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GENETIC DIFFERENCE IN *LEUCOSTOMA* CANKER RESISTANCE IN A DIVERSE PEACH POPULATION

by

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Abstract

Genetic Difference in Leucostoma Canker Resistance in a Diverse Peach Population

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Progress was made towards identifying and characterizing resistance to Leucostoma canker caused by Leucostoma persoonii in peach. High levels of resistance to both field and excised shoot inoculation were identified in open-pollinated progeny of the Russian Plant Introduction "Yennoh" and "NJ672017002".

Eight peach clones that represented 3 distinct groups, susceptible, intermediate, and resistant in terms of Leucostoma infection, were selected to measure the levels of xylem dysfunction induced by infection. The resistant clones were able to maintain adequate water transport through the canker infection zone during the summer in contrast to susceptible clones. Surviving vascular cambium evident in resistant clones subsequently differentiated new xylem and phloem to replace the damaged tissues.

The quantity of lignin formed in response to wounding of the bark and 3-4 mm depth of wood was measured using an assay of ligninthioglycolic acid (LTGA). The lignin content in the resistant clones was twice that of susceptible clones providing evidence that lignification was the mechanism of disease resistance in dormant trees.

Heritability of resistance to Leucostoma canker was quantified on a diverse range of peach genotypes by (1) partitioning the variance components in the least squares statistical method, and (2) determining parent-offspring regression. The two methods provided very similar estimates of the narrow sense heritability for canker necrotic length; 0.65 and 0.72, respectively. The estimates indicate that superior resistant genotypes can be identified in a breeding population to increase Leucostoma resistance.

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Guidance committee:

The journal paper format was chosen for this thesis in accordance with departmental and university regulation. The thesis is divided into five chapter. Chapter 1 was published in the Journal of the American Society of Horticultural Science. Chapter 2 has been published in HortScience. Chapter 3 and Chapter 4 will be submitted to Plant Disease. Chapter 5 is intended for publication in HortScience.

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INTRODUCTION

Leucostoma canker has been recognized as the most serious disease reducing peach tree life in Michigan and other northern peach growing areas. The fungal pathogens involved, Leucostoma persoonii Fr. (Nits.) Hohn and Leucostoma cincta (Pers, ex Fr.) Hohn, infect through dead or damaged tissues. Symptoms include cankering of the trunk and branches, branch dieback, progressive weakening, and ultimately death of the tree. Usually the combined influence of low temperature stress and Leucostoma canker is greater than the effect of either acting alone.

Although peach cultivars differ in their resistance to Leucostoma canker, no highly resistant selections have been identified in previous studies. This is due to the lack of a large-scale effort to identify resistance to Leucostoma in a broad-based population of peach. Therefore, we initiated a program to identify resistant selections which could be used for peach breeding. Because cold injury predisposes peach trees to Leucostoma infection, cold hardy cultivars might not only decrease the Leucostoma infections but also increase the capacity to limit disease progression.

Chapter 1 describes an evaluation of a broad

collection of peach germplasm, for Leucostoma resistance following inoculation, and for cold hardiness in the Michigan climate. The goal was to identify useful genetic material for breeding peach for Leucostoma resistance.

The screening procedures described in chapter I required an effort spanning 7 months. To avoid this long-term time commitments, and concomitant introduction of copious infections into the plantings, a quick screening technique to evaluate Leucostoma canker resistance in-vitro was developed. This is described in chapter II.

Attempts have been made to understand the causes of death of branches following Leucostoma infection. The levels of xylem dysfunction due to the Leucostoma invasion into the wood were measured in the study described in chapter III. The susceptibility to Leucostoma was related to the plants failure to maintain sufficient water transport though the zone of infection. this restriction on transport consequently caused wilting and death of branches.

The cultivars differed in their levels of resistance to Leucostoma. However, the mechanism of host resistance in the highly resistant cultivars was unknown. Therefore an investigation was undertaken of selections of Yennoh (1-39) and NJ672017002 (1-8) which had been identified as highly resistant to Leucostoma infection. These genotypes were examined to quantify the accumulation of lignin in response

to wounding. Significant differences in lignin content in selected clones and peach cultivars were measurable during cold temperature acclimation. Chapter IV describes these new findings indicating that lignification is the mechanism of host resistance functioning during the peach dormant season.

The purpose of the study described in Chapter V was to estimate the heritability of Leucostoma resistance on this diverse peach germplasm collection. The estimate of the narrow sense heritability was about 0.65 which could be used to discriminate superior genotypes resistant to Leucostoma infection and select useful breeding materials.

CHAPTER ONE

Leucostoma persoonii Tolerance and Cold Hardiness among Diverse Peach Genotypes

Abstract

Open-pollinated progeny from 15 peach (Prunus persica) cultivars, two peach x P. kansuensis hybrids, and one peach almond (P. amygdalus) hybrid were evaluated for their cold hardiness and for tolerance to Cytospora canker following artificial inoculation with Leucostoma persoonii. Winter hardiness was negatively correlated with canker necrotic length (r = -0.26**) and positively correlated with canker rating (r = 0.26**), as indicated by qualitative ratings. The half-sib families differed for canker necrotic length following fall inoculation, indicating that individuals with increased tolerance to L. persoonii canker could be selected from the population. Progeny from the cultivar 'Yennoh' exhibited the shortest canker necrotic length following fall inoculation, and all the inoculated branches were visually healthy. 'Yennoh', a plant introduction from Russia, may have a higher tolerance to Leucostoma than has previously been found in U.S. germplasm.

