

Temporal Changes in the Infection Court After Wounding of Peach Bark and Their Association with Cultivar Variation in Infection by *Leucostoma peroonii*

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ABSTRACT

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Peach bark wounds 0, 7, 9, 11, 13, and 15 days old were inoculated with mycelium of *Leucostoma peroonii*, and colonization frequency and extent were determined. Six peach cultivars ranging in their relative susceptibility to *L. peroonii* were studied to test for the presence and extent of cultivar variation in wound response and the association of variation in wound response with susceptibility to fungal infection. Uninoculated wounds of similar ages and in close proximity to inoculated wounds also were sampled and examined histologically for morphological and histochemical changes associated with nonspecific plant defense reactions, especially the

formation of suberized wound periderm. Peach cultivars varied in the rates at which they accumulated suberin, and suberin accumulation rate was correlated with the relative susceptibility of peach cultivars to infection by *L. peroonii*. By calculating the number of days to maximum resistance for each cultivar, that is, the point when the infection court was no longer susceptible to infection, it was estimated that the infection court of the most susceptible cultivar would remain receptive to inoculum approximately 19 days longer than the infection court of the most resistant cultivar used in this study.

Peach canker, caused by *Leucostoma cincta* (Pers. & Fr.) Höhn. and *L. peroonii* (Nits.) Höhn., is a major limiting factor in peach production in the northern portions of the region favorable for deciduous-tree fruit production in North America. Wounds created by leaf abscission, pruning, and winter injury are major routes of entry for peach-canker fungi (22).

In many host-pathogen interactions, the boundary-setting process in the wound (the infection court) confers resistance to infection (9,16,20). Many researchers have demonstrated that wounds become increasingly less susceptible to infection with age (8,10,11,17,18), and peach bark wounds are no exception (5). This type of resistance to infection is thought to be related to nonspecific plant responses leading up to and including formation of primary lignosuberized tissues and secondary wound (or necrophylactic sensu Mullick) periderm (14,15); major structural components of these tissues are lignin and suberin (2,3,21). Definitive proof of the role and importance of lignosuberized tissue and wound periderm in disease resistance in trees has never been presented.

Although it is well known that wounds of many woody plants become less susceptible to infection with time, it is not known whether this phenomenon is subject to genetic variation within a species. We have been able to demonstrate in the field that suberin accumulation in peach bark wounds is highly cultivar dependent, and that a cultivar's rate of suberin accumulation generally is indicative of that cultivar's past field performance in resisting

Leucostoma spp. (7). Although several researchers have demonstrated that events that take place after formation of an infection court influence the host-pathogen interaction (8,11,12,17,18), no one has shown that intraspecific variation in wound response acts on a temporal level to influence relative susceptibility within a species. Given that intraspecific variation exists in peach for suberin accumulation (7), the objective of this study was to examine the relationship between suberin accumulation and resistance in peach to infection by *Leucostoma* species.

MATERIALS AND METHODS

Plants. Two-year-old, nursery-grown peach trees (*Prunus persica* (L.) Batsch) were dug in November 1986 and placed in commercial storage (Mori Nurseries, Ltd., Virgil, Ontario, Canada) until the first week of January 1987. Five of the six cultivars used in this study, cultivars Redhaven, Vanity, Candor, Madison, and Earlired, were from the commercial nursery. The sixth genotype was clonal selection V68101, which was propagated and stored at the Horticultural Research Institute of Ontario under conditions similar to the commercial nursery. Five trees of each cultivar or clone, transplanted into Vineland silt loam:peat:sand (20:9:6) in 30-cm-diameter clay pots, were pruned to provide 50 cm of clear stem and 10 growing shoots per plant. Average length of the new shoots was approximately 10 cm at the beginning of the experiment. Greenhouse temperature was maintained within the range of 21–33 C. The choice of cultivars and clonal lines used in this study was based on relative susceptibility data collected in the

field over several years (7).

Inoculation. On 12 February 1987, all trees were sponged with distilled water to remove extraneous soil and debris on the main stem. Each plant stem was measured and marked off to provide seven 7-cm segments. Each segment on each tree was randomly designated to receive one of seven wound-age treatments: inoculated at 0, 7, 9, 11, 13, or 15 days postwounding and an uninoculated wounded control. These times were chosen based on previous experiments with the cultivar Loring (5). On the appropriate number of days before inoculation, the bark was wiped with a cloth moistened with 70% ethanol, and a sharpened 4-mm-diameter cork borer was used to remove a portion of bark down to the xylem. Care was taken not to physically injure underlying xylem tissues while wounding. Wounds on each segment were done in pairs, each directly on the opposite side of the stem from the other, for a total of 14 wounds per stem.

