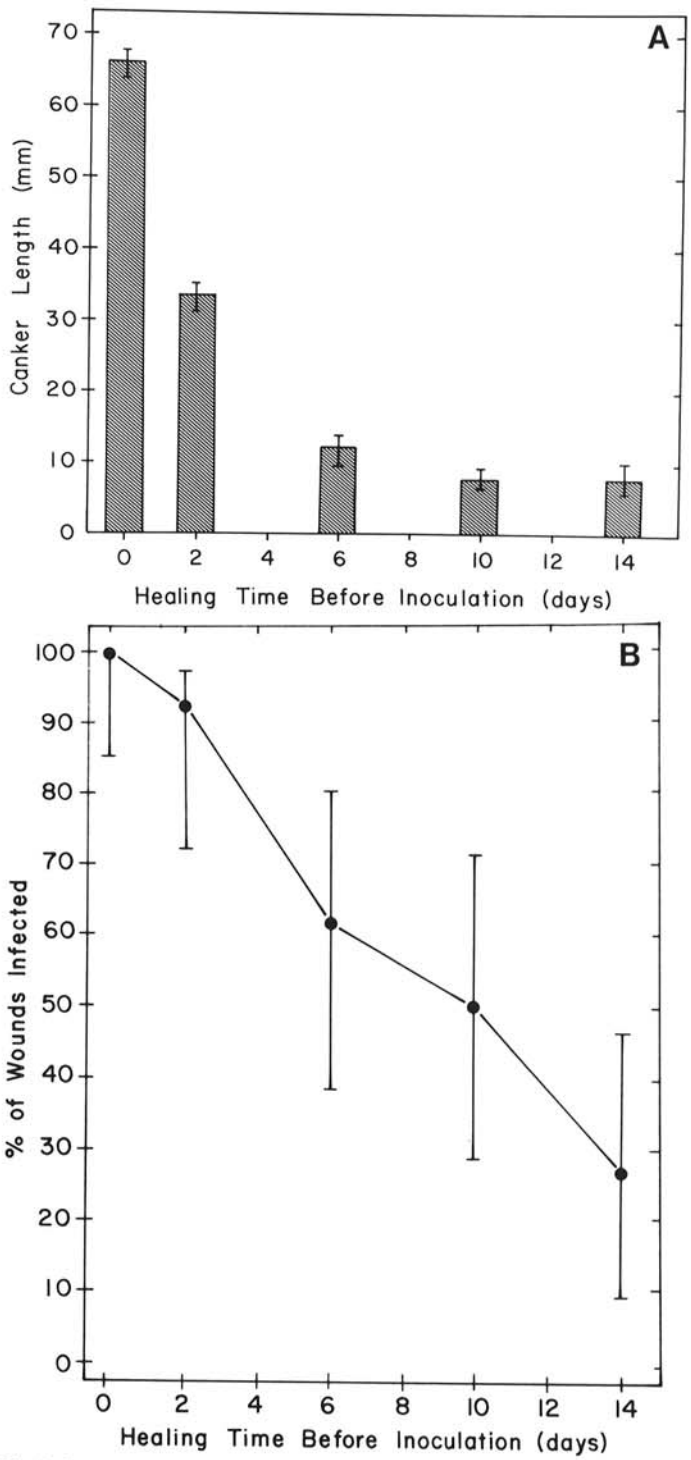

Fig. 3. Ceratocystis canker development in fresh or aged wounds in stems of potted Carmel almond trees in the lathhouse. **A,** Canker size 4 wk after inoculation. Standard errors of the means are indicated. **B,** Percentage of wounds infected. Confidence intervals for a 0.05 significance level are indicated (n = 26 for each sampling time).

developing from the cambium usually had a peridermlike layer three cells thick that connected with the hypertrophied layer. Cell walls in the hypertrophied layer stained with phloroglucinol/HCl (Fig. 4C) and Sudan black B (Fig. 4D). Phloroglucinol/HCl-reactive material also accumulated in the walls of the browned cells adjacent to the hypertrophied layer close to the wound periderm and in crushed tissues in the same area. In most of the wounds aged for 8–10 days, organized cell files indicative of a developing wound periderm were apparent (Fig. 4D). Some wounds had a nearly complete wound periderm after 10 days of aging, whereas others of the same age still lacked hypertrophied cells in the cortex near the outer periderm. In all sections examined, wound periderm

