

HORTSCIENCE 20(5):903-905. 1985.

Suberin Deposition as a Measure of Wound Response in Peach Bark

A.R. Biggs¹ and N.W. Miles¹

Agriculture Canada, Research Station and Horticultural Research Institute of Ontario, Vineland Station, Ont., Canada LOR 2E0

Additional index words. impervious tissue, lignification, *Prunus persica*, *Cytospora* canker

Abstract. Seven peach clones were assessed for wound response using suberin deposition, lignification, and lignification + suberization as criteria. Suberin and lignin were measured fluorometrically using a new technique that permits unimpeded observation of suberin autofluorescence. Suberin deposition was first observed in cells of the impervious tissue which forms prior to phellogen generation at the wound. Suberin deposition measured as early as 7 days postwounding was negatively correlated with peach canker incidence.

The fungi which cause peach canker, *Leucostoma cincta* (Pers. ex Fr.) Hohn. and *L. persoonii* (Nits.) Hohn, are considered weak pathogens because they require wounds to initiate infection. The importance of wounds in the peach canker pathosystem was realized originally by Willison (9), and Weaver's studies (7) on defoliation provided evidence that peach varieties may possess different inherent levels of wound response. The work of Shigo and Marx (5), Shigo and Wilson (6), Wilson (10), and Wilson et al.

(11), has rekindled interest in wound response, particularly with regard to peach. A thorough understanding of wound response could provide insight into processes of pathogenesis and disease resistance, especially in weak parasite/wound interactions (3).

Wilson (10) suggested that selection toward efficient xylem compartmentalization would increase disease resistance in peach. Although xylem responses are significant, bark tissue responses also must be considered. Bark responses, generally described by researchers as "wound closure" or "callusing", are quite complex and should be included with studies on compartmentalization in an organismal approach to disease resistance. This research investigated clonal differences in suberin deposition in impervious tissue and necrophyllactic phellem at wounds and evaluated the use of suberin deposition to assess quickly and quantitatively nonspecific host plant responses associated with resistance.

Three trees each of 7 peach clones (V-68101, V75011, 'Ellerbe', HW233, 'Candor', and 2 Boone Co. seedlings) thought to represent a range of susceptibility to peach canker fungi were selected for wounding (N. Miles, unpublished data; R.E.C. Layne, personal communication). The field grown trees were of variable age, so an effort was made to wound only tissues of similar age (about 5 years old).

A 4-mm diameter cork borer was used to injure the phellogen and cortex to a depth of about 2 mm. Five wounds, 10 cm apart, were placed in a semi-spiral pattern on one scaffold limb per tree so that one wound was not directly above or below another wound. After 3, 7, 10, 14, and 17 days, the wounded areas were removed with a larger diameter cork borer to the depth of the cambium. Non-wounded tissues were taken at the beginning of the experiment and on the sample dates. Each bark disk was halved longitudinally, placed in FAA, dehydrated in t-butyl alcohol, embedded in paraffin, and sectioned with a rotary microtome at 8 μ m thickness. Ribbons were affixed to glass slides, deparaffinized in xylene, and mounted in glycerol or stained with toluidine blue O and mounted in Pro-Texx (Lerner Laboratories, New Haven, CT 06513).

Suberin autofluorescence was determined using methods described in detail elsewhere (1, 2). Briefly, tissues stained with toluidine blue O were examined under ultraviolet epillumination (340-380 nm excitation, 430 nm suppression). Toluidine blue O quenched lignin autofluorescence when used in conjunction with ultraviolet excitation. Residual autofluorescence under these conditions was due solely to suberin (2). Autofluorescence intensity was measured at 430 nm using a Leitz MPV compact microscope photometer with a HBO IOOW mercury burner and stabilized power supply. Photometer measure-

Received for publication 26 Nov. 1984. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Research Scientist.

