Comparative Anatomy and Host Response of Two Peach Cultivars Inoculated with *Leucostoma cincta* and *L. persoonii*

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ABSTRACT

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Stems of 2-yr-old peach cultivars, Candor (susceptible) and Sunhaven (relatively resistant), were inoculated in the greenhouse with mycelium of either *Leucostoma cincta* or *L. persoonii*, and sampled for histological study and pathogen reisolation at 14, 28, and 56 days postinoculation. Additional plants were wounded and sampled at 6, 8, 10, 14, 28, and 56 days postwounding. The cultivar Candor was initially colonized by both fungi more extensively than the cultivar Sunhaven. With time, cultivar differences were less important than differences in fungal virulence. Discontinuities or points of weakness in new periderms were detected in both wounded and inoculated samples. Sites of periderm circumvention and ingress by fungi into previously healthy tissues included the juncture of wound periderm with healthy periderm and with primary phloem fibers, and the nonsuberized centripetal surface of juvenile callus tissue. The expansion and coalescing of gum ducts caused the rupture of suberized xylem parenchyma boundaries, thus providing entry sites for the pathogens. Suberization of extant xylem ray parenchyma internal to wounds was observed in all wounded samples but in only one of nine inoculated samples. The results suggested that peach cultivars varied in their ability to establish anatomical barriers in bark and xylem and that *Leucostoma* spp. inhibited suberin deposition in xylem barrier zones.

Peach canker, caused by *Leucostoma cincta* (Pers. & Fr.) Hohn., (anamorph = *Cytospora cincta* (Pers.) Fr.) and *L. persoonii* (Nits.) Höhn. (anamorph = *C. leucostoma* (Pers.) Fr.) is a major limiting factor in peach production in the northern portions of the North American deciduous tree fruit region. The disease, manifested as perennial cankers on trunks, scaffold limbs, and branches, has proven difficult to control. Certain cultural practices, however, can reduce its incidence (5). All of the currently grown peach cultivars are susceptible to the pathogens (7,17).

The pathological anatomy of 1-yr-old diseased peach shoots has been described for both fungi (19,23). Goring (8) described *L. persoonii* infection and canker formation on sweet cherry. Hampson and Sinclair (9) described xylem dysfunction induced by *L. persoonii* on peach, and Helton and Randall (10) examined cambial gummosis of Italian prune caused by *L. cincta*.

Evidence for colonization of xylem tissues by *L. cincta* and *L. persoonii* in advance of visibly necrotic bark tissues is conflicting. Hildebrand (11) found both fungi only within the boundaries of the externally visible cankered zone, although Willison (21) had earlier stated that the fungi may extend beyond these limits. Wisniewski et al (23) found *L. persoonii* to be a very poor invader of xylem tissues, although the fungus was present up to 1 cm in advance of visible bark necrosis in the gum duct region and subjacent xylem. Tekauz and Patrick (19) described colonization patterns in peach twigs infected with *L. cincta* and *L. persoonii* and reported that pathogenesis was similar for both fungi. They concluded that if larger limbs were colonized in patterns similar to 1-yr-old shoots, then the peach canker fungi could be classified as sapwood parasites sensu Hubbes (12).

Knowledge of the patterns of tree tissue colonization by fungal pathogens is useful in making recommendations for pruning or canker surgery. In addition, pathogen tissue preferences, if they exist, could provide clues regarding disease resistance and the relationship, if any, between xylem and bark response to fungal invasion (3). The purpose of this study was to compare tissue colonization patterns of *L. cincta* and *L. persoonii* under similar conditions and to examine nonspecific host responses associated with mechanical wounding and delimitation of pathogen colonization in both xylem and bark.

MATERIALS AND METHODS

Two-year-old nursery-grown peach trees *Prunus persica* (L.) Batsch cv. 'Sunhaven' and 'Candor' were dug in November 1983 and stored commercially (Mori Nurseries, Ltd., Virgil, Ontario, Canada) until the first week of January 1984. Forty trees of each cultivar were transplanted into a soil mix (20:9:6, Vineland siltloam, peat, sand) in 30-cm-diameter clay pots, pruned to provide 45 cm of clear stem and 10 growing shoots per plant. Average length of the new shoots was approximately 9 cm at the beginning of the experiment. Cultivars Candor and Sunhaven are considered highly susceptible and relatively resistant, respectively, to the peach canker fungi (R. E. C. Layne, *personal communication*).

On 1 March 1984, before inoculation, all plants were sponged with distilled water to remove extraneous soil from the main stem. Eighteen plants of each variety were inoculated 15 cm above the graft union by treating the bark with 70% ethanol and mechanically wounding the stem using a sharpened sterile 7-mm-diameter cork borer to remove, down to the xylem, a portion of bark. Care was taken not to physically injure underlying xylem tissues. A 7-mm malt agar disk of either *L. cincta* or *L. persoonii* was placed into the wounded area of nine plants of each cultivar. Inoculum was secured for seven days with cellophane tape. Preparation of the inoculum was as described previously (2). Control trees were wounded in an identical manner and sterile malt agar was used instead of mycelium.

Samples for histological examination were taken at 0, 14, 28, and 56 days postinoculation. Control trees were sampled similarly and, in addition, at 6, 8, and 10 days postwounding to examine cultivar differences in rate of phellogen regeneration and xylem response to wounding. Three plants per cultivar were sampled at each time by removing a stem segment with the entire wound or infection plus 4 cm of nonaffected tissues on either side of the visible canker or wound margin. All stem segments were then halved longitudinally through the wound or inoculation site with a

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sterile razor blade. One half was divided into 1 cm lengths, fixed in formalin-acetic acid-50% ethanol (5:5:90) (FAA) (13), and paraffin embedded as described previously (2). The other half was subjected to isolations at 1-cm intervals from the pith, 2-yr-old zylem, 1-yr-old xylem, current season's xylem, and bark tissue.

Wounded tissue. Bark and xylem reaction zone tissues, which formed after mechanical wounding, were tested histochemically for lignin (phloroglucinol-HCl reaction) (13) and suberin (residual autofluorescence test) (1) to determine if wound response could be related to cultivar differences in disease susceptibility (4). Microtome sections, 8 μm thick, were assessed quantitatively using a Leitz MPV compact microscope photometer to measure the intensity of the histochemical reactions. Phloroglucinol-HCl-reactive tissues in the bark cortical region and in the 1983 xylem (approximately 500–800 μm internal to the wound surface) were measured using percentage of transmission at 540 nm. In the cortex, the reaction was measured at 250× magnification using a 200 μm diameter photometer aperture. The xylem reaction was measured at 100× magnification with a 500 × 950 μm rectangular photometer aperture. The photometer was adjusted to 100% transmission using nonwounded tissues.