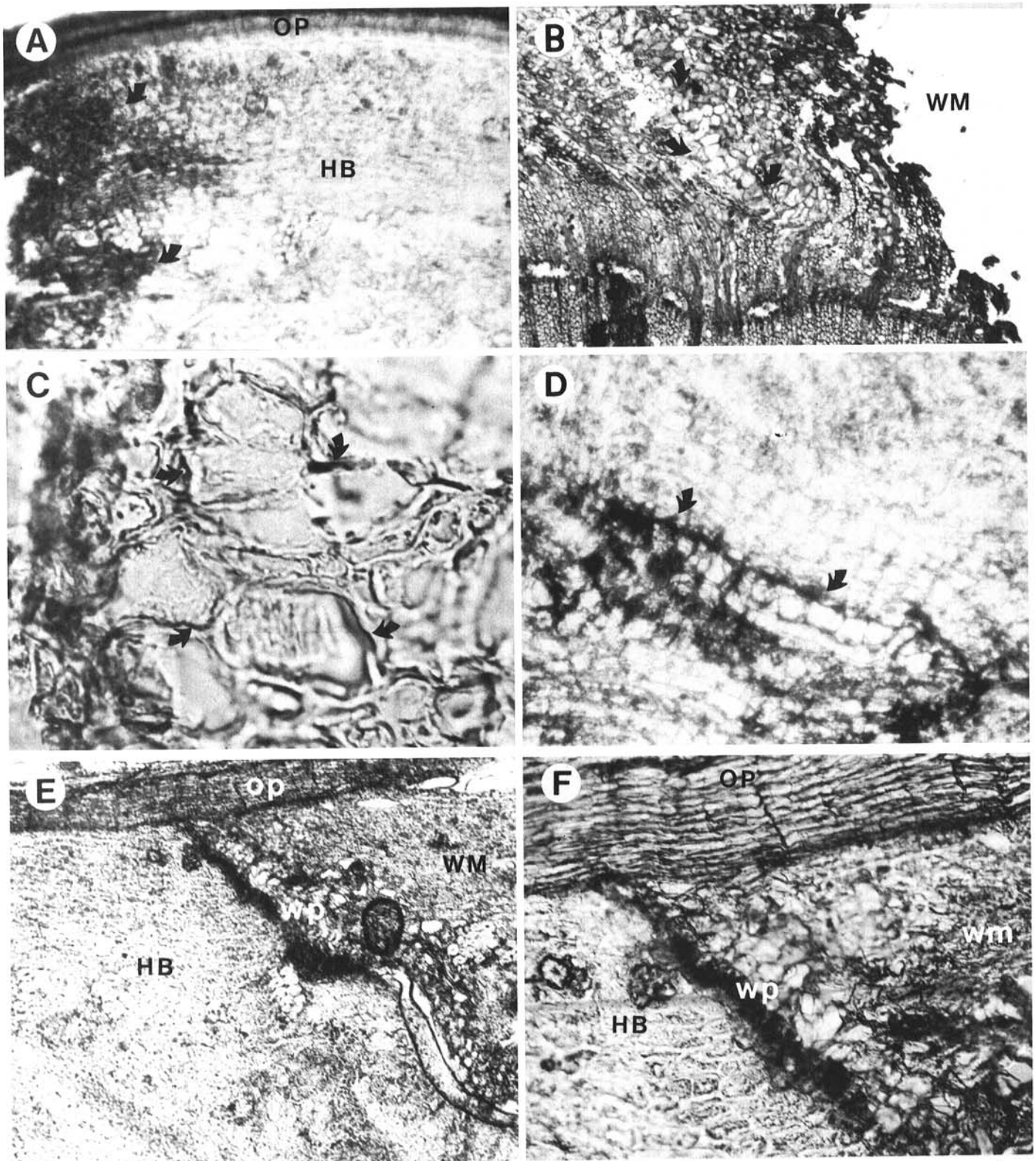

Fig. 4. Transverse sections from the lateral margins of aged bark wounds from Nonpareil almond branches. **A,** Aged 2 days, unstained. Wound margin on left, dieback and browning (arrows), healthy bark (HB), and outer periderm (OP). ×168. **B,** Aged 6 days, stained with toluidine blue. Wound margin (WM), zone of hypertrophied cells (arrows). ×168. **C,** Aged 6 days, stained with phloroglucinol/HCl. Areas of lignified cell walls (arrows). ×1,480. **D,** Aged 8 days, stained with Sudan black B. Cell files and area of extensive suberin deposition (arrows). ×500. **E,** Aged 14 days, stained with Sudan black B. Outer periderm (OP), wound periderm (WP), wound margin (WM), healthy bark (HB). ×210. **F,** Higher magnification of E, showing wound periderm at junction with outer periderm. ×560.

development appeared slower where the wound edges crossed the long axis of the branch than where the wound edges paralleled the vascular flow. By 14 days after wounding, all wounds examined had a well-developed wound periderm connected to the outer periderm by one to five cell layers that extended over the callus developing from the cambial region (Figs. 4E and 4F). The wound periderm was interrupted along the fiber bundles. The wound-induced materials that reacted strongly with phloroglucinol/HCl reacted weakly or not at all with the Maule reagent. Cells in the wound periderm reacted with phloroglucinol/HCl and Sudan black B after extraction of the tissue with methanol.

Effect of Parafilm on resistance of aged wounds. Cankers developed in 50% (11 of 22) of the wounds aged for 14 days, then wrapped with Parafilm for 2 days after inoculation, and in 80% (8 of 10) of those wrapped for 3 days. Infected wounds had small cankers with an average length of 2 mm (2-day wrap) and 5 mm (3-day wrap) by 4 wk after inoculation. No cankers developed in any of the same age wounds wrapped with Parafilm for 1 day after inoculation. All wounds that were inoculated immediately after wounding became infected, with an average canker length of 34 ± 2 mm.

Effect of wound type on resistance to canker development. Analysis by a stepwise logistic regression program showed no significant differences ($P = 0.05$) in the infection rates between methods of wounding over the time course of the experiment (Table 3).

Effect of reinjury on resistance of aged bark wounds. All wounds that were rewounded and immediately inoculated after 14 days of aging became infected, with an average canker size of 35 ± 4 mm 4 wk after inoculation. Eleven of the 12 wounds inoculated after 14 days but not rewounded were not infected; a 3-mm canker developed in the remaining wound.