Table 1. Clonal differences in suberin autofluorescence intensity at the site of phellogen generation in mechanically wounded peach bark cortical tissue.^z

Clone	Time postwounding (days) ^y				
	3	7	10	14	17
V68101	0.08 a ^x	5.19 a	9.42 a	14.08 a	18.49 a
V75011	0.00 a	1.76 ab	7.38 ab	11.98 a	16.27 a
Boone Co.-1	0.09 a	1.09 ab	4.88 ab	9.29 ab	14.78 a
Boone Co.-2	0.06 a	0.99 ab	3.81 b	11.20 ab	14.52 ab
Ellerbe	0.06 a	1.08 ab	4.10 b	6.93 bc	13.91 ab
HW233	0.14 a	0.44 b	2.72 b	5.96 bc	11.01 b
Candor	0.34 a	0.47 b	2.10 b	5.48 c	10.86 b

^zAutofluorescence intensity measured over a circular area with 272 μm diameter, each measured area contained about 100 cells. Values represent autofluorescence intensity of boundary zone (impervious tissue) or boundary zone plus necrophylactic phellem depending on wound age. Nonwounded bark autofluorescence intensity = 0.

^yEach value represents the pooled data from 3 replications (June, July, and August) with 3 trees per clone and 5 measurements per tree.

^xDifferent letters in columns denote significant differences separated using orthogonal comparisons ($P = 0.05$).

ments were taken to quantitate total boundary zone and suberin autofluorescence. Nonsuberin (presumably lignin) autofluorescence was obtained by subtraction.

The wound series on the 7 clones was repeated 3 times (12 June, 10 July, and 1 Aug. 1984). Each repetition was performed on a different scaffold limb on the same 3 replicate trees of each clone. Each photometer observation represented an average of 5 measurements taken on serial sections from one slide. Final autofluorescence intensity was calculated by subtracting the fluorescence intensity of nonwounded control tissue from the fluorescence intensity of the wounded tissue.

All scaffold limbs proximal to the wound series were free of *Cytospora* cankers. Cankers distal to the wound sites were effectively compartmentalized during the growing season and presumed not to have a significant influence on wound response. Total numbers of cankers distal to the wounds were counted at the end of the experiment and used to rank the 7 clones. The 7 clones were also ranked at each wound age according to the pooled data for suberin and nonsuberin autofluorescence intensity and Spearman's r was used to assess the correlation between autofluorescence intensity and number of cankers. The autofluorescence intensity data were

evaluated using a 3 factor analysis of variance with replication x treatment interactions pooled as an estimate of error. Single degree of freedom sums of squares (orthogonal comparisons) were calculated to clarify interpretation of significant interaction effects.

For total boundary zone autofluorescence, suberin autofluorescence, and lignin autofluorescence, the main effects (except replication), first order interactions, and the 2nd order interaction were significant ($P = 0.01$). Examination of wound date (month) interactions with postwounding time (wound age) and clone revealed the importance of increased intensity of lignin and suberin autofluorescence during August, particularly at 10, 14, and 17 days postwounding. The role of clone in the 2nd order interaction was apparent where V68101 showed significantly greater autofluorescence intensities relative to 'Candor' during August when compared to June or July. Wound date (month) was not associated with any significant shifts in relative clonal response; therefore, the data were pooled for presentation in Tables 1 and 2.

Clonal differences in suberin autofluorescence intensity were observed at 7 days postwounding, with clone V68101 exhibiting greatest suberin deposition (Table 1).

Table 2. Clonal differences in non-suberin (lignin) autofluorescence intensity at the site of phellogen generation in mechanically wounded peach bark cortical tissue.^z

Clone	Time postwounding (days) ^y				
	3	7	10	14	17
V68101	1.64 a ^x	15.31 ab	24.74 a	28.41 a	29.94 ab
V75011	1.60 a	14.07 ab	25.68 a	25.75 a	27.15 ab
Boone Co.-1	1.73 a	16.42 a	24.64 a	26.41 a	32.81 a
Boone Co.-2	1.94 a	13.83 ab	20.71 a	26.59 a	34.53 a
Ellerbe	2.56 a	9.95 ab	17.70 a	19.84 a	24.14 ab
HW233	1.56 a	8.83 ab	19.13 a	19.03 a	21.99 bc
Candor	1.42 a	5.91 b	15.87 a	19.28 a	21.67 c

^zAutofluorescence intensity measured over a circular area with 272 μm diameter, each measured area contained about 100 cells. Values represent autofluorescence intensity of boundary zone (impervious tissue) or boundary zone plus necrophylactic phellem depending on wound age. Nonwounded bark autofluorescence intensity = 0.

