Heritability of Suberin Accumulation in Wounded Peach Bark

A. R. Biggs, N. W. Miles, and R. L. Bell

West Virginia University, University Experiment Farm, P. O. Box 609, Kearneysville 25430; Horticultural Research Institute of Ontario, P. O. Box 7000, Vineland Station, Ontario, Canada L0R 2E0; and USDA-ARS, Appalachian Fruit Research Laboratory, Kearneysville, WV 25430.

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ABSTRACT

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Controlled reciprocal crosses among peach clones V68101, V68051, and New Jersey Cling 95 (NJC95) were performed during spring 1985. Parental clones and seedlings from the crosses were planted at two sites in May 1986. Wounding studies were conducted during June 1989 to quantify suberin accumulation in healthy phloem/cortex tissues adjacent to the wound site. Differences in suberin accumulation among the three parent clones were significant, whereas all environmental sources of variation and their interactions with parent clones were not significant. Suberization of V68101 was significantly greater than that of NJC95 and V68051, which did not differ from each other. Suberization responses in the three families involving V68101 were not significantly different from each other, and the remaining three families also were not significantly different from each other. The full-sib family heritability of 0.93 $\pm$ 0.31 reflects the significance of differences among full-sib family means. For hybrids, the individual tree heritability of 0.56 $\pm$ 0.24 indicates that approximately 55% of the phenotypic variability is accounted for by genotype. Genetic factors are sufficiently large to allow for selection for increased suberization response.

Woody plants are susceptible to injury and subsequent infection by a variety of microorganisms. In the deciduous tree fruit-growing region of northern North America, a canker disease of peach (*Prunus persica* (L.) Batsch), caused by the fungi *Leucostoma persoonii* Höhn. (anamorph = *Cytospora leucostoma* Sacc.) and *L. cincta* (Fr.:Fr.) Höhn. (anamorph = *C. cincta* Sacc.), is particularly destructive. The disease appears as perennial cankers on trunks, scaffold limbs, and branches, and it causes crop losses mainly through reduction in bearing surface and premature tree death. All currently grown peach cultivars are susceptible to these pathogens (13), and no known treatment will prevent infection over the long term (4). The pathogens initiate disease in wounds created by pruning, leaf abscission, winter injury, and insect damage (18).

Interest in wound responses of trees is a reflection of the importance of canker and decay diseases that are considered to be major limiting factors in forest, urban, and orchard tree management and production throughout the world. Improving insect and disease resistance of woody plants through traditional breeding is a viable, yet long-term, supplement to chemical crop protection and silvicultural methods. However, traditional breeding methods with trees often are hampered by several factors, including the long-term nature of tree life cycles, the labor-intensive methods of data collection, and land-intensive requirements for progeny testing. In addition, durable long-term resistance in trees is most likely to be polygenic (9), thus confounding efforts of both traditional and molecular breeding

approaches. Nevertheless, there has been renewed interest in recent years in the development of trees with increased genetic resistance to fungal and bacterial pathogens. One aspect of tree defense mechanisms that appears to be under genetic control is the wound compartmentalization process (sensu Shigo: 14) that occurs after injury to the xylem tissues (8,10,11,15). The wound response process is particularly important in regard to pathogens that invade the healthy plant through wounds or natural openings. Peach canker is only one of many wound diseases that can potentially be controlled or managed by improved use of host resistance related to the deposition of structural barriers to pathogen ingress.

A rapid, reliable, and economically efficient method to detect pathogen resistance would expedite development of new peach cultivars with resistance to *Leucostoma* spp. Recent research has shown that suberin deposition rate in mechanical wounds of bark cortex tissue is significantly correlated with the known relative susceptibility of peach cultivars and clones to the peach canker fungi (6). In addition, suberin accumulation rate in bark cortex was shown to be correlated with the relative susceptibility of peach cultivars and clones in inoculation studies with *L. persoonii* (5). It is clear that intraspecific variation exists in peach for suberin accumulation and that this variation is related to relative susceptibility to the peach canker pathogens. Knowledge of the natural variation and the degree to which suberin accumulation rate is transmitted from parent to progeny is essential if this character is to be considered part of a breeding program for increased resistance to peach canker disease. However, there are no references in the literature on the heritability of this trait. Therefore, the objective of this study was to determine the heritability of suberin accumulation rate in wounded peach bark tissue.