After measurements of percentage of transmission for the lignin reaction, phloroglucinol-HCl-treated tissues were examined further using ultraviolet excitation (Leitz HBO 100W mercury lamp, stabilized power supply, fluorescence filter block A) to detect suberin (1). Fluorescence intensity of suberin in the bark cortex was measured at 250× magnification using a 200 μm diameter photometer aperture. Xylem ray parenchyma suberin was measured at 630× magnification with an 80 μm diameter photometer aperture. Fluorescence intensity of nonwounded tissue was used to adjust the photometer to zero before examination of wounded tissues. Five measurements were made on each of three plants per cultivar at each of the seven sampling times to determine both percentage of transmission (lignin) and fluorescence intensity (suberin). Each plant was assessed in both the longitudinal and transverse orientation and the observations pooled. Photometric data were analyzed at each postwounding time with unpaired Student's t tests (equal variances assumed) to determine cultivar differences.

In addition, two 2 × 2 chi-square analyses were used to determine if there were cultivar differences in whether lignin (test 1) or suberin (test 2) was preferentially deposited in xylem or bark. At each of the six postwounding times, the photometric data for lignin or suberin were averaged and the cultivar with the higher mean was assigned a value of 1 (versus 0 for the lower mean). The assigned values for bark and xylem were summed by cultivar for the six postwounding times and the adjusted chi-square value was determined.

Inoculated tissues. In inoculated tissues, necrophylactic (wound) periderm (14) development was determined by counting the number of new phellem cells at the junction of the new periderm with the original periderm. Data were analyzed using a factorial analysis of variance (cultivar, fungus species, postinoculation time, and tissue orientation) using a completely randomized design.

Canker length and horizontal width (percentage of stem circumference colonized) were monitored weekly for 8 wk. The initial response of the two cultivars to fungal inoculation was evaluated at 7 days postinoculation using a two-factor (cultivar and fungus species) analysis of variance and a completely randomized design. In addition, the weekly data on canker length and width were used to determine colonization rates using simple linear regression. Regression parameters were tested for homogeneity using an F test in an analysis of covariance. Paired Student's t tests were used to test for homogeneity of slopes when the analysis of covariance yielded a significant F value ($P = 0.05$ or less).

RESULTS

Colonization, canker enlargement, and reisolation. At 7 days postinoculation, the cultivar Candor had longer cankers than the cultivar Sunhaven for both *L. persoonii* and *L. cincta* ($F = 3.54$, $P = 0.07$)(Fig. 1). Fungus species and the interaction between cultivar and fungus species did not influence initial canker length. Colonization by *L. persoonii* was more extensive around the stem in both cultivars relative to *L. cincta* ($F = 17.5$, $P = 0.01$)(Fig. 2 at 7 days). Cultivar and the interaction between cultivar and fungus species did not influence canker horizontal width.

Regression analyses of the relationship between postinoculation time and canker length showed that Candor was colonized by *L. persoonii* more aggressively than the other cultivar/pathogen combinations ($F = 5.71$, $P = 0.01$). Sunhaven was colonized by *L. persoonii* more aggressively than either cultivar inoculated with *L. cincta* (Fig. 1). In both cultivars inoculated with *L. persoonii*, there was a second rapid increase in canker length and colonization rate between days 28 and 35.

Analyses of canker width over time showed that *L. persoonii* colonized both cultivars faster than L. cincta ($F = 7.78$, $P = 0.01$) (Fig. 2). There were no cultivar differences in canker width over

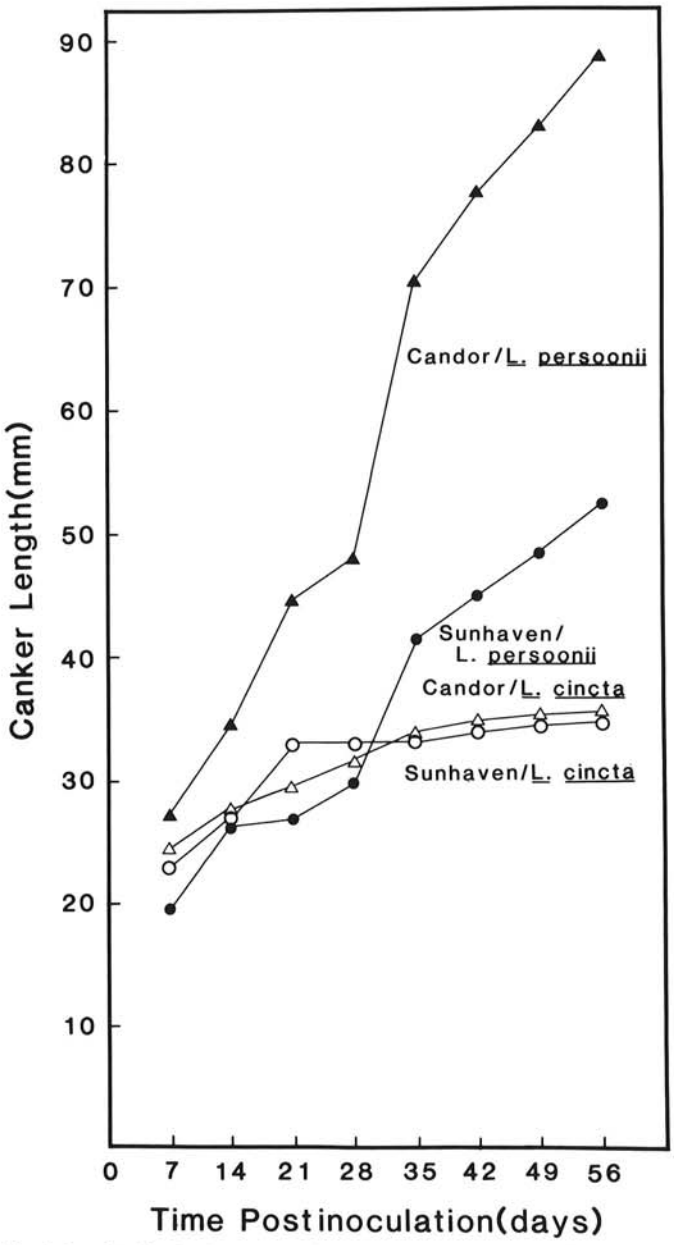


Fig. 1. Longitudinal enlargement of cankers on peach cultivars Candor and Sunhaven inoculated with *Leucostoma cincta* and *L. persoonii*. Trees inoculated in the greenhouse and cankers measured weekly for 8 wk (number of trees measured per treatment per week decreased because of random destructive sampling, i.e., weeks 1 and 2, n = 9; weeks 3 and 4, n = 6; weeks 5–8, n = 3).

time. Sharp increases in canker width were observed between days 28 and 35 for both cultivars inoculated with *L. persoonii*.