Introduction

Cytospora canker, caused by Leucostoma persoonii Fr. (Nits.) Hohn. and Leucostoma cincta (Pers. ex Fr.) Hohn., is

the most serious disease reducing peach life in Michigan, New York, New Jersey, and Colorado (7). Symptoms include cankering of the trunk and branch dieback, progressive weakening, and, ultimately, death of the tree. Leucostoma is a pathogen that enters through dead and damaged tissues. Pruning cuts that do not callus properly are ideal Leucostoma entry sites. Winter injury that results in dead and damaged tissue predisposes that tree to Leucostoma invasion (1). Usually the combined influence of low temperature stress and Cytospora canker is greater than the effect of either acting alone.

There has been no large-scale effort to identify genetic resistance or tolerance to Leucostoma in a broad-based population of peach (4). Cultivars do, however, differ in their level of tolerance to Leucostoma, but no highly tolerant selections have been identified (2,6). Various inoculation techniques have been investigated for screening peach for Leucostoma tolerance, but these techniques have not been used to screen a diverse peach germplasm collection (5,8).

Because cold injury predisposes peach trees to Leucostoma infection, it is important to determine the hardiness of the trees inoculated in a screening program. Well-acclimated, hardy cultivars not only should have reduced infection, but also have increased capacity to

combat disease progression. The objectives of this study were to evaluate a broad collection of peach germplasm for L. persoonii disease development following inoculation and for cold hardiness in the Michigan climate to identify useful genetic material to breed for L. persoonii tolerance.

Materials and Methods

In Spring 1984, open-pollinated peach seedlings from 15 peach clones of diverse background, two peach x P. Kansuensis hybrids, and one peach almond (P. amygdalus) hybrid (Table 1) were planted in a completely randomized design at the Horticultural Research Center, East Lansing, MI. Open-pollinated progeny, instead of clones, were used to maximize the genetic diversity in the population to be screened. These seedlings represent half-sib families with progeny numbers ranging from 3 to 73. On 22 Oct. 1985, a 2-year-old branch on each seedling was inoculated with 20 ul of a suspension of 10^7 Leucostoma persoonii conidia per milliliter derived from isolates collected from cankers on peach at Clarksville and Hartford, MI. A wound-freezing inoculation technique developed by Scorza and Pusey (8) was followed. A second branch on each seedling was inoculated the following day.

On 21 Nov., two 1-year-old shoots were collected from each seedling and placed in a freezing chamber and chilled

Table 1. Parents of the 18 clones used in the Leucostoma/cold-hardiness screening program.

Clone	Parents
Babygold 8	PI35201 x Ambergen
Canadian Harmony	Redskin x Sunhaven
Elberta	Chinese Cling (open-pollinated)
Glohaven	(J.H. Hale open-pollinated) x Kalhaven
Harken	Redskin x Sunhaven
Loring	Frank x Halehaven
Reliance	(Minn. PH04559 x Meredith) open-pollinated
Red Hale	Unknown
Yennoh	Plant Introduction from Russia
B8-11-147	(K82 x Sunrise) x [(Red C x NJ191) x Okinawa]
B8-20-171	(5110417 x Ta Tao 3) x C2R31T45
B8-21-20	Orange Cling x RR65-1
C2-28-89	Kasna Dupnishka open-pollinated
C4-11-97	peach x almond
NJ257	Honeydew Hall x Jefferson
NJN69	(NJN55 x NJC68) x Marzochella
NJ672017002	(PI35321 x Cherryred) x Prunuskansuensis
RR37-15	NJ174 x Prunuskansuensis

to a critical temperature of -22C at a cooling rate of 4C/hr. The critical temperature was determined the previous week as the temperature required to cause cambium death of 50% of two 1-year-old shoots from 13 'Redhaven' trees. One week later, the cambium from each shoot was examined and rated as dead (0) or alive (1). Three additional hardiness evaluations were made on 9 Jan., 24 Feb., and 4 Apr. 1986, with critical temperatures of -29.5C, -28C, and -23C, respectively.

On 19 May, when the trees were beginning to leaf out, all the inoculated branches were measured for canker necrotic length (length of necrotic area distal to the point of inoculation) and branch diameter. Additionally, inoculated branches were rated visually for canker using a scale where 1 = dead, 2 = severe wilting of expanding leaves, 3 = weak growth and slight wilting of expanding leaves and 4 = healthy.

On 26 May 1986, two branches on each seedling were inoculated with L. persoonii as described earlier. The resulting cankers were evaluated for canker necrotic length and canker necrotic length/branch cross-sectional area on 19 Sept. 1986.

A second fall inoculation was made on 8 Oct. 1986 and evaluated on 30 apr. 1987. A cold-hardiness evaluation using two 1-year-old shoots per seedling as described earlier was

performed on 18 Feb. 1987 (critical temperature = -25C). A second spring inoculation was made on 26 Apr. 1987 and evaluated on 21 Sept. 1987.

For the inoculation and hardiness experiments, all the progeny from all 18 families were evaluated; however, because equal progeny numbers are required to statistically conduct mean comparisons, from each family with 20 or more progeny, 20 progeny were chosen at random for use in the statistical analysis, which was a nested design. Since the canker and cold-hardiness rating was nonparametric data, the Kruskal and Wallis test (9) was used to test for significant differences among families. Mean comparisons of these nonparametric data are not appropriate.

Result and Discussion

Canker necrotic length was positively correlated with canker necrotic length/branch cross-sectional area (r = 0.60**). for the 693 open-pollinated peach seedlings following inoculation on 22 Oct. 1985 (Table 2). Therefore, only canker necrotic length data will be presented in Tables 3 and 4. Both canker necrotic length and canker necrotic length/branch cross-sectional area were negatively correlated with canker rating. Canker necrotic length and canker length/branch cross-sectional area were also negatively correlated with cold hardiness on all four

Table 2. Correlation coefficients between Leucostoma infection canker necrotic length and canker rating[y] following inoculation on 22 Oct. 1985 and cold hardiness in a population of 693 open-pollinated peach seedlings from 18 cultivars. Cold hardiness[x] was evaluated on the following dates: 20 Nov. 1985, 9 Jan., 24 Feb., and 3 Apr. 1986.

