Association of Suberin Formation in Uninoculated Wounds with Susceptibility to *Leucostoma cincta* and *L. persoonii* in Various Peach Cultivars

A. R. Biggs and N. W. Miles

Research scientists, Agriculture Canada, Research Station, and Horticultural Research Institute of Ontario, respectively, Vineland Station, Ontario, Canada L0R 2E0.

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ABSTRACT

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Branches of field-grown peach cultivars were mechanically wounded and sampled for histological study after 0, 3, 7, 10, 14, and 17 days. Cell counts and tissue thickness measurements were used to determine the extent of formation of ligno-suberized boundary tissue and suberized wound periderm. Suberin autofluorescence intensity also was measured with a microscope equipped with a photometer/fluorometer. The experiments were conducted twice at two sites from 1985 to 1987 and included wound series from the months of April, May, June, July, September, October, and November. Cultivars were ranked after each wound series according to the degree of formation of wound tissues or intensity of suberin autofluorescence. These ranks were compared to cultivar disease susceptibility ranks, based on field performance history, using Spearman's rank correlation test. There were no consistent relationships between relative susceptibility to disease and phellem cell numbers or suberized tissue thickness. However, the rate of suberin accumulation during May and June was correlated in both years with the known relative susceptibility of the cultivars to the peach canker fungi.

Peach canker, caused by *Leucostoma cincta* (Pers. & Fr.) Hohn. (anamorph = *Cytospora cincta* (Pers.) Fr.) and *L. persoonii* (Nits.) Hohn. (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. Wounds created by pruning, leaf abscission, winter injury, and insect damage are the major avenues of entry for the peach canker fungi (24). The disease, manifested as perennial cankers on trunks, scaffold limbs, and branches, has been difficult to control. Although certain cultural practices can reduce peach canker incidence (15), all of the currently grown peach cultivars are susceptible to the pathogens to some degree (16,19).

Breeding peach trees for resistance to the canker pathogens has been difficult. Problems have included regional variation in cultivar performance (19), cultivar by year variation in susceptibility (16), lack of standardized inoculation procedures (20), lack of knowledge regarding pathogen variation, lack of known resistant cultivars, and the land-intensive and labor-intensive nature of conventional fruit tree breeding. It is clear that a rapid, reliable, and economically efficient method to detect disease resistance would facilitate development of new, *Leucostoma*-resistant peach cultivars.

Histochemical and ultrastructural studies of wounds and fungal infections of peach (2,4–6,8,14) and other tree barks (1,3,4) have shown that suberin is an important cell wall component in new tissues that form after wounding and before the delimitation of infections by wound pathogens. Studies have shown that formation of suberized tissues is related spatially and temporally to inhibition of fungal colonization (2,6,12). Suberin occurs as lamellae on the inner walls of cells, present at the time of wounding, which differentiate after wounding into a primary ligno-suberized layer (14). Suberin occurs also as the major component of phellem cell walls in both normal and "pathological," or wound-induced, periderms. Several functions related to disease resistance have been attributed to suberin, including: barrier to diffusion of pathogen enzymes or toxins into living tissues, structural barrier to pathogen ingress, and biochemical barrier to microbes due to the high proportion of phenolic materials incorporated into the suberin polymer (17).

Suberin can be detected easily in injured or infected tissues of many tree species because it autofluoresces in the violet-blue range when examined with ultraviolet epi-illumination (1,3). The autofluorescence is probably due to the phenolic constituents of suberin. We have found that suberin autofluorescence measurements are useful for making quantitative relative comparisons of genotypic or experimental treatment effects (7,9,11).

In 1984, we initiated field experiments to test the hypothesis, originally proposed by Weaver (22), that peach cultivars show different responses to wounding and that these differing responses were correlated with resistance to the peach canker fungi. The objective of the present study was to examine the correlative relationship between wound-induced suberin accumulation in several different peach cultivars and clonal selections and their field susceptibility to peach canker. The results of the initial experiments were published in 1985 (13). A preliminary report on the present study has been published (10).