The extent of fungal invasion, as determined by isolation, varied with time and the particular cultivar/pathogen combination. At 14 days postinoculation, all cultivar/pathogen combinations showed approximately equal invasion of bark and the small amount (about 200 μm in thickness) of extant 1984 xylem. Fungi were not recovered from 1983 or 1982 xylem or pith.

By day 28, Candor inoculated with *L. persoonii* yielded this fungus from 1983 and 1982 xylem both under the point of inoculation and 1 cm proximally and distally from this point. Pathogen colonization in extant 1984 xylem was similar to that in bark. By day 56, *L. persoonii* could be isolated from all tissues including the pith. Colonization in 1982 xylem and pith exceeded the visible canker margin in the bark by 4 cm proximally and 1 cm distally.

The extent of colonization in Sunhaven inoculated with *L. persoonii*, and in Candor inoculated with *L. cincta*, was similar. Pathogens from both cultivars were isolated once in 1982 xylem directly under the inoculation point. In 1983 xylem and extant 1984 xylem, *L. persoonii* was equally advanced relative to bark symptoms in Sunhaven. In Candor, *L. cincta* was in xylem in advance of visible bark symptoms 3 cm proximally and 1 cm distally.

Isolations of *L. cincta* from Sunhaven were limited to bark and extant 1984 xylem. Xylem colonization was ahead of that in bark by 1 cm proximal to the visible canker margin but equal to that in bark distally.

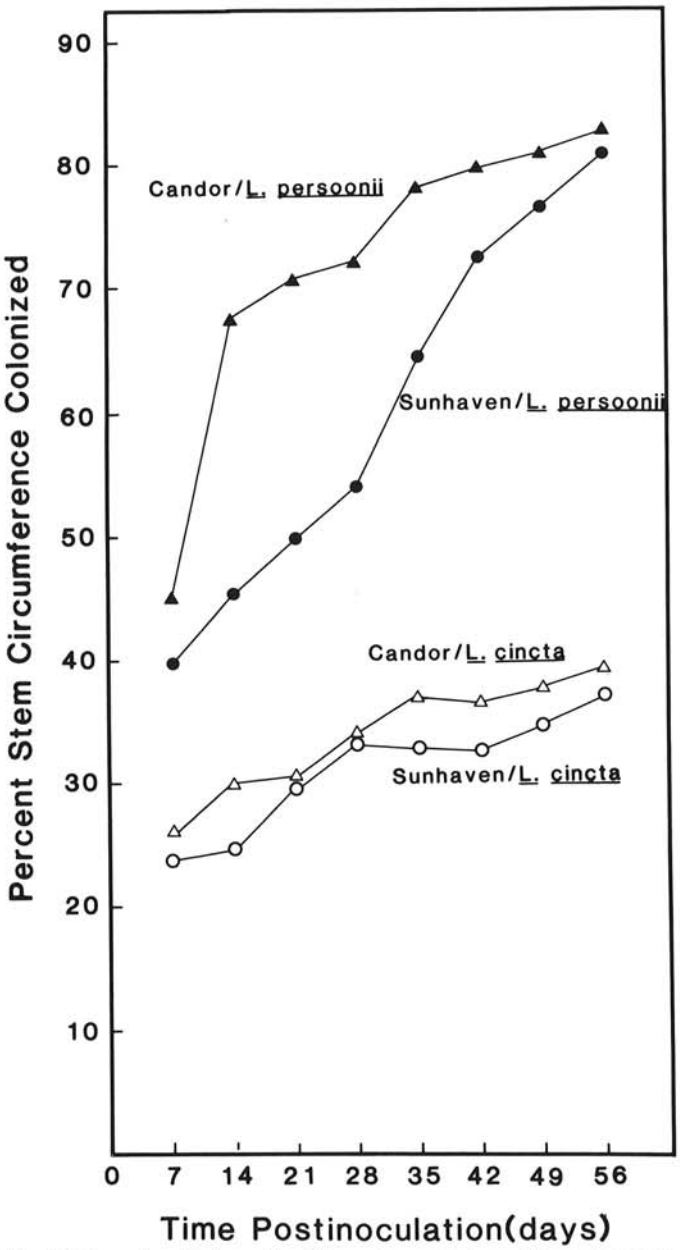


Fig. 2. Circumferential growth of *Leucostoma cincta* and *L. persoonii* on peach cultivars Candor and Sunhaven after inoculation. Trees inoculated in the greenhouse and cankers measured weekly for 8 wk (number of trees measured per treatment per week decreased because of random destructive sampling, i.e., weeks 1 and 2, n = 9; weeks 3 and 4, n = 6; weeks 5–8, n = 3.)

Quantitation of lignin and suberin in wounded and inoculated stems. The location and pattern of suberin deposition around wounds or cankers was determined for each cultivar or cultivar/pathogen combination. Suberized cells in wounded and infected bark were of two basic types. Primary suberization occurred in tissues that were present in the wound reaction zone or in advance of colonization. The tissue was comprised of dedifferentiated cells with lignified walls lined with lamellar suberin. Cell division was not involved in forming these cells. Primary suberization was limited to the impervious boundary zone of the bark in the inner periderm, cortex, and primary and secondary phloem (Figs. 4 and 5). Secondary suberization in the bark occurred after cell division, resulting in generation of new cork cambium and its suberized derivatives. Secondary suberized cells were part of the necrophylactic periderm formed in the inner exophylactic periderm, cortex, primary phloem, and secondary phloem (Figs. 4 and 5).

Xylem tissues also became suberized in response to wounding or infection. Suberin was observed in ray parenchyma cells of 1983 or 1984 xylem present at the time of wounding or inoculation (Figs. 6–9) and in 1984 xylem formed after inoculation and at a distance from the inoculation point. In the latter case, suberization was limited to xylem parenchyma in the gum duct zone (Figs. 10 and 11).

The number of necrophylactic phellem cells at the junction of the necrophylactic and exophylactic periderms for the cultivar/pathogen combinations is presented in Figure 3.