Length/branch Trait	Canker symptom rating	Canker necrotic length	necrotic section area	Date of cold treatment Nov. 1985	Jan. 1986	Feb. 1986	Apr. 1986
Canker rating							
Canker necrotic length		-0.42**					
Canker necrotic length/branch x section area		-0.34**	0.60**				
Nov. 1985		0.05	-0.14**	-0.06			
Jan. 1986		0.15**	-0.16**	-0.06	0.11**		
Feb. 1986		0.19**	-0.16**	-0.06	0.09**	0.34**	
Apr. 1986		0.18**	-0.17**	-0.19**	0.04	0.16**	0.14**
x over four cold treatments		0.26**	-0.26**	-0.15**	0.35**	0.70**	0.70**	0.61**

[x] The critical temperatures were: -22C, -29.5C, -28.5C, -23C, respectively; 0=dead, 1=alive.

[y] Visual rating: 1=dead, 2=severe wilting, 3=weak growth and slight, 4=healthy.

[z] **Significant at P=0.05 and 0.01%, respectively.

sampling dates, confirming the negative association between canker development and cold hardiness. The sum of the cold hardiness values from the four dates was positively correlated with cold-hardiness values on each of the individual dates. The correlations obtained from the Feb. 1987 hardiness evaluation and the 9 Oct. 1986 inoculation (data not presented) were similar to those presented in Table 2.

There were significant differences between half-sib families for canker necrotic length and canker rating following fall inoculation, and for cold hardiness (Table 3). The data are presented as means over 2 years, since the year by half-sib family interaction was not significant. Progeny half-sib family mean values for canker necrotic length following the October inoculations ranged from 8.8 to 14.4 cm. Progeny from C2-28-89 and 'Loring' had the longest mean canker necrotic length and one of the lowest cold-hardiness ratings. Progeny from 'Baby-gold 8' had the shortest mean canker necrotic length and the largest number of healthy branches following inoculation. The data presented represent half-sib family means; however, within some of the more-tolerant families, individuals with small canker necrotic lengths and healthy branches following inoculation could be selected. For example, 'Babygold 8' open-pollinated progeny number 1-30 had a mean canker

Table 3. Mean values and coefficients of variation for L. persoonii canker necrotic length and combined canker ratings and winter hardiness ratings from artificial inoculations in the fall of 1985 and of 1986 of 20 open-pollinated progeny of 15 peach selection.[y,z]

Trait	Canker necrotic length (cm)		Canker rating[x,w]	Cold hardiness rating[w,v]
	x	cv		
C2-28-89	14.4	58	1.64	0.38
Loring	15.2	62	1.61	0.57
NJN69	13.1	65	1.88	0.56
NJ257	11.8	62	1.71	0.67
Elberta	10.8	40	1.61	0.77
Harken	10.8	63	1.91	0.92
Canadian Harmony	10.4	30	1.79	0.61
B8-21-20	10.1	60	2.05	0.66
RR37-15	9.5	52	2.21	0.68
Red Hale	9.8	36	1.97	0.72
NJ672017002	9.4	65	2.72	0.91
C4-11-97	9.2	75	2.33	0.89
B8-20-171	9.1	38	2.13	0.82
B8-11-147	8.8	37	2.66	0.84
Babygold 8	8.8	81	2.88	0.82
LSD 5%	1.8			

[z] inoculation dates and harvest dates were respectively; Year 1, 22-23 Oct. 1985 and 19-20 May 1986; year 2, 6 Oct. 1986 and 12 Apr. 1987.

y The data represent identical trees inoculated both years.

[x] Data for 2 years were combined. Rating scale: 1=dead; 2=severe wilting; 3=weak growth; 4=healthy.

[w] Means were significantly different at the 1% level using the Kruskal and Wallis test (9).

[v] Hardiness tests were performed in February. Data for 2 years were combined. Rating scale: 0=dead; 1=alive.

necrotic length for 1986 and 1987 of 4.6 cm and a canker rating of 4.0.

For the spring inoculation date, the half-sib families and progeny within half-sib families responded similarly in both years for canker necrotic length and canker necrotic length/branch cross-sectional area (data not presented). However, the peach seedlings in half-sib families responded differently to the two inoculation times (spring vs. fall). Following spring inoculations, host callus tissue was deposited and mean canker necrotic lengths were significantly smaller (ranging from 4.8 to 6.6cm) than following fall inoculation. Cytospora canker development on peaches has been observed as being restricted during the summer (3). In northern peach growing areas, delaying pruning until trees are near bloom is recommended (7). At this time, trees are actively growing and pruning wounds will heal more rapidly, allowing less time for invasion by Leucostoma spp.

When the half-sib families include 'Yennoh', 'Reliance', 'Glohaven', 'Harken', 'Canadian Harmony', 'Elberta', and 'Loring' were ranked for mean canker necrotic length, the rankings generally agreed with a previous ranking of some of the parent clones using the wound-freezing technique (8) Table 4. In general, 'Reliance' and 'Harken' are most to tolerant to Leucostoma infection,

Table 4. A comparison of published Leucostoma rankings from wound-freeze inoculated peach cultivars compared to rankings from open-pollinated progeny.

Cultivar	Open-pollinated Progeny		Data of Scorza and Pusey (8)[y,x] (cm)
	No. trees	Mean length[z] (cm)	
Yennoh	3	6.2	
Reliance	5	8.7	2.2 a
Glohaven[w]	3	9.0	--
Harken	22	10.4	3.8 ab
Canadian Harmony	22	10.1	5.5 bc
Elberta	20	10.8	10.2 d
Loring	22	13.1	7.3 c

[z] Length of necrosis on 2-year-old limbs, values represent 2-year means (1986 and 1987).