MATERIALS AND METHODS

Orchard sites. Peach (*Prunus persica* (L.) Batsch) orchards were established at the Agriculture Canada experimental farm in Jordan Station, Ont., (Jordan) and at the experimental farm of the Horticultural Research Institute of Ontario in Vineland Station, Ont., (Victoria) in May of 1984 and 1985, respectively. The Jordan farm is about 3 km east of the Victoria farm. Soils on both farms are imperfectly drained Vineland fine sandy loam and are typical of peach orchard soils in the Niagara Peninsula. The Jordan orchard was initiated from 1-yr-old, virus-tested, canker-free nursery stock on cultivar Bailey rootstock. Cultivar Boone County rootstocks were used at the Victoria site, except for the combination of cultivar Madison on Halford. Trees on the Victoria farm were not virus tested. Tree spacing was 1.5 × 4.0 m and 3.0 × 5.0 m at the Jordan and Victoria farms, respectively. Trees were managed with clean cultivation in the spring followed by a rye grass cover crop planted in July. Fungicides and insecticides were applied as needed to control peach leaf curl, brown rot, and oriental fruit moth (ferbam, captan, and phosmet, respectively). The cultivars planted at each orchard site are listed in

Tables 1 and 2. Cultivars were selected to represent a range of susceptibility to *Leucostoma* spp. based on historical field performance ratings with visual assessment and a 1 to 10 numerical rating system: 1 = no canker observed; 2 = trace in 1- or 2-yr-old wood; 3 = canker very light in major limbs of tree; 4 = canker light to moderate in trunk, crotch, and lower scaffold; 5 = canker moderate in trunk, crotch, and lower scaffold, but severe in minor branches; 6 = canker severe in one of trunk, crotch, or scaffold; 7 = canker severe in two of trunk, crotch, or scaffold; 8 = tree alive but canker likely to become major cause of death, disease severe in trunk, crotch, and scaffold; 9 = tree dying, canker severe and judged to be the major cause of dying; 10 = tree dead, canker severe and judged to be the major cause of death (R. E. C. Layne, *personal communication*). None of the cultivars selected for these experiments was immune to the disease because known sources of immunity are not available. Therefore, the cultivars selected range from highly susceptible to relatively resistant.

Wound treatments. Experiments were initiated in each orchard the year after planting. Six wounds per tree on 1-yr-old branches were inflicted at internodes, located near the midpoints of the branches, with a 4-mm-diameter cork borer inserted through the bark to the xylem. The excised bark disk was removed and discarded. One wound per tree per postwounding time, on three (Victoria farm) or five (Jordan farm) replicate trees, was sampled for histological analysis of wound response after 0, 3, 7, 10, 14, and 17 days. Dates when wounds were made are given in Tables 1 and 2.

Histology. Wound samples were collected by removing the twig with hand-held pruning shears, trimming away the excess tissue to 0.5 cm above and below the wound, and cutting, with a razor blade, the remaining tissue transversely and longitudinally through the wound. Tissues were placed immediately in Formalin:acetic acid:50% ethanol (FAA, 5:5:90) and fixed for a minimum of 7 days. Samples were dehydrated in a tert-butyl alcohol series, embedded in Paraplast Plus (Sherwood Medical, St. Louis, MO), and sectioned in the transverse orientation at 8 μm thickness on a rotary microtome. Sections were mounted on glass slides with a gelatin adhesive and dried overnight. The paraffin was removed with xylene; sections were hydrated to water in a graded ethanol series, stained for 2 min in 0.1% aqueous toluidine blue 0, and partially air-dried; and coverslips were mounted in Pro-Texx (Lerner Laboratories, New Haven, CT).