Inoculations were made on 27 February when all five replicate trees of each cultivar had received six pairs of wounds ranging from fresh to 15 days old. One wound of each pair was inoculated with a 4-mm-diameter malt agar disk of *L. personii* and covered with cellophane tape for 7 days. The seventh wound pair (also made on 27 February) was used as an uninoculated control; one of the wounds received a plain malt agar disk covered with cellophane tape. Preparation of the inoculum was as described previously (1). Length of cankers was recorded at 7, 14, and 21 days postinoculation in the manner described previously (5).

Histology. The second (uninoculated) wound of each pair was excised on 27 February with a sterile razor blade, halved transversely, and placed immediately into Formalin:acetic acid:alcohol (12). The tissue was processed and embedded in paraffin as described previously (1). Longitudinally oriented rotary microtome sections 8 μ m thick were assessed quantitatively for suberin in the lignosuberized boundary and necrophylactic periderm by using the suberin autofluorescence method (2,3) and a Leitz MPV compact microscope photometer (Ernst Leitz Wetzlar GmbH, Wetzlar, West Germany). For autofluorescence intensities, three measurements were taken from serial sections on each slide at each of the six wound-age treatments. Additional data were collected on the number of necrophylactic phellem cells across the longitudinal axis of the new periderm (measured at its junction with the original periderm) and the total thickness of new suberized layers. The experiment was a completely randomized design and was performed two times.

Data analysis. Error variances for the two experiments were homogeneous (Bartlett's test, $P \leq 0.05$) and, therefore, data were pooled for analysis (19). Values of suberin autofluorescence

intensity were regressed against time to determine the suberin accumulation rate (b_1) for each plant of each cultivar:

$$Y = b_0 + b_1(X) \quad (1)$$

where Y = suberin autofluorescence intensity (in millivolts), X = time postwounding (in days), and b_0 and b_1 are undefined regression parameters. Similar equations, where Y = number of phellem cells or thickness of suberized tissue, were used to calculate rates of increase for these anatomical variables.

For each cultivar, the length of cankers (in millimeters) at 14 and 21 days postinoculation was regressed against wound age (in days) to determine the rate of canker length limitation (b_1) by application of equation 1 where Y = canker length, X = age of the infection court, and b_0 and b_1 are undefined regression parameters.

Estimated days to maximum resistance (X) was calculated for each cultivar with equation 1, where $Y = 6$ (the length of an inoculated infection court that was no longer susceptible to infection), $b_0 = 59.3$ (the mean canker length [millimeters] for 0-day-old infection courts), and b_1 = the rate of canker length (millimeters) inhibition calculated previously.

Differences among cultivar regression lines for each dependent variable, that is, suberin accumulation, canker length inhibition, phellem cell accumulation, and suberized tissue thickness, were determined with analysis of covariance and the F -test (19). Differences among regression lines were determined with paired t -tests when the analysis of covariance indicated that lines were heterogeneous. Spearman's nonparametric rank correlation test was used to examine relationships among variables (19).

RESULTS

Rates of suberin accumulation exhibited significant differences due to cultivar ($P \leq 0.001$) (Table 1). Clone V68101 accumulated suberin most rapidly, followed by cultivars Redhaven, Candor, Vanity, Madison, and Earlired, respectively. Cultivar ranks for suberin accumulation rate were correlated significantly and negatively with the known field performance ranks ($r = -0.94$, $P \leq 0.01$) (Table 2). Cultivars that accumulated suberin more rapidly in these experiments had past field performance histories that indicated greater resistance to the peach canker fungi.

Rate of canker length inhibition is an estimate of the rate of limitation in canker length (in millimeters per day) that was observed as infection court age increased. The cultivars in this study were separated easily into three groups after analysis of covariance ($P \leq 0.001$) (Table 1). Ranks for rate of canker length

TABLE 1. Suberin accumulation rate, canker length inhibition rate, estimated days to resistance, phellem cell accumulation rate, suberized tissue thickness rate, and field performance rank for six peach cultivars inoculated with *Leucostoma personii* at 0, 7, 11, 13, and 15 days postwounding

Cultivar	Suberin accumulation rate (mV/day) ¹	Canker length inhibition rate (mm/day) ²	Estimated days to resistance ³	Phellem cell accumulation rate (cells/day) ⁴	Suberized tissue thickness rate (μ m/day) ⁵	Field performance rank ⁶
V68101	0.94 a ⁷	-3.14 a	17.0	0.58 a	1.51 b	1
Redhaven	0.80 b	-3.42 a	15.6	0.62 a	1.70 a	2
Vanity	0.73 bc	-1.83 c	29.1	0.43 b	1.39 b	3
Candor	0.78 bc	-2.32 b	23.0	0.62 a	1.04 d	4
Madison	0.72 bc	-2.32 b	23.0	0.65 a	1.20 cd	5
Earlired	0.69 c	-1.51 c	35.3	0.62 a	1.28 c	6

¹ b_1 parameter from the regression equation $Y = b_0 + b_1X$, where Y = suberin autofluorescence intensity and X = days. Value is the mean from 10 plants.