[y] Length of necrosis of inoculated wounds minus that of control wounds on young budded trees.

[x] Means within a column sharing a letter in common are not significantly different (P = 0.05).

[w] Only 1986 data.

'Canadian Harmony' is intermediate, and 'Elberta' and 'Loring' are the most susceptible. The similarity in ranking between the open-pollinated progeny means plus the parent cultivar data suggests that the differences in Leucostoma tolerance observed in this study are heritable.

Because the 'Reliance' and 'Yennoh' families had only five and three progeny respectively, these progenies were only included in Table 4. Progeny from 'Reliance' had a small canker necrotic length; however, many of the branches were wilting and would eventually die. Although 'Reliance' is considered to support less canker growth than other cultivars, cankers do develop under field conditions. A canker rating of 2.58, indicating wilting and eventual death of the inoculated branches, confirms that the destructive effect of L. persoonii on 'Reliance' is also observed on its progeny.

In contrast, although there were only three progeny of 'Yennoh', the six branches that were inoculated had the smallest mean canker necrotic length (Table 4) and showed no symptoms of wilting, having a canker rating of 4.0, 'Yennoh', a plant introduction from Russia, may have a higher tolerance to Leucostoma canker development than previously reported for peach. 'Yennoh' ripens late-season and has white-fleshed fruit.

In conclusion, fall inoculations with L. persoonii

permitted detection of genetic differences between open-pollinated half-sib families for L. persoonii tolerance. The host-pathogen response differed between spring vs. fall inoculations; however, it is the fall inoculation that resulted in the best discrimination among the seedlings for L. persoonii tolerance. Genetic material useful for breeding for L. persoonii tolerance has been identified in this study. However, further evaluations are being conducted to determine which - 'Babygold 8' seedling, 'Yennoh' seedling, or the 'Yennoh' cultivar itself - would be the best parent for incorporating genes for L. persoonii tolerance and cold hardiness into a breeding program. Additionally, this tolerance material must be screened for its reaction to the other Leucostoma species, L. cincta.

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CHAPTER TWO

Excised-shoot Assay for Tolerance of Peach to *Leucostoma persoonii*

Abstract

Dormant, excised shoot segments from peach [Prunus persica (L.) Batsch] seedlings previously identified as tolerant, intermediate, or susceptible to L. persoonii (Nits) Hohn. were evaluated for longitudinal canker necrotic length after incubation in contact with a culture of L. persoonii growing on clarified oatmeal agar. The differences in seedling canker necrotic lengths were significant and corresponded with field ratings of disease susceptibility. Seedlings Yennoh 1-39 and NJ672017002 1-8 were the most tolerant, whereas Loring 14-20 and Elberta 8-25 were the most susceptible. The excise shoot assay is sufficiently quick, reliable, and related to field disease reaction to be used as a screening procedure in the breeding of peach cultivars tolerant to L. persoonii.

Introduction

L. persoonii (Nits) Hohn. [-Valsa leucostoma (Pers. ex Fr.) Fr.], one causal agent of Cytospora canker in peach, usually colonizes cold-injured or dead tissue during cool weather when the trees are not actively growing. In the spring, the fungus progressively invades adjacent healthy

tissue, forming cankers and eventually plugging the xylem vessels and causing wilting and death of the infected twigs, ranches or limbs (Willison, 1933; Tekauz and Patrick, 1974; Biggs, 1984; Wisniewski et al., 1984).

Previous work (Chang et al., 1989) has shown that open-pollinated progenies from 'Yennoh', a peach introduction from the Soviet Union, and NJ672017002, a peach x Prunus kansuensis Rehd. hybrid, are tolerant of L. persoonii based on 2 years of field evaluation using the screening technique of Scorza and Pusey (1984). Since fungicides and cultural practices inadequately control canker caused by Leucostoma spp., the best control approach is through breeding and selection for host resistance (Luepschen, 1981). The field screening procedure used to identify seedlings of 'Yennoh' and NJ672017002 as L. persoonii tolerant was time consuming in the inoculation and evaluation, and required 7 months (October to May) for symptom development to occur. Moreover, large amounts of inoculum were introduced into the seedling plot during inoculation. Therefore, the objective of this work was to develop an excised-shoot assay for L. persoonii tolerance in peach which would be efficient, reliable, less destructive to the seedling plot and correlated with disease reaction in the field.

Excised twig assays have been used previously with

apple to determine the pathogenicity of isolates of Phytophthora spp. (Jeffers et al., 1981) and for studying the infection of apple rootstocks with different isolates of Phytophthora cactorum (Sewell and Wilson, 1959). Sewell and Wilson (1959) suggested that dormant twig assays in the laboratory could substitute for field-inoculated screening trials.

Materials and Methods

In the spring of 1984, open-pollinated peach seedlings from 15 peach clones, 2 peach x Prunus kansuensis hybrids, and one peach x almond (P. dulcis Webb) hybrid were planted in a completely randomized design at the Horticultural Research Center, East Lansing , Mich. Open-pollinated seedlings were used to maximize the genetic diversity in the population to be screened. These seedlings, representing half-sib families with progeny numbers ranging from 3 to 73, were evaluated twice for canker necrotic length following 2-year-old branch inoculation with L. persoonii using the technique developed by Scorza and Pusey (1984). Branches for the 2 experiments were inoculated in October, 1985 and 1986, and evaluated in May, 1986 and 1987, respectively. Two branches per seedling were each inoculated with 20 ul of a suspension of 10^7 L. persoonii conidia per milliliter derived from a mixture of isolates collected in Hartford and

Clarksville, Mich. (Hammar, 1988). Canker necrotic length was measured as a length of the necrotic area distal to the point of inoculation. Dhanvantari and Dirks (1983) found with Leucostoma that linear canker extension following artificial inoculation was related to visual ratings of the incidence of naturally induced cankers over 1 10-year period.