The number of necrophylactic phellem cells was significantly less for Candor in the longitudinal orientation relative to the other cultivar × orientation combinations ($F = 5.60$, $P = 0.05$) (Fig. 3). At 56 days postinoculation, tissues colonized by *L. cincta* possessed significantly more necrophylactic phellem relative to the other fungus species × time combinations ($F = 5.53$, $P = 0.05$). Fungus species alone accounted for the greatest amount of the observed variation in necrophylactic phellem cell number (29.3%), followed by the cultivar × orientation interaction (14.1%). Trees inoculated

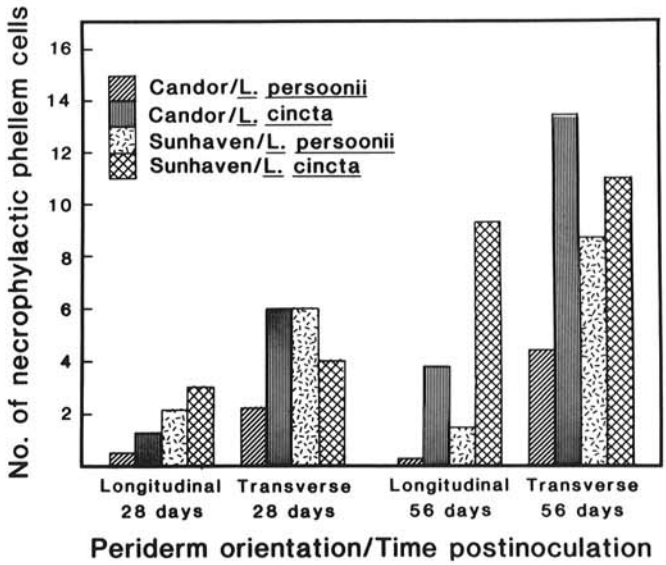
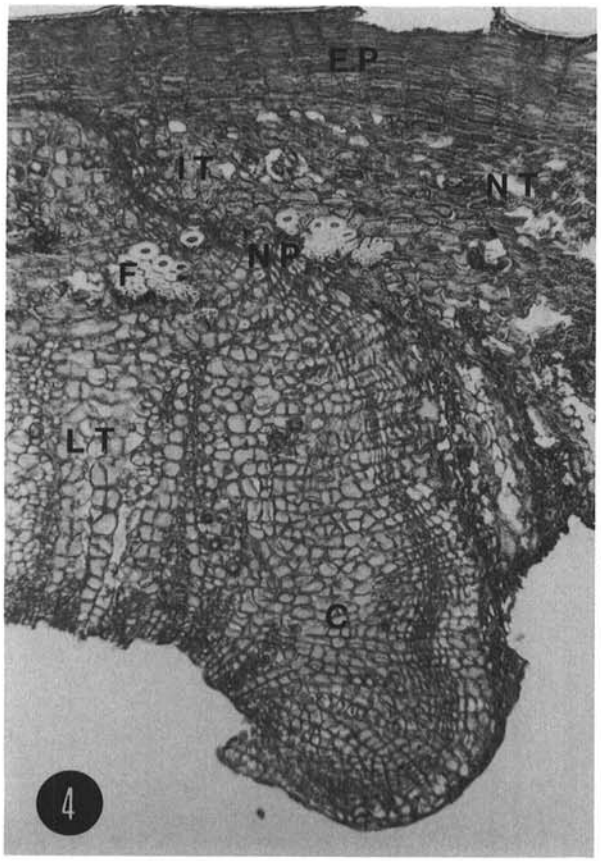
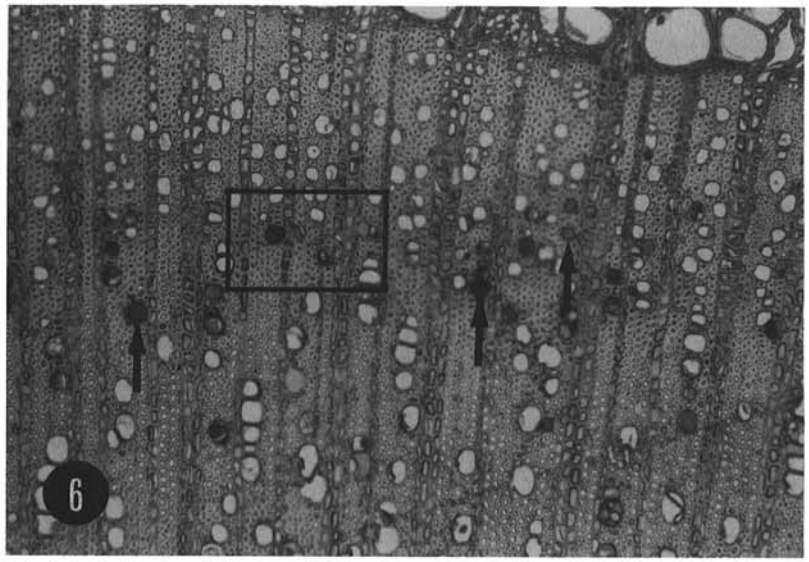
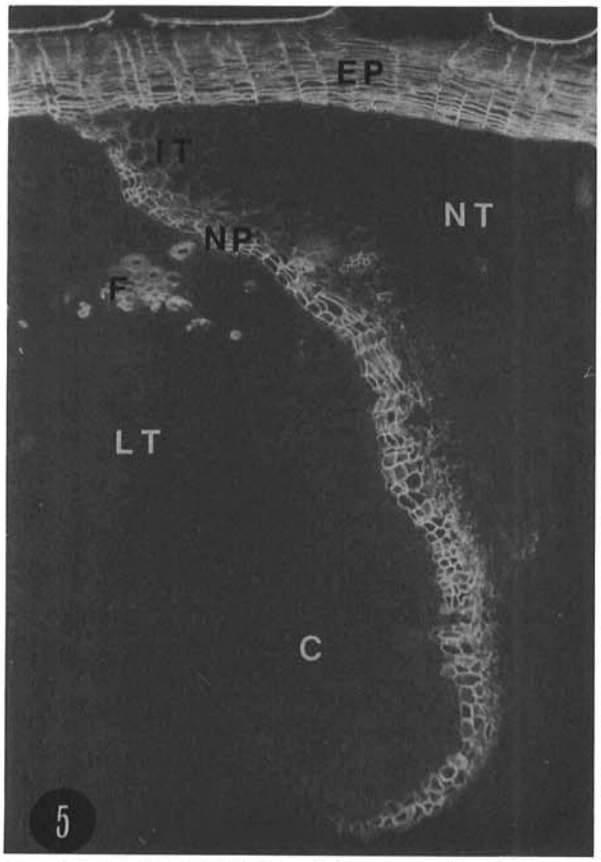
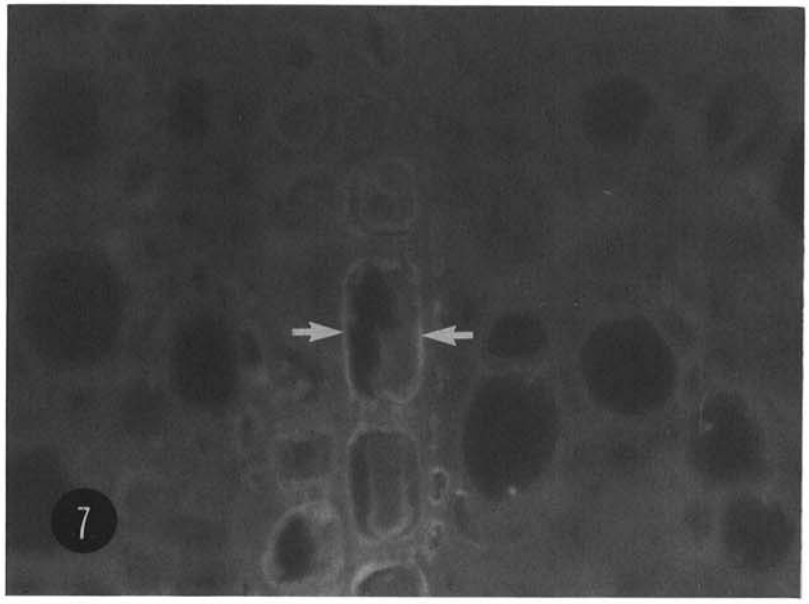