Stained sections were examined for residual autofluorescence due to suberin (1,3) with a Leitz Ploemopak and fluorescence filter block A (340–380-nm excitation, 430-nm barrier filters). The intensity of residual autofluorescence (in millivolts), hereafter referred to as suberin autofluorescence, was measured at 430 nm with a Leitz MPV compact microscope photometer with an HBO 100W mercury burner and stabilized power supply. Autofluorescence was measured with a circular area photometer diaphragm at 25× magnification and included approximately

TABLE 1. Suberin accumulation values and ranks (in parentheses) for wound experiments conducted at the Jordan farm in 1985 and 1986 and the Spearman's rank correlation coefficients for the relationship between each cultivar's susceptibility to peach canker, based on its field performance history, and suberin accumulation[a]

Cultivar (rank)[b]	Wound date							
	8 May 85	11 June 85	14 July 85	22 Apr 86	5 May 86	3 June 86	9 Sept 86	7 Oct 86
Sunhaven (1)	0.64 (1)	0.74 (4)	1.02 (4)	0.54.(5)	1.87 (2)	3.04 (1)	2.96 (4)	1.42 (5)
Redhaven (2)	0.58 (2)	0.82 (2)	1.06 (3)	0.77 (2)	1.86 (3)	2.49 (3)	3.02 (2)	1.44 (4)
Garnet Beauty (3)	0.46 (4)	1.11 (1)	1.08 (2)	0.58 (4)	2.08 (1)	2.86 (2)	3.07 (1)	1.52 (2)
Loring (4)	0.37 (5)	0.59 (5)	0.97 (5)	0.73 (3)	1.48 (6)	2.29 (5)	2.65 (5)	1.36 (6)
Vanity (5)	0.49 (3)	0.78 (3)	1.13 (1)	1.08 (1)	1.80 (4)	2.44 (4)	2.99 (3)	1.46 (3)
Candor (6)	...[c]	...	...	0.39 (6)	1.78 (5)	2.25 (6)	2.50 (6)	1.58 (1)
Spearman's r_s	0.70	0.10	−0.18	0.03	0.60	0.89	0.49	−0.54
t-value	1.70	0.17	−0.32	0.06	1.74	3.82	1.12	−1.28
Significance	0.10	NS[d]	NS	NS	0.09	0.01	NS	NS

[a] Suberin autofluorescence accumulation values are the slope parameters from the linear regression where Y = suberin autofluorescence intensity and X = numbers of days postwounding. Tissues from each cultivar were sampled at 3, 7, 10, 14, and 17 days after wounding with three replicate trees per sampling time. Degrees of freedom = 13, except for V68101 where degrees of freedom = 3.
[b] Cultivar rankings for canker resistance least susceptible (1) to most susceptible (6). Ranks for suberin accumulation are from highest (1) to lowest (6).
[c] Data not available.
[d] NS = not significant.

TABLE 2. Suberin accumulation values and ranks (in parentheses) for wound experiments conducted at the Victoria farm in 1986 and 1987 and the Spearman's rank correlation coefficients for the relationship between each cultivar's susceptibility to peach canker, based on its field performance history, and suberin accumulation[a]

Cultivar (rank)[b]	5 May 86	3 June 86	9 Sept 86	7 Oct 86	21 Apr 87	19 May 87
V68101 (1)	1.67 (1)	2.43 (3)	...[c]	...	1.58 (3)	4.05 (1)
V68051 (2)	1.37 (4)	1.89 (10)	2.42 (7)	1.39 (9)	1.84 (2)	3.96 (2)
Veeglo (3)	1.47 (3)	2.45 (2)	3.12 (1)	1.69 (7)	1.29 (9)	3.30 (6)
Babygold 5 (4)	1.57 (2)	2.48 (1)	2.46 (5.5)	1.91 (4)	1.16 (10)	3.70 (4)
Vanity (6)	1.00 (8)	2.38 (4)	2.36 (8)	2.06 (3)	1.42 (5)	3.85 (3)
Redhaven (5)	1.29 (5)	2.11 (7)	2.46 (5.5)	2.32 (2)	1.91 (1)	2.94 (7)
Candor (7)	1.20 (7)	2.16 (6)	2.62 (3)	2.64 (1)	1.57 (4)	2.53 (10)
Madison (8)	0.98 (9)	2.30 (5)	2.50 (4)	1.89 (5)	1.41 (6)	2.89 (8)
Vivid (9)	0.68 (10)	2.04 (8)	2.08 (9)	1.48 (8)	1.27 (8)	3.40 (5)
Earlired (10)	1.28 (6)	1.95 (9)	2.65 (2)	1.70 (6)	1.40 (7)	2.77 (9)
Spearman's r_s	0.82	0.44	−0.06	−0.15	0.30	0.71
t-value	4.05	1.39	−0.17	−0.40	0.89	2.85
Significance	0.001	0.10	NS[d]	NS	NS	0.02