Following the field inoculation experiments with L. persoonii, 8 peach seedlings which were identified as being tolerant (Yennoh 1-39, NJ672017002 1-8), intermediate (Reliance 3-1, Babygold 8 16-11, Canadian Harmony 6-19, B8-20-171 17-45) or susceptible (Loring 14-20, Elberta 8-25) to L. persoonii were selected (Chang et al., 1989). These open-pollinated seedlings are identified by the maternal parent, i.e. 'Yennoh', and the seedling clone number, i.e. 1-39. These seedlings were sampled on December 10, 1987 for excised-shoot experiments I and II, and on January 22, 1988 for experiment III. One-year-old peach branches were collected from each seedling.

Clarified oatmeal agar was prepared as follows: 75 g rolled oats were autoclaved for 5 min in 1 liter water, homogenized for 5 min in a Waring blender, and filtered through 3 layers of cheesecloth. The liquid was then centrifuged at 20,000 g for 30 min. The upper 200 ml of liquid was added to 800 ml H_2O with 20 g agar and 1 ml of

vitamin solution (Adams et al., 1987) and autoclaved for 30 min. The medium was cooled to 45C and 1 ml pure lactic acid and 200 mg streptomycin sulfate were added per liter prior to dispensing. The agar was dispensed into sterile 6.5 x 6.5 x 9.5 cm tissue culture boxes (Magenta Corp., Chicago, IL) to a depth of 1.5 cm.

The medium in each culture box was inoculated with a 0.7 cm diameter agar plug with mycelium of L. persoonii cut from a culture growth on clarified oatmeal agar. The L. persoonii culture was derived from the same mixture of isolates used for the field inoculation experiments. The control treatment consisted of non-inoculated medium. The mycelium covered the oatmeal agar surface of the inoculated boxes in approximately 14 days.

Shoot segments 10 cm long were cut from the lower central portion of the current seasons' growth, disinfected for 5 minutes in 0.6% NaOC1, rinsed in distilled sterilized water 3 times, and blotted dry. A 1-cm segment was cut off each end of the shoot segments to removed portions which may have absorbed the NaOC1 from solution. A sterilized razor blade was used to make one tangential 2.0 cm cut on the end of each shoot segment to expose the cambium and vascular tissue. One shoot segment from each of the 8 seedlings was inoculated in each test box with the cut end of each segment inserted to a depth of 1.5 cm in the culture medium.

For those shoots collected on December 10, 12 boxes (10 inoculated and 2 control) were placed at 25C under cool-white fluorescent lights (35 umoles s^{-1} m^2) and evaluated 24 days later (experiment I). Another set of 12 boxes (10 inoculated and 2 control) were placed in the dark at 4C for 60 days and then moved under the same lights at 25C for 10 days until evaluation (experiment II). For those twigs collected on January 22, 14 boxes (12 inoculated and 2 control) were placed at 25C under the same lights for 20 days until evaluation (experiment III).

For evaluation, the shoot segments were removed from the boxes and the bottom 1.5 cm portion which has been below the agar surface was cut off. The bark adjacent to the tangential cut was removed and the longitudinal length of the necrotic tissue was measured. When the excised shoot segments were completely necrotic , tissue was surface sterilized for 1 min. in 0.6% NaOCl and the fungi were reisolated into Difco potato dextrose agar (Difco Lab., Detroit, MI) to verify the pathogens. Tissues rotted by fungi other than L. persoonii were treated as missing data. Three twig segments in experiment II (2 Loring 14-20 and 1 Elberta 18-25) were colonized by other fungi (Rhizoctonia Fomes-like organism) and treated as missing values. Canker necrotic lengths from previous field screening test (Chang et al., 1989) were compared with the results from the

excised twig assays.

The zonate longitudinal necrosis on most shoot segments was similar to that observed on shoots following field-inoculations with L. persoonii. L. persoonii was reisolated from the zonate necrotic margins confirming the association between the necrosis and this fungus. No necrosis developed on the shoots in the control boxes.

Results and Discussion

Data was analyzed by analysis of variance. Where a significant F-test was observed (5% level), means were separated with Duncan's multiple range test. The 2 field experiments were treated as replications.

The difference in canker necrotic length among the 8 seedlings was highly significant and the ranking of the seedlings for canker necrotic length was similar for all 3 experiments (Table 1). Excised-shoot canker necrotic lengths correlated well with the canker necrotic lengths following field inoculation ($r=0.84^{**}$, 0.95^{**}, and 0.89^{**}, for experiments I, II, and III, respectively).

Although the shoots for experiment III were collected a month later in the winter than those for experiment I, the results were very similar. In experiment II, the canker necrotic lengths were generally longer than in the 2 other experiments. The shoots in experiment II were kept in the

Table 1. A comparison of canker necrotic lengths for 8 peach seedlings following field inoculation with those on excised-shoots inserted into cultures inoculated with Leucostoma persoonii.

Seedling	Field inoculation mean canker necrotic length (cm)[z]	Excised Shoot Assays mean length of necrotic zone (cm)[y]		
		Expt. I	Expt. II	Expt. III
Loring (14-20)	11.0a[x]	5.05a	6.14a	4.91a
Elberta (8-25)	10.8ab	3.79a	5.28a	3.52b
Reliance (3-1)	8.6ab	3.27ab	3.84b	3.11bc
Babygold 8	8.0ab	4.53bc	3.66b	2.31c
Canadian Harmony	7.5ab	2.58cd	3.29b	2.36c
B8-20-171 (17-45)	4.9ab	2.60cd	2.79b	2.36c
NJ672017002	5.0c	1.66d	1.60c	0.85d
Yennoh (1-39)	4.8c	1.75d	1.28c	0.91d

x Each value is the mean of 4 observations.

y Each value is the mean of 10 observations for experiment I and II and 12 observations for experiment III.

z Mean separation in columns by Duncan's multiple range test (P-0.05).

cold longer than those in the other experiments; however, L. persoonii did not cause measurable canker necrosis development during the period at 4C.