² b_1 parameter from the regression equation $Y = b_0 + b_1X$, where Y = canker length and X = age of the infection court. Value is the mean from 10 plants.

³ Value of X from the equation $X = (Y - b_0)/b_1$, where $Y = 6$, $b_0 = 59.3$, and b_1 = mean canker length inhibition rate.

⁴ b_1 parameter from the regression equation $Y = b_0 + b_1X$, where Y = number of phellem cells and X = days postwounding. Value is the mean from 10 plants.

⁵ b_1 parameter from the regression equation $Y = b_0 + b_1X$, where Y = thickness of suberized tissue and X = days postwounding. Value is the mean from 10 plants.

⁶ Relative susceptibility to *Leucostoma* spp. based on field observations; 1 = least susceptible and 6 = most susceptible.

⁷ Means within columns followed by the same letter are not significantly different according to paired t -tests ($P \leq 0.05$) performed following a significant analysis of covariance ($P \leq 0.05$).

TABLE 2. Spearman's rank correlation matrix for the variables described in Table 1

	Canker length inhibition rate (mm/day)	Estimated days to resistance	Phellem cell accumulation rate (cells/day)	Suberized tissue thickness rate ($\mu\text{m/day}$)	Field performance rank
Suberin accumulation rate (mV/day)	-0.84**	-0.84**	-0.39	0.54	-0.94**
Canker length inhibition rate (mm/day)	-0.09	-0.52	0.76*
Estimated days to resistance	-0.09	-0.52	0.76*
Phellem accumulation rate (cells/day)	-0.46	0.58
Suberized tissue thickness rate ($\mu\text{m/day}$)	-0.71

** indicates significance at $P \leq 0.01$; * indicates significance at $P \leq 0.05$.

inhibition were correlated significantly with ranks for suberin accumulation rate ($r = -0.84$, $P \leq 0.05$) and ranks for field performance ($r = 0.76$, $P \leq 0.05$). Results were similar for rates calculated from either the 14-day or the 21-day postinoculation data. In the present study, cultivars that accumulated suberin more rapidly exhibited larger rates of canker length inhibition. The correlation between rate of canker length inhibition and field performance rank suggests that the greenhouse performance of the cultivars adequately represented their field performance.

Estimated days to resistance was calculated with the rate of canker length inhibition and the mean length of cankers from 0-day-old infection courts. The calculated value is, therefore, an estimate of the number of days postwounding when an infection court would cease to be susceptible to infection. For the peach genotypes in this study, estimates ranged from 15.6–35.3 days (Table 1). Estimated days to resistance was correlated significantly with rate of suberin accumulation ($r = -0.84$, $P \leq 0.05$) and field performance ($r = 0.76$, $P \leq 0.05$). Cultivars that were estimated to achieve resistance in a shorter number of days exhibited faster suberin accumulation rates and were known to have a prior history of being less susceptible to *Leucostoma* species.

Cultivars varied significantly in the production of phellem cells ($P \leq 0.10$) and in the thickness of suberized tissues ($P \leq 0.001$). However, neither of the two anatomical parameters were correlated with suberin accumulation, canker length, days to resistance, or field performance (Table 2).

DISCUSSION

Historical observations of relative susceptibility of peaches to *Leucostoma* in the field were shown previously to be correlated with rates of suberin accumulation in the months of May and June (7). This relationship has to do, in part, with the fact that the more resistant plants become less susceptible more quickly after wounding than the susceptible plants. This type of resistance represents different levels of infection risk for different cultivars based on the duration of infection court susceptibility and the likelihood of an infection period occurring while the infection court is susceptible. Bostock and Middleton illustrated this last point in their study of *Ceratocystis* canker of almond (8), although they examined the wound reaction in only one cultivar. This study is the first to show that hosts vary significantly in the infection court and that this variation is related to disease resistance.

Data from this study and from recent work (7) suggest that host resistance is not due solely to rates of wound periderm regeneration after wounding. Rather, our data from both field (7) and greenhouse studies show that suberin accumulation is more important than the actual number of new phellem cells or the thickness of the new suberized layers. Therefore, it would be possible for a heavily suberized periderm composed of relatively few cells in thickness to be a more effective barrier to pathogen ingress than a periderm with more cells that is less heavily suberized. Mechanisms by which suberin could impart disease resistance have been suggested by Kolattukudy (13), including a barrier to diffusion of pathogen

enzymes or to 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.

Results of this study have potential for future practical application for control of peach canker disease. Knowledge of the duration of wound susceptibility, as influenced by host genotype and local environmental conditions (4), could be incorporated into a model designed to estimate the time required for fungicide protection of the infection court. Given that most woody plants examined in our laboratory respond to wounding in a manner similar to peach (3,6), the present demonstration of genetic variation in infection court properties may be relevant to investigations with other woody plant host-pathogen systems.

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