[a] Suberin autoflorescence accumulation values are the slope parameters from the linear regression where Y = suberin autofluorescence intensity and X = numbers of days postwounding. Tissues from each cultivar were sampled 3, 7, 10, 14, and 17 days after wounding with three replicate trees per sampling time. Degrees of freedom = 13, except for V68101 where degrees of freedom = 3.
[b] Cultivar rankings for canker resistance indicate least susceptible (1) to most susceptible (10). Ranks for suberin accumulation are from highest (1) to lowest (10). Ranks for suberin accumulation are from highest (1) to lowest (10).
[c] Data not available.
[d] NS = not significant.

75–100 cells over an area 272 μm in diameter. The fluorescence intensity of stained, unwounded tissue, measured about 1.0 mm distal or proximal to each wound site, was used as a baseline for each measurement. Each photometer value, for statistical purposes, represented the mean of three measurements taken on serial sections from one slide. Slides were coded alphanumerically so that the histotechnologist was blind to specimen identity.

The following additional data about wound-related tissues were collected from these samples: total thickness (μm) of suberized tissues, including primary ligno-suberized zone and new phellem; and the mean number of cells in the phellem of the new periderm, when present. All measurements were made in the outer cortex just internal to the healthy, outer periderm.

Data analysis. Suberin autofluorescence values were subjected to simple linear regression and analysis of covariance to determine the homogeneity of regression coefficients (21). The regression line slope parameters, termed suberin accumulation rates, were used to rank the cultivars after each of the 16 wound experiments. These ranks were compared with the ranks of the peach genotypes (based on historical field performance data supplied by the coauthor and R. E. C. Layne, *personal communication*) with the nonparametric Spearman's rank correlation procedure (21).

Data on tissue thickness and number of phellem cells were subject to simple linear regression and analysis of covariance for each wound series. Regression line slopes for rate of phellem production and rate of tissue thickness increase were ranked and compared with field susceptibility and suberin accumulation ranks with Spearman's procedure. Rank correlation tests also were used to assess the relationship between suberin autofluorescence and the two anatomical parameters. For all rank correlation tests, a 10% level of significance was chosen because of the relatively small number of cultivars used in these experiments as necessitated by constraints in orchard space and the time consumed by the histological procedures (21).

Data describing the time of initiation of new periderms in the wounded cortex were assessed in two ways: Observations at 3, 7, and 10 days after wounding were made for the percentage of wounds within each cultivar possessing a complete ligno-suberized layer in contact with the original periderm; and the regression equations derived from the suberin autofluorescence data were used to obtain the X intercept, the approximate number of days to initiation of suberization. The ranks of the percentage and the X intercepts for cultivars were compared to the cultivar ranks for canker susceptibility.

RESULTS

Suberin accumulation. Linear regressions for each cultivar for each of the 16 wound experiments were conducted to determine the relationship between suberin autofluorescence (Y) and days postwounding (X). The data for May 1986 (Table 3) represent the linear regressions from experiments in May and June. Analysis of covariance was used to examine cultivar variation in the rate of suberin accumulation (Table 4). Regression slopes (Table 3) were found to be heterogeneous and demonstrated that cultivars differed in the rate of suberin accumulation. Of the seven experiments conducted in May and June 1985 to 1987, suberin accumulation rate was different among cultivars in six of them (Table 4). In months other than May and June, cultivars did not differ in suberin accumulation.

The association of suberin accumulation rate with cultivar susceptibility to the peach canker fungi is summarized in Tables 1 and 2. Of the seven experiments conducted in May and June, six showed a significant ($P \leq 0.10$) correlation between suberin accumulation and field performance for the cultivars examined. Those cultivars that accumulated suberin faster after wounding were more likely to be ranked as less susceptible to the canker-causing fungi. There were no significant relationships between suberin accumulation rate and field performance history in months other than May or June.