In summary, the excised-shoot assay for tolerance of peach to L. persoonii is rapid, reproducible, and gave relative responses to those in the field. The laboratory evaluation was completed in one month in contrast to the 7 months required for the field screen. The laboratory method avoids the introduction of L. persoonii into the orchard. There was good agreement between the results of laboratory and field experiments suggesting that Yennoh 1-39 and NJ672017002 1-8 are tolerant to L. persoonii. The identification of a high degree of tolerance to L. persoonii and the development of a rapid screening technique will facilitate the breeding of peach cultivars tolerant to L. persoonii.

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CHAPTER THREE

Hydraulic Conductance in Susceptible vs. Resistant Peach Clones infected with *Leucostoma persoonii*

Abstract

Eight open-pollinated peach families (Prunus persica.L) were selected from a germplasm collection which was screened for resistance to Leucostoma persoonii following field inoculation (Chang et al., 1989). The eight peach families could be divided into three distinct groups; susceptible, intermediate, and resistant to L. persoonii infection based on canker length measurements. Following artificial inoculation, measurements of hydraulic conductance per unit length (K_h) were made on the branch segments from the eight clones, and safranin dye was used to mark the conductive xylem pathways. For the peach clones resistant to L. persoonii, the K_h of the canker branch segments was significantly greater than that for the susceptible peach clones. The inoculated branch segments from the resistant peach clones maintained about 20-30% of water transport and infected seedlings survived in the field. Safranin dye ascents and descents indicated that the conductive xylem tissue was almost completely blocked in the susceptible infected peach segments. However, branch segments from the resistant peach clones infected with L. persoonii often had an incomplete xylem growth ring with part of the cambium

surviving and the fungus could not be isolated from the wood beneath the cambium. It is concluded that xylem dysfunction is correlated with reduction in K_h and that the resistant peach clones are better able to maintain water transportation. Additionally, the surviving vascular cambium present in resistant peach clones is capable of producing new xylem vessels and phloem.

Introduction

In the 1950s, peach (Prunus persica) orchards in the northeastern United States were expected to remain productive for at least 30 years. Now, the life of a peach orchard averages 15 years or less. Cytospora canker, caused by Leucostoma persoonii and L. cincta, is the most serious disease reducing peach tree life in central and northern United States (Hildebrand, 1947; Gairola and Powell, 1970; Jones and Luepschen, 1982; Luepschen, 1981) and in eastern peach growing regions of Canada (Cline, 1982; Dayne, 1976; Wensley, 1964).

Histopathological investigations of Leucostoma classified the canker pathogen as a facultative bark parasite (Wisniewski et al., 1984; Biggs, 1984, 1986) or as a sapwood parasite (Tekauz and Patrick, 1974; Banko and Helton, 1974). These histopathological investigations concentrated on fungal growth in the bark; if, however, the

pathogen grew beyond the bark and into the xylem, there could be a noticeable reduction in water transport measured as hydraulic conductance. Hydraulic conductance is the measured rate of a fluid divided by the pressure differential. Reductions in hydraulic conductance have been associated with death of branches in American chestnut blight (Ewers et al., 1989) and peach phony disease (Evert, 1987). Hampson and Sinclair (1973) inoculated five peach cultivars grown in a greenhouse with a canker causing pathogen and reported that there were no significant differences among these peach cultivars for xylem dysfunction following pathogen invasion. However, the pathogen infection reduced the xylem function in the inoculated trees in comparisons with healthy ones.

Recently, peach clones resistant to L. persoonii following field inoculation were identified (Chang et al., 1989). The objective of this work was to determine if the level of xylem dysfunction were associated with resistance or susceptibility to L. persoonii. In addition, stem segments were sectioned and L. persoonii isolations were made to determine the depth of invasion of the fungus into the xylem and wood.

Materials and Methods

Peach (Prunus persica (L.) Batch) seedlings were

planted in 1984 at the Horticultural Research Center, Michigan State University, East Lansing, MI. In 1985, two-year-old branches on healthy trees were randomly selected for inoculation with spores of Leucostoma persoonii (Nits.) Hohn. Following the procedure described previously (Chang et al., 1989). these open-pollinated peach seedlings were evaluated twice for canker necrotic length following 2-year-old branch inoculation with L. persoonii using the technique developed by Scorza and Pusey (1984).

For hydraulic conductance measurements, eight open-pollinated peach families were selected based on the previous studies as susceptible, intermediate, or resistant (Chang et al., 1989), and three seedlings within each of these peach families were chosen for the experiments. Two branches per seedling were each inoculated with 20 ul of a suspension of 106 L. persoonii conidia per milliter as previously described (Chang, et al, 1989). Two healthy branches within each tree were used as the non-inoculated control.

To measure hydraulic conductance, the branches were trimmed to similar lengths (approximately 0.25m) with a bandsaw and stored under water. Both ends of each stem segment were shaved until smooth with a fresh razor blade. Vinyl tubing was then firmly clamped to the distal end. The stem surface was vacuum infiltrated at -87 Kpa for 5 minutes

prior to the conductivity measurement. Pressure was applied with a distilled water column and the rate of flow measured with a stopwatch and pipet (Zimmermann, 1978). The hydraulic conductance per unit stem length (K_h) was calculated as the rate of (m^3/s) divided by the applied pressure gradient ($MPam^{-1}$). Filtered oxalic acid (0.1M, PH 2.0) was used to minimize possible artifacts caused by swelling of pit membranes (Sperry et al., 1988). Three readings were used to calculate a mean K_h of each stem segment. Data was recorded as specific conductivity and percent specific conductivity. Specific conductivity (specific K_h) was calculated as K_h divided by cross-sectional area. The percent specific K_h was the specific K_h of the experimental stem segments divided by the specific Kh of the control stem segments.