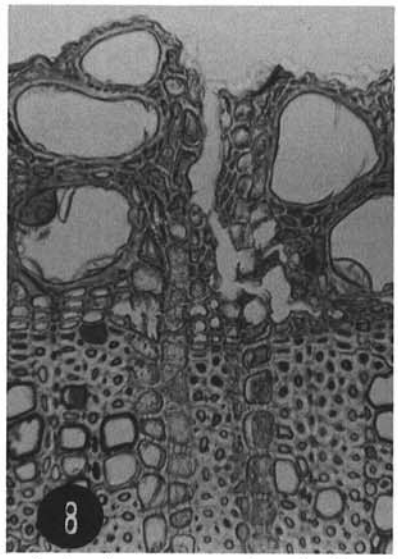
Fig. 3. Thickness in number of cells of the necrophylactic periderm located at longitudinal and transverse canker margins of peach cultivars Sunhaven and Candor inoculated with *Leucostoma cintca* and *L. persoonii*. Data collected from three plants of each cultivar/pathogen combination.

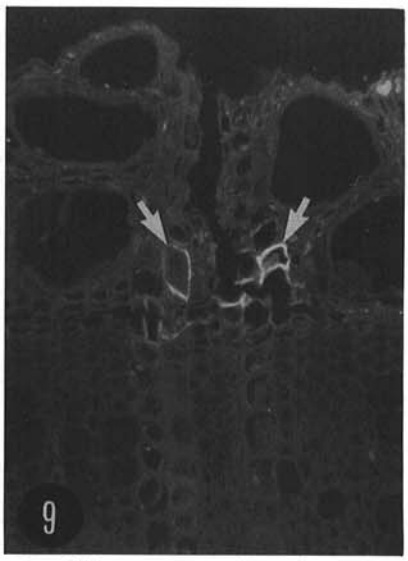




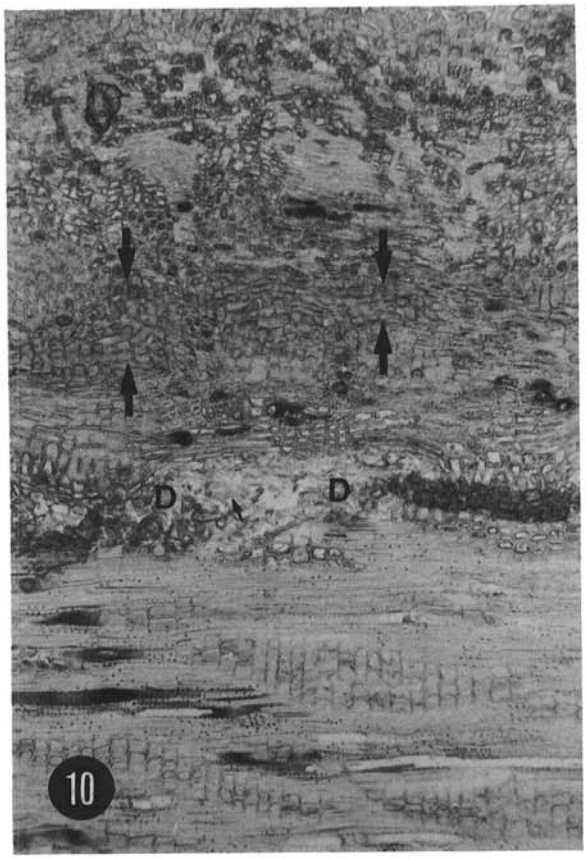


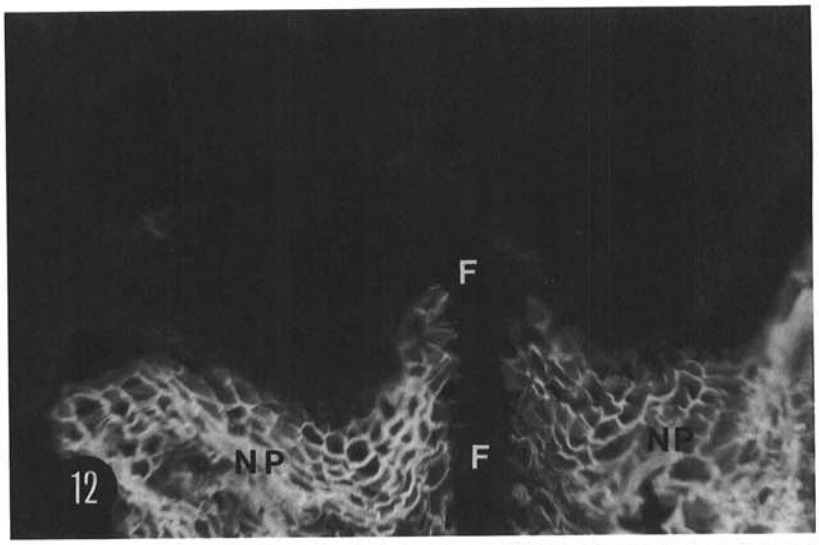


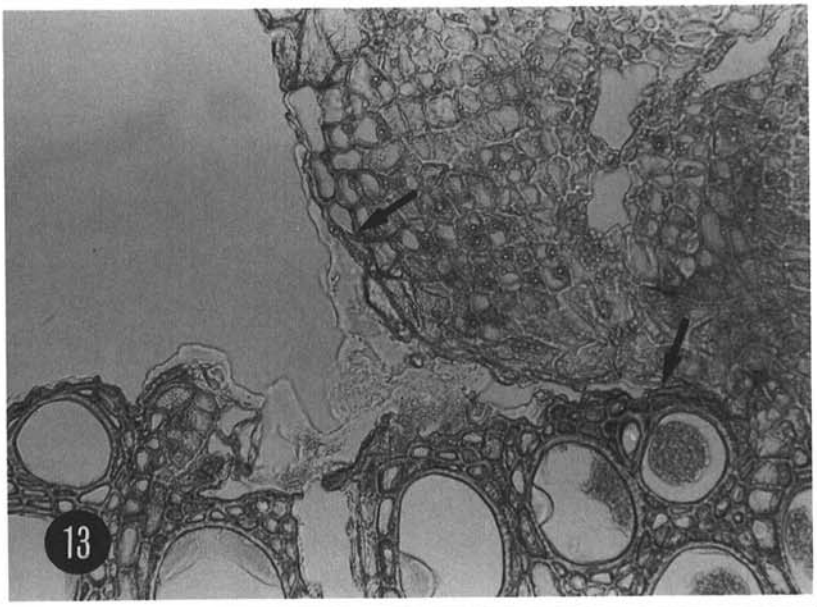


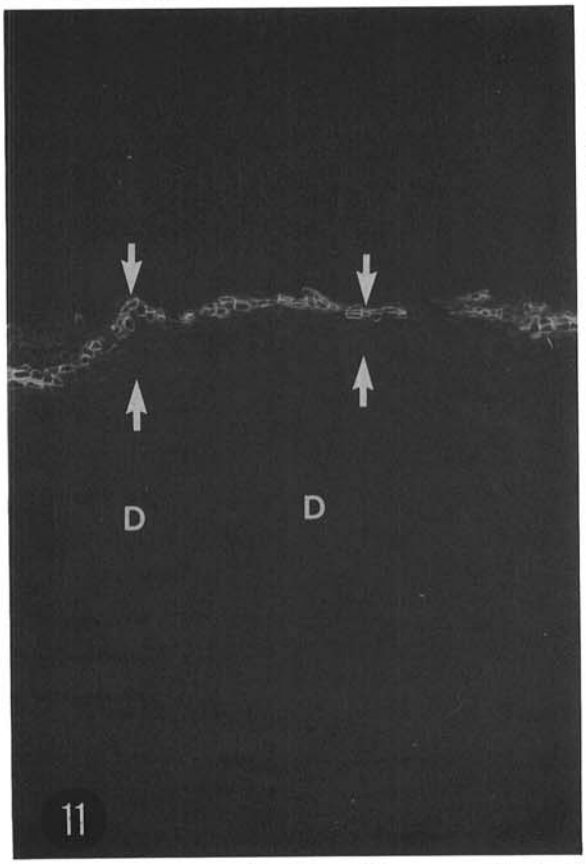


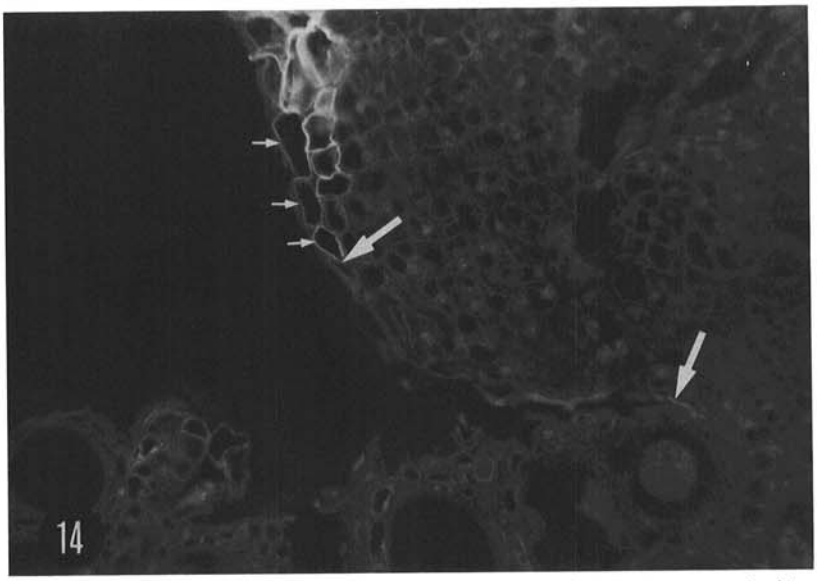
Figs. 4–9. 4 and 5, Bright field and fluorescence illumination, respectively, of transverse section of cultivar Candor, 28 days postwounding stained with phosphine GN showing necrophylactic periderm (NP) formed internal to a ligno-suberized impervious boundary (IT). Code: necrotic tissue (NT), living tissue (LT), exophylactic periderm (EP), primary phloem fibers (F), callus (C). 256×. **6,** Transverse section of Candor xylem tissue located immediately internal to the site of mechanical wounding and stained with phloroglucinol HCl. Note the dark deposits of phloroglucinol chromophore in 1983 xylem vessels and tracheids (arrows). 256×. **7,** Higher magnification and fluorescence illumination from boxed area in Fig. 6 showing residual autofluorescence due to suberin in 1983 xylem ray parenchyma. 800×. **8 and 9,** Transverse section of Candor xylem tissue at the surface of a 14-day-old mechanical wound treated with phloroglucinol HCl and examined with fluorescence optics. Note residual autofluorescence due to suberin in 1984 xylem ray parenchyma (arrows). 800×.