Anatomical parameters. Cultivar variation in phellem cell accumulation and increase in boundary layer thickness could not be demonstrated consistently by analysis of covariance (data not shown). A significant cultivar difference in phellem cell accumulation was found in only one of two October experiments (1986, Victoria). Significant rank correlations of phellem cell accumulation with cultivar performance were observed in only three of seven experiments conducted in May and June and in one experiment conducted in September (Table 5). In these four experiments, when the new phellogen of one cultivar produced suberized phellem more rapidly than the phellogen of another cultivar, the first cultivar was more likely to be ranked as less susceptible to the disease. Data from histological examination of wounded tissues were not reliable for predicting cultivar field performance.

Rate of increase in the thickness of the new suberized boundary layers was correlated with field performance history in four of seven experiments conducted in May and June and in one experiment conducted in September (Table 5). In these experiments, cultivars that exhibited greater rates of increase in suberized tissue thickness tended to be less susceptible to the disease than cultivars that exhibited slower rates of increase.

Relationships between suberin formation and anatomical parameters. The relationship between the ranks for histological

TABLE 3. Regression analysis of the relationship of days postwounding (X) and suberin autofluorescence intensity (Y) for 10 peach cultivars[a]

Cultivar	r^2	P	Regression coefficients: Intercept (b_0)	Regression coefficients: Slope (b_1)	Slope rank	Canker susceptibility rank[b]
Babygold 5	0.846	0.0001	−7.55	1.57	2	4
Candor	0.846	0.0001	−5.58	1.20	7	7
Earlired	0.792	0.0001	−6.33	1.28	6	10
Madison	0.810	0.0001	−3.21	0.98	9	8
Redhaven	0.792	0.0001	−5.31	1.29	5	5
V68051	0.757	0.0001	−6.89	1.37	4	2
V68101	0.941	0.0132	−7.25	1.67	1	1
Vanity	0.722	0.0002	−3.96	1.00	8	6
Veeglo	0.828	0.0001	−6.69	1.47	3	3
Vivid	0.689	0.0001	−2.46	0.68	10	9

[a] Tissues from each cultivar were sampled at 3, 7, 10, 14, and 17 days after wounding, with three replicate trees per sampling time. Degrees of fredom = 13, except for V68101, where degress of freedom = 3. Experiment initiated on 5 May 1986 at the Victoria farm.

[b] Cultivar rankings for canker resistance indicate least susceptible (1) to most susceptible (10). Ranks for suberin autofluorescence accumulation are from the highest (1) to lowest (10).

TABLE 4. F-statistic and level of significance from analysis of covariance tests for homogeneity of regression line slopes for suberin accumulation for 13 wound series experiments[a]

Suberin accumulation in:	Jordan 1985	Jordan 1986	Victoria 1986	Victoria 1987
April	...[b]	$F = 1.81$ NS[c]	...	...
May	$F = 4.93$ $P = 0.01$	$F = 1.29$ NS	$F = 2.35$ $P = 0.02$	$F = 2.08$ $P = 0.05$
June	$F = 2.45$ $P = 0.05$	$F = 5.36$ $P = 0.01$	$F = 1.68$ $P = 0.10$	... ...
July	$F = 0.99$ NS	...	...	...
September	...	$F = 0.63$ NS	$F = 0.54$ NS	...
October	...	$F = 0.31$ NS	$F = 0.62$ NS	...

[a] For linear regresions, Y = suberin autofluorescence intensity (mv), and X = number of days after initial wounding.

[b] Experiment not performed at this site/month/year combination. Data from experiments in April 1986 and 1987 and November 1986 were not analyzed because tissues were present only on the last sample date or were completely absent.

[c] NS = not significant.

parameters and suberin accumulation was examined. Rate of increase in suberin autofluorescence was correlated with rate of increase in number of suberized phellem cells in only two of 12 experiments (5 May 1986, Victoria, and 9 September 1986, Jordan, Table 6). A significant correlation would have indicated a positive association between suberin autofluorescence and the number of suberized cells. This relationship was not demonstrated in 10 of 12 experiments. Rate of increase in thickness of new suberized tissue was correlated with rate of increase in suberin autofluorescence in only three of 12 experiments (5 May 1986, Victoria, and 9 September 1986, both sites). In these three experiments, suberin autofluorescence increased in association with increases in tissue thickness.