^yEach value represents the pooled data from 3 replications (June, July, and August) with 3 trees per clone and 5 measurements per tree.

^xDifferent letters in columns denote significant differences separated using orthogonal comparisons ($P = 0.05$).

Subsequent postwounding assessment showed that this clone continued rapid suberin deposition relative to other clones, particularly 'Ellerbe', HW233, and 'Candor'.

Table 2 shows clonal differences in non-suberin (lignin) autofluorescence intensity. Lignin deposition showed fewer significant differences among clones than suberin deposition because of increased variation. At 7 days postwounding, Boone County seedling source No. 1 exhibited the highest autofluorescence intensity for lignin; whereas 'Candor' exhibited the lowest. Clone V68101 was consistently highest in suberin autofluorescence intensity. For lignin autofluorescence intensity, no one clone was consistently high. The clones which supported highest lignin autofluorescence intensity generally supported the highest suberin deposition at 7, 10 and 14 days postwounding ($r = 0.86, 0.82, 0.82$, respectively, $P = 0.05$). This relationship was not significant at 3 or 17 days postwounding.

The total numbers of cankers distal to the wound treatments for each clone were ranked and compared to the clonal rankings of suberin and nonsuberin autofluorescence intensity (Table 3). The correlation between suberin autofluorescence intensity and canker number is negative ($P = 0.01$) when measured at 7, 10, or 17 days after wounding. Lignification, as measured by nonsuberin autofluorescence intensity, was not correlated with the canker rating, except at 10 days postwounding ($P = 0.05$).

The rate of impervious tissue formation and of phellogen generation at wounds could be useful indicators of cultivar susceptibility to the peach canker fungi. These findings and those of Wensley (8) agree that wound closure was related to field performance. Wound response assessments using suberin autofluorescence can be made over a shorter time period than that used in previous studies where wound closure (callus formation) was assessed.

Clone V68101, which suberized rapidly, has Chinese parentage [(New Jersey Cling 1 x Hui Hun Tao) open pollinated.] A vigorous examination of Asian germplasm is needed, and conservation of this large source of genetic diversity is essential (4).

Table 3. Spearman's correlation coefficient for pooled suberin and nonsuberin autofluorescence intensity versus number of cankers on peach scaffold limbs distal to wound treatments.^z

Wound age (days)	Spearman's r	
	Nonsuberin autofluorescence intensity	Suberin autofluorescence intensity
3	- 0.803 ^y	- 0.807
7	- 0.793	- 0.921**
10	- 0.878*	- 0.950**
14	- 0.835	- 0.907*
17	- 0.779	- 0.921**

^zRanks for nonsuberin and suberin autofluorescence intensity by wound age for seven peach clones versus ranks for total number of cankers on all wood distal to the wound treatment area.

^ySpearman's r ; *** = significant correlation at $P = 0.05$ and 0.01.

The experimental approach described in the present study must not be confused with pathogen inoculation studies. The rapid wound response of clone V68101 relative to HW233, for example, demonstrates that one clone rapidly reestablishes a preformed barrier responsible for disease resistance. Where the time the wound is open (infectible) is decreased, the probability for infection is decreased, and this is reflected in fewer cankers. This argument presumes that both a susceptible clone and a less susceptible clone, in the absence of wounds, would appear resistant. The literature does not contradict this view. Reestablishment of suberin continuity as measured by suberin autofluorescence intensity in impervious tissue and necrophylactic periderm, could be an effective measure of plant susceptibility to peach canker fungi. Studies are in progress to determine the relationship, if any, between xylem and bark responses. Xylem ray cell suberization, as-

sessed with techniques described herein, may provide an accurate measure of compartmentalization, where gumming and discoloration are too variable to be useful.

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