MATERIALS AND METHODS

Controlled reciprocal crosses among clones (Vineland clones are designated with the prefix V) of V68101, V68051, and New Jersey Cling 95 (NJC95) were performed during spring 1985. Trees of the three clones from which crosses and selfs were obtained were covered with tents to insure the occurrence of purebred lines. Parental clones were asexually propagated by budding onto Boone County rootstocks. Seeds from parents and crosses were collected in August 1985, embryo-cultured in the laboratory (16), transferred to peat pots, and grown in the greenhouse for about 7 wk (16-h photoperiod, 25–27 C during the day, 18–20 C at night). The seedlings, approximately 46 cm in height, were hardened in a cold frame for 8 to 10 days in preparation for field planting in late May. Seedling orchards, including the asexually propagated parental clones, were established at the Agriculture Canada experimental farm in Jordan Station, Ontario (Jordan), and at the experimental farm of the Horticultural Research Institute of Ontario in Vineland Station, Ontario (Victoria), in May 1986. 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. Tree spacing was 1.4 × 4.0 m at both locations. Fungicides and insecticides were applied at labeled rates uniformly at both locations as needed to control peach leaf curl, brown rot, and oriental fruit moth (ferbam, captan, phosmet, respectively). The seedlings and parental clones were planted in four randomized complete blocks with four replicate full-sib seedlings, for each of the nine crosses (families) and three parental clones, per block at each orchard site. The four seedlings of each cross were planted together as a plot within each block. Because some crosses yielded a high proportion of infertile seeds, some plots were established with fewer than four replicate full-sib seedlings. Empty spaces were filled in with trees of other crosses; data from those trees are not presented here.

Wounding studies were conducted during June 1989 to quantify suberin accumulation in healthy phloem/cortex tissues adjacent to the wound site. Wounds to the depth of the xylem were created with a 4-mm-diameter cork borer on 1-yr-old twigs. Samples for suberin measurements were taken 14 days after wounding. A portion of the stem supporting the wound was removed with pruning shears and was trimmed to about 5 mm above and below the wounded area. The trimmed stem portion then was divided into four pieces through the wound site with longitudinal and transverse razor blade cuts, and all pieces were placed in Formalin/acetic acid/alcohol (FAA) fixative (5). Only one of the four pieces was used for further examination. Tissues fixed in FAA were dehydrated and embedded in paraffin in transverse orientation (1). Sections were affixed to glass slides, the paraffin was removed with xylene, and the sections were stained with toluidine blue O and mounted in a nonautofluorescent medium (2). Sections were examined with fluorescence microscopy, and the total residual autofluorescence intensity (in millivolts) after staining, due to suberin, was determined in the outer bark cortex with a Leitz MPV compact microscope photometer (2). Suberin measurements were taken from three serial sections per slide, and these were averaged to obtain the final suberin value for the sample.

Data on suberin accumulation were analyzed with the general linear models (GLM) procedure assuming a random effects model and type III sums of squares for unequal cell sizes (12). Orthogonal contrast comparisons were used to test the significance of parental reciprocity in the initial analyses. Homogeneity of error variances among families and parents were tested using Bartlett's test (17). The test rejected the hypothesis of equal variances, but log and square root transformations failed to result in homogeneity. Therefore, nontransformed data were used in subsequent analyses.