Initiation of boundaries. A significant relationship between timing of boundary initiation and canker susceptibility was observed only once. In the June 1986 experiment at the Victoria site, the percentage of cultivar replicates with complete ligno-suberized boundary layers was correlated with canker susceptibility ($r = 0.74$, $t = 3.14$, $P \leq 0.05$, data not shown). The X intercept, used to estimate the timing of suberin initiation, was not a useful criterion for rating cultivar susceptibility (data not shown).

DISCUSSION

The potential for disease resistance as a control measure for peach canker was first suggested in Willison's early studies on canker incidence (24,25). These studies focused on leaf scars as infection sites, and it was shown that slow rates of tissue healing in wounds incurred late in the season were susceptible to infection by *Leucostoma* spp. Wensley (23) examined the rate of wound closure (callus formation) in peach branches and its relation to disease resistance. He found that the wound closure rate varied with date of wounding, age and diameter of the branch, the age of the tree, and cultivar. The most rapid wound closure occurred on the most canker-resistant cultivar, Elberta, when wounds were inflicted in June or July. Wensley concluded that faster wound closure was a characteristic of cultivars that were relatively resistant to *Leucostoma* spp.

In the present study, we have confirmed an association between postwounding tissue changes in peach tree bark and resistance to *Leucostoma* spp. The repeatable nature of these experiments in the months of May and June, the fact that the experiments were performed over 2 yr at two sites, and the association between suberin accumulation and field susceptibility occurring during months when suberin accumulation was significantly heterogeneous indicate the presence of a correlation between suberin accumulation and field susceptibility.

By measuring suberin autofluorescence, we estimated the accumulation rate of a biopolymer associated with regeneration of preformed barriers to pathogen ingress. This is a more pertinent measure of host response than callus formation, given that the latter has not been demonstrated to play a role in disease resistance in this pathosystem (6). Suberized phellem is thought to serve as a resistance barrier in several host-pathogen systems, particularly in woody plants and storage roots (18).

The absence of a repeatable relationship between suberin autofluorescence and tissue anatomy is interpreted as showing that anatomical changes such as phellem cell production and tissue thickness may be less important than the actual biopolymeric structure of the new tissues. This interpretation supports the hypothesis that the phenolic constituents of suberin could be responsible for its fungistatic or fungitoxic effects (17).

Although the data presented herein demonstrated a correlation between suberin accumulation and disease resistance, the direct role of suberin in resistance has not been proven conclusively. The absence of correlations between suberin accumulation and resistance in months other than May and June could be interpreted as evidence that suberin does not have a role in resistance. However, when trees are wounded during November through April, periderm generation is extremely slow, with the majority of new tissues formed in May (7). Wounds created during these

TABLE 5. Spearman's rank correlation coefficients (r_s) and t-values for the relationship between each cultivar's susceptiblity to peach canker, based on its field performance history, and rates of increase in numbers of phellem cells in the new periderm and rates of increase in thickness of the suberized tissue[a]

	Jordan		Victoria	
Wound date	Phellem cells	Tissue thickness	Phellem cells	Tissue thickness
8 May 85				
r_s	0.70	0.10	...[b]	...
t	1.70*[c]	0.17	...	...
11 June 85				
r_s	0.90	0.70	...	...
t	3.58**	1.70*	...	...
14 July 85				
r_s	0.20	0.60	...	...
t	0.35	1.30	...	...
5 May 86				
r_s	−0.09	0.62	0.41	0.44
t	0.18	1.58*	1.27	1.39*
3 June 86				
r_s	0.49	0.54	0.14	0.33
t	1.12	1.29	0.40	0.99
9 Sept 86				
r_s	0.54	0.14	0.78	0.67
t	1.28	0.28	3.34***	2.37**
7 Oct 86				
r_s	0.46	0.37	−0.32	−0.05
t	1.04	0.80	0.89	0.13
19 May 87				
r_s	...	...	0.70	0.62
t	...	...	2.77**	2.26**

[a] Tissues assessed in this experiment were not present in samples collected when wounds were made in April 1986 and 1987 and November 1986.
[b] No data were collected at this wound date/site combination.
[c] Asterisks indicate significant t-value (one-tailed test) at $P = 0.10$ (*), 0.05 (**), and 0.01 (***).