0.5% Safranin-O dye was poured into the vinyl tube immediately after finishing K_h measurements. The height of the dye in the tubes was kept constant for each stem segment for a 1.5 hour period. The dye was used to mark the functional xylem vessels as a comparison of the tissue area capable of water transport.

Various precautions were taken to minimize the error in the hydraulic conductance measurements. To reduce the lateral transport of water, lateral twigs were removed from the branches and wounds were immediately sealed with nail polish. To minimize the introduction of embolisms, branches

were harvested from the trees at 7-8 am, the basal ends immediately immersed in water, and recut under water. All hydraulic conductance measurements were done within 12 hours of the initial collection time.

Attempts were then made to isolate L. persoonii from the wood to determine the depth of the fungal invasion. The stem segments containing the inoculated wound were trimmed to 6 mm lengths with a bandsaw, de-barked, and surface sterilized with 95% ethanol. 1 mm deep sections of wood were excised with a razor blade from stem segments beginning at the surface margins of necrosis and proceeding downward to the pith and beyond through the stem in 1 mm increments. The 1 mm sections of tissue layers were incubated on Leonian medium (Leonion, 1921). The culture plates were stored at 25°C under cool-white fluorescent light for 2 weeks and scored for the presence or absence of L. persoonii.

Results and discussion

Following field inoculation in the fall of 1986 and 1987, there were significant differences in response to L. persoonii infection among the diverse peach populations (Chang et al., 1989). The open pollinated Yennoh seedlings numbered 1-31, 1-39 and 4-11, and open pollinated NJ672017002 seedlings numbered 1-8, 2-32, and 4-16 had the shortest longitudinal length of necrosis (cankers) following

Table 1. Mean values and coefficients of variation for L. persoonii canker length and canker ratings following artificial inoculations in the fall of 1986 and 1987 of open-pollinated progeny of 8 peach selection.[u]

Selection	Canker length (cm) 1986	c.v.	1987	c.v.	Canker ratings 1986	1987
Loring (2-7; 11-27, 11-32)	15.7a[v]	19	19.0a	23	0.0	1.7
Elberta (6-32, 11-34, 11-38)	17.0a	33	11.8ab	50	0.0	1.0
Canadian Harmony (3-17, 3-20, 5-9)	13.1abc	18	8.7cd	27	0.5	1.7
Harken (2-6, 2-29, 2-44)	11.6abc	19	15.0ab	54	0.5	1.7
Reliance (1-33, 1-41, 15-16)	13.8ab	46	7.8cde	27	0.3	0.8
Babygold 8 (2-13, 2-14, 3-10)	12.8abc	43	11.2bc	50	0.5	1.0
NJ672017002 (1-8, 2-32, 4-16)	7.0c	71	5.4de	71	2.3	3.0
Yennoh (1-31, 1-39, 4-11)	7.1bc	51	3.2e	39	2.5	3.0

[u] Data is the mean of three progeny per selection.

[v] Different letters are significantly different according to LSD at 0.05 level.

Table 2. Specific hydraulic conductance per unit length (specific K_h)[u] in 10^{-5} $m^2MPa^{-1}s^{-1}$[w] and percent specific K_h of experiment/control K_h (% specific K_h)[v] for Leucostoma canker infection from artificial inoculations in the fall of 1986 and 1987 of open-pollinated progeny of peach.[y]

Selection	Specific K_h[w] 1986	c.v.	1987	c.v.	% Specific K_h 1986	c.v.	1987	c.v.
Susceptible seedlings								
Loring (2-7; 11-27, 11-32)	0.58c[z]	49	1.02b	48	5.9cd	49	8.8bc	48
Elberta (6-32, 11-34, 11-38)	0.92bc	76	1.52ab	69	6.0cd	65	17.9bc	70
Canadian Harmony (3-17, 3-20, 5-9)	1.81abc	68	0.78b	44	14.2abcd	42	5.9c	47
Harken (2-6, 2-29, 2-44)	0.63c	20	0.82b	73	5.4d	41	11.4bc	80
Intermediate seedlings								
Reliance (1-33, 1-41, 15-16)	1.18bc	85	0.93b	69	10.9bcd	41	11.8bc	70
Babygold 8 (2-13, 2-14, 3-10)	1.81abc	56	2.81ab	34	20.3a	67	20.7ab	47
Resistant seedlings								
NJ672017002 (1-8, 2-32, 4-16)	3.10a	54	3.44a	80	15.2abc	101	21.4ab	62
Yennoh (1-31, 1-39, 4-11)	2.48ab	48	3.16a	61	18.5ab	29	33.9a	38

[u] Hydraulic conductance (K_h) = volume of water (m^3) x length of segment (cm) / seconds (s) x height of segment (cm) x gravity gradient (MPa/m). The S.I. unit of Kh is $m^4s^{-1}MPa^{-1}$. Specific K_h = Kh /cross sectional area of segment (m^2) = X ($m^2s^{-1}MPa^{-1}$).

[v] Percent Specific K_h = (Specific K_h of experimental stem segment/specific K_h of control stem segment) x 100.

[y] Data is the mean of three progeny per selection.

[z] Different letters are significantly different according to LSD at 0.05 level.

inoculation and the highest canker rating (Table 1). These seedlings were classified as resistant clones. Likewise the open-pollinated progeny from Loring, Elberta, Canadian Harmony, and Harken vs those from Reliance and Babygold 8 were classified as susceptible and intermediate tolerance, respectively.