TABLE 1. Sources of variation and expected mean squares for the random effects model describing suberin accumulation in 14-day-old wounds on 1-yr-old peach bark tissues

Source of variation	Expected mean squares
Site	$\sigma^2 + k_1\sigma^2_{gb(s)} + k_2\sigma^2_{gs} + k_4\sigma^2_{b(s)} + k_5\sigma^2_{s}$
Block (site)	$\sigma^2 + k_1\sigma^2_{gb(s)} + k_4\sigma^2_{b(s)}$
Genotype[y]	$\sigma^2 + k_1\sigma^2_{gb(s)} + k_2\sigma^2_{gs} + k_3\sigma^2_{g}$
Genotype × site	$\sigma^2 + k_1\sigma^2_{gb(s)} + k_2\sigma^2_{gs}$
Genotype × block (site)	$\sigma^2 + k_1\sigma^2_{gb(s)}$
Residual error[z]	σ^2

[y] Genotype (g): either parental clones, or selfed or hybrid families, depending on the analysis.
[z] The nature of the residual error varies according to the nature of the genotype source of variation.

TABLE 2. Analyses of variance for suberization for parents and selfed and hybrid families[z]

	Parents				Selfs				Hybrids			
Source of variation	df	MS	Var	$P>F$	df	MS	Var	$P>F$	df	MS	Var	$P>F$
Site	1	7.22	0.18	0.98	1	1.72	0.00	0.50	1	37.78	0.35	0.10
Block (site)	6	3.18	0.00	0.56	6	2.40	0.07	0.29	6	7.39	0.23	0.13
Genotype	2	17.38	0.59	0.04	2	16.88	0.54	0.10	2	44.07	0.86	0.07
Genotype × site	2	0.67	0.00	0.84	2	2.06	0.00	0.34	2	3.36	0.00	0.42
Genotype × block (site)	12	3.80	0.37	0.20	12	1.72	0.03	0.49	12	3.59	0.34	0.05
Residual error	65	2.76	2.68		64	1.78	1.74		132	1.95	1.89	

[z] Degrees of freedom (df), mean squares (MS), variance components (Var), and probability (P) of significance for random effects models describing suberization in 1-yr-old peach bark 14 days after mechanical wounding in May 1989.

There was no apparent relationship in within-family variances between selfed and hybrid families.

The data set of seedling families included hybrids and those which resulted from self-pollination of the parents. Parental clones were analyzed separately. Because variance components for selfed and full-sib (i.e., hybrid) families have different genetic expectations (7), heritabilities were estimated separately for these two groups. The data were analyzed with the SAS GLM procedure, and variance components were estimated with the SAS variance components (VARCOMP) procedure (12). The sources of variation and their expected mean squares are given in Table 1. All sources of variation were assumed to be random. SAS type III sums of squares were computed because of unequal numbers of observations in the location-replication-population cells. Means were separated with the Waller-Duncan *k*-ratio procedure (12).

Clonal repeatability of parental means (7) was estimated from the variance components as

$$r_c = V_c/[V_e + V_{cs} + V_{cb(s)} + V_c],$$

where r_c is the clonal repeatability computed on a single tree basis, V_e is the variance due to individual parental trees within each plot, V_{cs} is the variance due to parental clone × site interaction, $V_{cb(s)}$ is the variance due to the interaction of clone × block within site, and V_c is the variance due to differences among parents. Clonal repeatability is a measure of the proportion of total phenotypic variability due to differences among parental clones and is an estimate of the total genetic variance.

Heritability was computed from the variance components as

$$h^2 = 2V_f/[V_t + V_{fs} + V_{fb(s)} + V_f],$$

where h^2 is the heritability computed on a single tree basis (7), V_t is the variance due to individual seedling trees within each block (this includes components due to genetic differences among seedlings, environmental differences within plots [estimable only from clonal data], and covariance due to common environment), V_{fs} is the variance due to family × site interaction, $V_{fb(s)}$ is the variance due to the interaction of family × block within site, and V_f is the variance due to differences among families. Standard errors of heritability estimates were computed according to Falconer (7).

The heritabilities of full-sib and selfed family means were computed from the equation

$$h^2 = V_f/[V_f + V_{fs}/s + V_{fb(s)}/bs + V_t/nbs],$$

where n is the number of seedlings per family, b is the number of blocks, and s is the number of sites (19).