TABLE 6. Spearman's rank correlation coefficients (r_s) and t-values for the relationship between rates of increase in suberin accumulation intensity and rates of increase in numbers of phellem cells in the new periderm and rates of increase in thickness of the suberized tissue[a]

	Jordan		Victoria	
Wound date	Phellem cells	Tissue thickness	Phellem cells	Tissue thickness
8 May 85				
r_s	−0.05	0.10	...[b]	...
t	0.09	0.17	...	...
11 June 85				
r_s	0.20	0.60	...	...
t	0.35	1.30	...	...
14 July 85				
r_s	0.60	0.30	...	...
t	1.30	0.54	...	...
5 May 86				
r_s	0.03	0.14	0.49	0.54
t	0.06	0.28	1.59*[c]	1.82*
3 June 86				
r_s	0.49	0.43	0.31	0.18
t	1.12	0.95	0.92	0.52
9 Sept 86				
r_s	0.66	0.89	0.37	0.82
t	1.75*[b]	3.82***	1.05	3.80***
7 Oct 86				
r_s	0.47	0.49	−0.21	−0.04
t	1.06	1.12	0.57	0.11
19 May 87				
r_s	...	...	0.3 6	0.19
t	...	...	1.09	0.55

[a] Tissues assessed in this experiment were not present in samples collected when wounds were made in April 1986 and 1987 and November 1986.
[b] No data were collected at this wound date/site combination.
[c] Asterisks indicate significant t-value (one-tailed test) at $P = 0.10$ (*), 0.05 (**), and 0.01 (***).

months are considered susceptible to disease (26). Periderm generation in July, August, and early September also may be sufficiently rapid to exclude invading fungi (26). Lack of significant cultivar differences in suberin autofluorescence in September and October were surprising in light of previous work relating leaf fall to canker resistance (22). Given that early initiation of abscission followed by rapid defoliation has been linked with resistance, we expected to observe cultivar variation in suberin accumulation during September and October. However, during these months, cultivars were homogeneous with respect to the histological parameters measured. It is likely that the wound system employed in these experiments inadequately represented events associated with abscission. The role of wound response in leaf scars and the variable temporal nature of abscission rate in different cultivars are currently being investigated in our laboratory.

Several explanations are plausible for the observed correlation of suberin accumulation in May and June with disease resistance. Although vascular cambial activity is greatest during May and June, phellogen regeneration is strongly influenced by temperature and, therefore, occurs most rapidly in July and August (7). In the spring, peach cultivars may vary in their ability to synthesize and incorporate suberin into the cell walls of the new phellem. This variation could be related to cultivar differences in phytohormone-regulated processes such as early-season emergence from dormancy, blossom production, fruit set, or carbohydrate metabolism (27). This early-season variation in suberin accumulation is lost around mid-June when all cultivars appear to accumulate it at similar rates. Most orchard pruning in the Niagara peninsula is conducted in May.

The correlation between suberin accumulation rate and canker resistance has epidemiological implications regarding the interaction of the influence of time and cultivar on the receptivity of the infection court. One would expect that, as the infection court becomes less susceptible to fungal colonization with time, cultivars that form wound-related suberized tissues more rapidly would be less susceptible than cultivars responding less rapidly. With this in mind, the risk of infection of the more heavily suberized cultivars would be reduced relative to those that are less suberized. We have preliminary data that support this hypothesis. The relative susceptibility in the field of the cultivars used in this study could be partially explained by variations in infection risk at the infection court.

The relationship of suberin accumulation rate with disease resistance may prove useful in peach breeding programs that have resistance to *Leucostoma* spp. as an objective. The concepts described here also may be applicable to other pathosystems where wounds serve as the primary infectible sites.

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