DISCUSSION

Fewer and smaller cankers were observed with increase in time between wounding and inoculation. Infection rate declined from 100% in fresh wounds to 0% in all Nonpareil orchard trees when wounds aged 14 days before inoculation and the Parafilm was removed within 1 day after inoculation. These changes were related to changes observed histochemically in the wound over time. By 14 days after wounding, all wounds had a suberized and lignified wound periderm several cell layers thick and extending from the outer periderm to the cambium. The most rapid decline in susceptibility appeared to occur during the first 6 days after wounding, however. The principal changes visible histochemically by 6 days after wounding were the appearance of hypertrophied cells with walls that reacted to both Sudan black B and phloroglucinol/HCl, indicating the presence of suberin and lignin. The deposits that yielded a diffuse phloroglucinol/HCl-positive reaction in the area of browning did not react to the Maule test. This indicates that these deposits are deficient in syringyl groups that normally characterize angiosperm lignin. Recently, Doster and Bostock (13) reported that significant increases in lignin accumulation in margins of almond bark wounds could be detected using thioglycolic acid as soon as 1–2 days after wounding, with a substantial increase by 6 days. These findings confirm that lignin is formed very soon after wounding and during the period of most rapid decline in wound susceptibility. In wounds aged for 6–14 days before inoculation, the cankers that developed were not significantly different in size, altbough the infection rate continued to decline (Figs. 2 and 3). Both resistance to infection and inhibition of canker expansion were abolished by excision of the tissue immediately surrounding the wound, indicating these to be localized effects. These findings are consistent with the hypothesis that the age-related resistance to infection by *C. fimbriata* in almond bark injuries results at least in part from the formation of the lignosuberized tissues of the developing wound periderm. Analysis of the orchard experiments suggests that protective measures applied to fresh bark wounds should remain effective for 14 days to prevent infection without hindering formation of the defensive barriers. Treatments applied after this period would be unlikely to provide an additional benefit for the grower.