Figs. 10–14. 10 and 11, Bright field and fluorescence illumination, respectively, of longitudinal sections of Candor inoculated with *L. cincta* treated with phloroglucinol HCl showing the inner bark (top), 1984 xylem produced post-inoculation (middle), a colonized gum duct (D) with fungal hyphae (arrows), and xylem present at the time of inoculation (bottom). Note the dark phloroglucinol chromophore in xylem vessels and pits. Suberized cells (Fig. 11, arrows) in 1984 xylem appear to separate the gum duct region from more recently derived xylem tissue. 256×. **12,** Longitudinal section of phloroglucinol HCl-treated Sunhaven bark inoculated with *L. cincta* and showing residual autofluorescence of suberized necrophylactic periderm (NP) adjacent to primary phloem fibers (F). This region is often the site of mycelial wedge advancement through bark tissues. 800×. **13 and 14,** Bright field and fluorescence illumination of wounded phloroglucinol HCl-treated transverse sections of Sunhaven callus tissue and subjacent xylem 14 days postwounding showing the nonsuberized centripetal region of the new callus (between large arrows). In Fig. 14 note the residual autofluorescence due to suberin (small arrows). 800×.

with *L. cincta* exhibited a necrophylactic periderm with nearly twice the number of cells as those inoculated with *L. persoonii* ($\bar{x}$ = 6.16 and 3.18, respectively, $F = 11.63$, $P = 0.01$). Mean number of necrophylactic phellem cells for the two extreme combinations (Candor/*L. persoonii* and Sunhaven/*L. cincta*) were significantly different in both orientations by day 56. The lack of significant increase in phellem cell numbers in the longitudinal orientation from day 28 to day 56 was related to fungal circumvention of the first-formed necrophylactic periderm followed by formation of a second periderm. This phenomenon was observed in both cultivars colonized by *L. persoonii*.