RESULTS AND DISCUSSION

Differences in suberin accumulation among the three parent clones were significant, whereas all environmental sources of variation and their interactions with parent clone were not significant (Table 2). Suberization of V68101 was significantly greater than NJC95 and V68051, which did not differ from each other (Table 3). The two parental clones from Vineland are both derived from a seedling of the cross NJC1 × Hui Hun Tao, hereafter referred to as NJC1HHT. V68101 resulted from open-pollination of NJC1HHT, and therefore, there is a 70% probability that V68101 is the result of self-pollination. V68051 is a seedling of Babygold7 × NJC1HHT. The difference in the response of these selections could have several alternative explanations. If V68101 resulted from outcrossing rather than self-pollination, then the original source of the increased suberization is an unknown pollen parent. If NJC1HHT possessed the responsible alleles, then the reduced response of V68051 must be due to segregation and pollen production. The clonal repeatability, expressed on a percentage basis, was estimated at 16% due to the large relative importance of differences among individual trees within plots. The standard errors of the parental clones were relatively large when compared with those of the seedling families (Table 3).

TABLE 3. Mean and standard error (SE) for suberin accumulation values (in millivolts) from 14-day-old cortex/phloem wounds on 1-yr-old peach twigs for nine families (reciprocal crosses and selfs) and three parental clones

Parent	*N*	Mean	SE	Family[y]	*N*	Mean	SE
V68101	32	7.9 a[z]	0.09	V68101 × NJC95	47	7.5 a	0.06
NJC95	27	6.9 b	0.12	V68101 selfed	28	7.4 ab	0.06
V68051	29	6.4 b	0.09	V68101 × V68051	49	7.3 ab	0.05
				NJC95 selfed	29	6.2 bc	0.06
				V68051 selfed	31	6.0 c	0.07
				V68051 × NJC95	60	5.8 c	0.04

[y] In an earlier analysis (data not shown), it was determined that parental reciprocity was not significant. Therefore, reciprocals were pooled for this analysis.

[z] Letters denote significant differences determined with Waller-Duncan's *k*-ratio *t* test.

In a preliminary analysis (data not presented), differences among reciprocal crosses were not significant and, therefore, their data were combined for subsequent analyses. Differences among families were significant, as was the interaction of family means with block within site (Table 2). The three V68101 families were not significantly different from each other in suberization response, and the remaining three families also were not significantly different from each other (Table 3). This reinforces the conclusion that the differences observed among the parents are genetic and are transmitted to seedling progenies.

The heritabilities of full-sib family means and selfed family means were calculated to be 0.93 ± 0.31 and 0.96 ± 0.42, respectively. These values are artificially high, probably due to the amount of inbreeding in all families. The magnitude is, however, indicative of the significance of differences among full-sib family means and among selfed family means. If selection is practiced among families, the rate of genetic progress would be great. However, in fruit tree breeding, individual or mass selection usually is practiced, and the heritability of individual tree values is preferred for practical purposes.

The individual tree heritabilities were 0.56 ± 0.24 and 0.47 ± 0.29 for full-sib and selfed families, respectively. The magnitudes of the standard errors are due, in part, to the small number of families used in this study. The estimates of heritability are to be considered preliminary and, as is customary, valid for only the populations produced for this study. These heritability estimates are biased upward by inbreeding. If the bias is not great, genetic factors are sufficiently large to allow selection for increased suberization response.

Other components of nonspecific defense mechanisms in trees have been demonstrated to be under genetic control. However, it should not be assumed that the various bark and xylem resistance mechanisms are controlled by similar sets of genes. Indeed, it was demonstrated previously that bark and xylem wound responses in peach appeared to be independent (3). In addition, the results of Shigo et al (15) suggested that wound closure is a separate process from xylem compartmentalization in hybrid poplar. Lowerts and Kellison (10) demonstrated that resistance to discoloration and decay in wounded yellow poplar was under moderately strong genetic control, with a family heritability estimated at 0.57. They suggested that the large amount of tree-to-tree variation and the moderately high single tree heritability estimate would allow the largest genetic gains to be made from a program of within-family selection. This observation also appears to be applicable to our findings regarding selection for resistance to bark pathogens.

The heritability of suberin deposition in wounded cortex/phloem tissues has been demonstrated for peach. Breeding and selection programs to develop superior peach cultivars could include this wound reaction among the selection criteria.

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