TABLE 3. Effect of wound type on development of resistance to *Ceratocystis fimbriata* on potted Carmel almond trees

Age of wound before inoculation (days)	Percentage of wounds infected[a]	
	Cork borer wound	Crushed wound
14	20	26
10	70	26
6	60	80
2	100	93
0	100	100

[a] Data were obtained from two separate experiments with 10 and 15 inoculations per sampling time for cork borer and crushed wounds, respectively. A stepwise logistic regression analysis (12) indicated that wound type did not significantly affect infection rate over the time course of the experiment ($P = 0.05$).

Leaving Parafilm on the wound for more than 1 day increased the infection rate and permitted the development of small cankers in aged wounds, suggesting that the microenvironment of the wound significantly influences its resistance. Although we did not investigate the basis for this, a likely explanation is an increase in the effectiveness of the inoculum resulting from a more favorable environment for germination and growth of *C. fimbriata*. This notion is consistent with our observation that canker size depends on inoculum level. However, a direct effect on the integrity of the wound periderm and associated anatomical barriers should not be excluded. Suberin formation in wounded potato tuber is most rapid at high relative humidities (>70%) but can be inhibited by low relative humidity or by excess moisture (16,29). If postinfection host responses such as induced lignin and suberin also contribute to the age-related resistance, it would be interesting to determine if these processes are compromised by excessive moisture in the wound environment.

The ability of trees to compartmentalize bark wounds through periderm formation has been related to resistance to fungal pathogens in several interactions (3–7,14,22,27). Our results suggest that *C. fimbriata* can breach the defensive barriers of partially closed wounds or weak areas of the developing wound periderm. Areas in histological sections where the wound periderm appeared thin were the junctions of the wound periderm with the vascular cambium and outer periderm and along fiber bundles. This has also been observed in wound periderm studies of bark in *Prunus persica* (L.) Batsch (3,4), *Castanea dentata* (Marsh.) Borkh. (14), and *Malus communis* DC. (27). During canker expansion, *Cytospora* spp., *Endothia parasitica* (Murr.) P. J. & H. W. And., and *Valsa ceratosperma* (Tode ex Fr.) have been observed to form mycelial aggregates that appear to penetrate these thin areas in developing periderm. Although similar structures have not been reported with *C. fimbriata*, earlier histopathological studies by DeVay et al (11) revealed that the fungus primarily advances along the cork cambium and newly formed phelloderm and along the cambium and newly formed xylem and phloem. In histological sections, these regions of the wound periderm appeared thinner than other regions.

The results of our study indicate that the changes progressing in almond bark after wounding are similar to those occurring in bark wounds of other *Prunus* spp. and tree species (3–7,14,21,24,27). The development of significant resistance in almond to *C. fimbriata* within 6 days of wounding is comparable to that reported in *Gleditsia triacanthos* L. to *Nectria cinnabarina* (Tode:Fr.) Fr. (1) and in *P. persica* to *Cytospora* spp. (7), in which a significant degree of resistance developed within 7 days of wounding. Although Biggs and Miles (7), using a photometric technique, found that the levels of both lignin and suberin formed in aged wounds were correlated with resistance to Cytospora canker development in peach clones, the correlation with suberization was highly significant. The deposition of lignin and suberin or other aliphatic components would contribute to the impervious character of aged wounds (21,28). The development and application of sensitive biochemical methods will complement

histological procedures for quantifying the aromatic and aliphatic polymers formed during the wound response (13). Such methods will facilitate comparative studies of tree bark responses to wounding and pathogens and may aid selection of cultivars with rapid wound closure rates.

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