At 8 days postwounding, Sunhaven bark showed higher mean suberin autofluorescence intensity relative to Candor ($\bar{x}$ = 4.28 and 2.48 millivolts, respectively, $t = 2.17$, $P = 0.05$). Only one-third of the Candor stems had suberized xylem rays at 8 days postwounding compared with Sunhaven, which showed xylem ray suberization in all plants. Suberin autofluorescence intensity was slightly higher in wounded Candor xylem ray parenchyma relative to Sunhaven. There were no significant cultivar differences in deposition of phloroglucinol-positive substances in either bark or xylem. In general, Candor showed higher levels of lignin in bark and Sunhaven showed greater depositions in xylem. Both cultivars displayed significant increases in bark and xylem phloroglucinol-positive substances and in suberin from day 6 to day 8. Lignin in bark and xylem and suberin in xylem increased until day 14. Suberin in bark continued to increase as new phellem was produced. When photometric values for lignin and suberin in bark and xylem were compared by cultivar, chi-square analyses showed that tissue deposition patterns for both lignin and suberin were independent of cultivar (chi-square = 24.8, df = 1). For example, Sunhaven formed more suberin in bark and more phloroglucinol-positive material in xylem than Candor. Moreover, Candor formed more lignin in bark and more intensely suberized xylem ray parenchyma than Sunhaven.

Discontinuities in suberized tissues. In both wounded and inoculated tissues, suberized primary and secondary cell types formed a boundary between living tissues and the external environment. Several discontinuities in bark and xylem boundaries were regularly observed and occurred in the absence of or as result of infection.

In transverse sections of wounded stems of both cultivars, suberin formed in tissues continuous with the exophylactic periderm, (i.e., in phelloderm and phellogen of the original exophylactic periderm), and continued through the cortex, primary phloem, and around the callus tissue derived from the secondary phloem. Suberized xylem ray parenchyma was observed at the point where callus and extant xylem parenchyma joined (Figs. 4 and 5). Toward the center of the wound (Fig. 6), progressively deeper cells of the ray parenchyma became suberized in the 1983 xylem (maximum depth about 700 μm). Suberized ray cells were adjacent to xylem vessels and fibers, which appeared plugged with phloroglucinol-reactive material (visible as a dark brown line in fresh dissections). Discontinuities in suberization at wounds were numerous and included: the juncture of the suberized tissue with the original periderm and with primary phloem fibers (Fig. 12); the centripetal surface of the callus as it grew across the exposed xylem (Figs. 13 and 14); and the presence of nonsuberized xylem vessels, tracheids, and axial parenchyma. Discontinuous suberin deposition in xylem and in bark adjacent to primary phloem fibers was associated with increased deposition of phloroglucinol-positive substances in these cells. The periderm juncture and callus discontinuities appeared time dependent and remained nonsuberized for 2 and 4 wk, respectively. In callus tissue, both primary and secondary suberized parenchyma eventually formed between callus parenchyma and new xylem (Fig. 15).

In inoculated tissues, suberization was less continuous than in wounds. With the exception of one Sunhaven/*L. cincta* stem, ray parenchyma of the 1983 xylem did not become suberized even though phlorglucinol-positive substances were deposited in the vessels and tracheids of a visible barrier zone. Suberization patterns in infected bark appeared similar to those in wounds, although necrophylactic phellem cell shape was altered in some samples (Fig. 16). Altered phellem appeared thin-walled and rounded compared with the relatively thick, rectangular phellem at wounds. Where bark tissues were colonized by fans of fungus mycelium, the junction of the suberized tissue with the exophylactic periderm and the primary phloem fibers were points of discontinuity or weakness. These areas often served sites of boundary circumvention by both pathogens. However, in Sunhaven/*L. cincta trees*, the periderm appeared effective in limiting colonization (Figs. 17 and 18).

The nonsuberized centripetal region of the callus also was readily colonized by both pathogens in the early stages of callus formation. Later, as callus tissues matured, the suberized boundary comprised of 1984 xylem parenchyma was often ruptured from the inside by the coalescing of enlarged gum ducts (Figs. 19 and 20). This rupture provided hyphal access to the gum duct region, thus facilitating longitudinal invasion. In addition, the rupture allowed fungal access to parenchymatous tissues of the differentiated callus resulting in rapid circumferential spread of mycelium. Direct penetration of well-formed necrophylactic periderm (at least three cells thick) was observed only once. Separation of adjacent rows of phellem, parallel to the axis of the tissue, was occasionally observed where mycelium of both fungi gained access to the tissue via growth cracks or lenticels. Both fungi were observed penetrating layers of primary suberized boundary tissues and/or one to two cell layers of altered necrophylactic phellem.

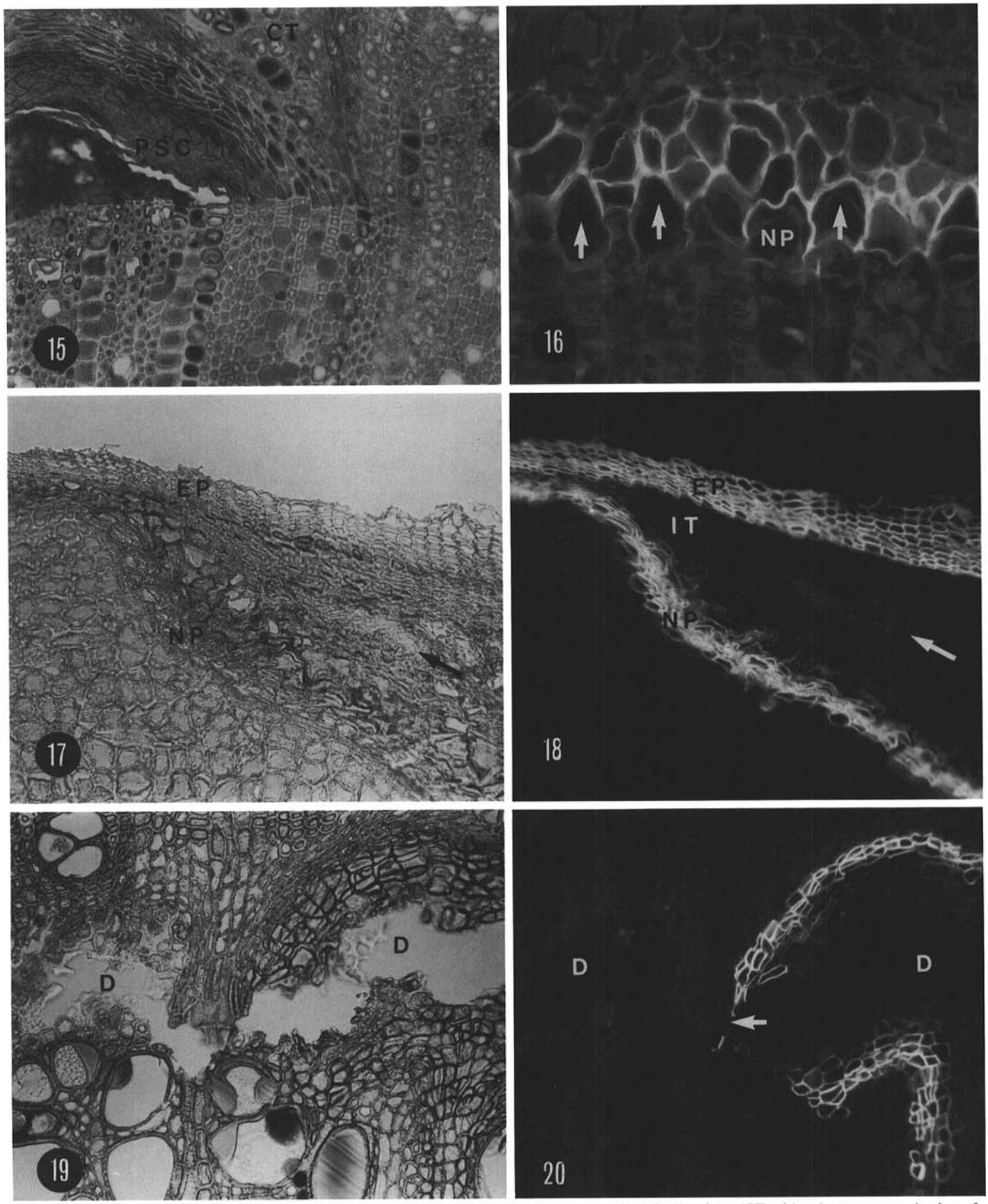
DISCUSSION

The cultivars Candor and Sunhaven appear highly susceptible and relatively resistant, respectively, to colonization by the peach canker fungi under field conditions (R.E.C. Layne, *personal communication*). Greenhouse inoculations of the two cultivars with both fungi provided data that compared favorably to field observations. However, the virulence of *L. persoonii* eventually overshadowed cultivar differences in susceptibility. The greater virulence of *L. persoonii* in this study may be related to greenhouse temperatures (6). There are no known sources of genetic resistance to *Leucostoma* spp. (17), although some Chinese germ plasm appears promising (4,15).

Cultivar wound response in the absence of infection may be related to nonspecific disease resistance (4,20). In the present study, Sunhaven displayed more rapid accumulation and consistently higher levels of suberin in bark relative to Candor. Deposition of suberin in noninoculated bark wounds has been correlated with field performance of other peach clones vis-a-vis peach canker (4). Xylem ray parenchyma cells in the cultivar Candor were more intensely suberized than in Sunhaven, although they were generally fewer and more widely dispersed. Sunhaven often exhibited large "sheets" of suberized ray parenchyma in longitudinal section, whereas this phenomenon was not observed in Candor. The type of xylem suberization in Sunhaven may provide a more effective barrier to fungal colonization than that observed in Candor.

The chi-square analysis showed independence between cultivars in their respective barrier zone patterns. For example, Sunhaven formed more suberin in bark and more phloroglucinol-positive substances in xylem relative to Candor. Alternatively, Candor formed lignified bark boundaries and suberized xylem ray parenchyma to a greater intensity (although to a lesser extent) than Sunhaven. These results are mainly of heuristic value and more rigorous tests to confirm them are required. These observations generally agree with Wilson's hypothesis (22) that the degree of compartmentalization sensu Shigo (18) could be a useful criterion for assessment of resistance in peach to canker fungi. However, bark responses, including rate of necrophylactic periderm formation may provide more useful information (4).

Suberin has been identified as a component of wall 4 of the CODIT model (18) in oak (16) and peach infected with *L. persoonii* (23). The present study is the first to demonstrate suberization of xylem ray parenchyma in tissue positions corresponding to walls 2



Figs. 15–20. 15, Transverse section of Sunhaven 28-day-old mechanical wound showing the junction of callus tissue (CT) with xylem present at the time of inoculation. The callus has an intact periderm (P) and an external surface of primary suberized cells (PSC). 400×. **16,** Portion of ligno-suberized boundary zone and new periderm (NP) adjacent to Candor tissue colonized by *Leucostoma persoonii* (top) showing altered phellem cell shape (arrows). 800×. **17 and 18,** Bright field and fluorescence illumination, respectively, of the longitudinal margin of a 56-day-old Sunhaven/*L. cincta* canker treated with phloroglucinol HCl and showing a complete intact necrophylactic periderm (NP) contiguous with the original exophylactic periderm (EP). Note the aggregation of fungus mycelium (arrow). This canker was not enlarging. 400×. **19 and 20,** Bright field and fluorescence illumination, respectively, of a phloroglucinol-HCl-treated transverse section showing coalescing gum ducts in Candor inoculated with *L. persoonii* (D) and the break in continuity of suberized tissues associated with coalescing (arrow). 800×.

and 3 of the CODIT model. Where xylem parenchyma exhibited suberized cell wall linings, the adjacent xylem vessels, tracheids, and pits appeared plugged with phloroglucinol-positive substances. Sparse xylem suberin depositions after wounding in Candor (versus extensive suberized ray "sheets" in Sunhaven), suggests a physiological compartmentalization deficiency may exist in some peach cultivars. In addition, nonspecific xylem responses may be inhibited by the peach canker fungi. Inhibition of nonspecific responses in peach bark by *L. persoonii* has been reported (2,23).

The present study demonstrated similar modes of pathogensis for the two peach canker fungi. Both fungi were found colonizing bark and cambial tissues, and xylem tissues in advance of visible canker margins. Both fungi colonized xylem beyond the visible margin at a greater distance proximally than distally. Colonization of 1982 xylem, primary xylem, and pith was observed at 56 days postinoculation only in Candor inoculated with *L. persoonii*. The combination of a highly susceptible host and the higher temperatures that may have favored *L. persoonii* probably account for the extensive colonization observed.

The present study agrees with the observations of Willison (21) who found hyphae beyond the visible margins of cankers, and contradicts Hildebrand's observation that hyphae occurred only within boundaries of the visible cankered zone (10). Tekauz and Patrick (18) observed *L. cincta* and *L. persoonii* in peach xylem beyond canker margins and characterized them as sapwood parasites. Wisniewski et al (23), in their analysis of *L. persoonii* infection of peach shoots, stated that there was no evidence of movement of the fungus from infected wood into living bark tissues. The present findings support Wisniewski et al (23) and expands on their conclusion to include both *Leucostoma* species as facultative bark parasites.

In summary, this study demonstrated similar modes of pathogenesis by the two peach canker fungi. In addition, it was observed that early pathogen growth in greenhouse-inoculated stems was related to field performance although, with time, differences in fungus virulence were more important. Peach cultivars differed in their abilities to establish anatomical barriers and the type and extent of xylem and bark responses appeared independent. Circumvention of necrophylactic periderm occurred by fungal penetration of natural and pathogen-induced discontinuities in suberized tissues. Inoculated trees did not display suberization of extant xylem ray parenchyma as was observed in wounded trees. Both fungi inhibited host-mediated nonspecific responses such as callus formation and periderm differentiation (2,23), and lignification and suberization in both bark and xylem.

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