The specific K_h calculated as K_h divided by cross-sectional area was significantly different among the seedlings of the 8 peach clones (Table 2). For the resistant peach clones, the % specific K_h was 20-30% of the healthy control branches while in susceptible peach clones, the % specific K_h was 5-18%. The variation in specific K_h and % specific K_h was quite large and probably attributed to the variation among the stems which could be due to size, age, vigor of the plants plus summing over the 3 seedlings which are genetically different in the experiment. Despite the variation in Table 3, K_h was clearly reduced by Leucostoma infection and the resistant plants were capable of transporting more water through the infected zone compared to the susceptible plants. This is probably why the resistant branches remained healthy 7 months after inoculation and infection. Also, the canker necrotic length and canker rating were correlated with % specific K_h ($r=0.74$, 0.75 respectively). The longer the canker extension and the less water transport and more severely wilting.

Vessel elements which stained with Safranin-O were visibly reduced in the inoculated branches compared to the non-inoculated control indicating that infection decreased the amount of conductive xylem tissue (Fig. 1). However, in the tolerant clones the extent of xylem damage following inoculation was less than that for the susceptible clones. In the susceptible clones the outer xylem ring was no longer functional while in the tolerant clones the outer and some of the inner xylem vessels remained functional (Fig. 2). Since the branches had been inoculated in October and evaluated in May, the presence of these functional xylem vessels would be critical during the growing period.

Above the necrotic zone of L. persoonii infection, there was no difference in K_h, specific Kh and quantity of dyed functional xylem vessels between the control and inoculated branches. This indicated that the vessel damage and subsequent reduction in K_h and specific K_h is fairly localized around the infected canker area.

The dramatically decreased water transport through the localized necrotic longitudinal zone following L. persoonii infection was associated with disease susceptibility. Where the vascular cambium was not damaged, new xylem and phloem could be produced to replace the damaged tissues and keep the plants alive. This is important to the survival of infected branches. It is clear that the resistant reaction

Table 3. Percent infection of open-pollinated progeny of peach as calculated as re-isolation of Leucostoma from the wounds of artificial inoculation.[u]

Selection	Percent infection of progeny	Coefficient of variation
Loring (2-7; 11-27; 11-32)	90a[v]	17
Elberta (6-32; 11-34; 11-38)	71[a]	52
Canadian Harmony (3-17; 3-20; 5-9)	97[a]	6
Harken (2-6; 2-29; 2-44)	95a	10
Reliance (1-33; 1-41; 15-16)	70[a]	35
Babygold 8 (2-13; 2-14; 3-10)	20a	12
NJ672017002 (1-8; 2-32; 4-16)	9[b]	133
Yennoh (1-31; 1-39; 4-11)	5b	109

[u] Data is the mean of three progeny per selection.

[v] Different letters are significantly different according to LSD at 0.05 level.

Figure 1. **Cross-sections of a pair of the disease susceptible** Can. **Harmony (3-17) peach stems used for hydraulic** conductance **measurements. The stem on the left was inoculated** with Leucostoma, **and the stem on the right was the control. Red areas indicated when safranin-O (0.5%) moved through the functional xylem area in the middle of inoculated wound.**

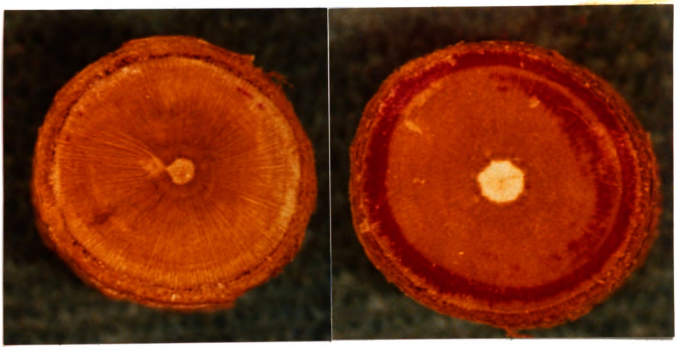

Figure 1.

Figure 2. Cross-sections of a pair of the disease resistant NJ672017002 (1-8) peach stems used for hydraulic conductance measurements. The stem on the left was inoculated with Leucostoma, and the stem on the right was the control. Red areas indicated when safranin-O (0.5%) moved through the functional xylem area in the middle of inoculated wound.

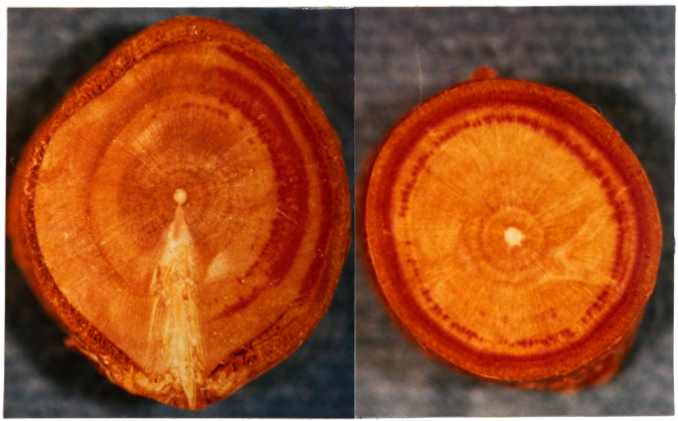

Figure 2.

of the clones investigated is in part due to the production of new xylem and phloem, and in part due to the capability of the inner xylem to maintain water transportation.

To understand the involvement of the L. persoonii fungus in disrupting xylem flow, attempts were made to determine the depth of L. persoonii invasion into the xylem area. In susceptible clones, it was possible to isolate the fungus from the margin of the surface necrosis down to the pith area. For the resistant clones, the fungus was isolated only as deep as the secondary layer (approximately 2mm) beneath the margin of the surface necrosis. Percent infection was determined by isolation of Leucostoma from the wounds following artificial inoculation. Yennoh and NJ672017002 seedling clones had less than 10% infection, however, the susceptible ones had more than 90% infection (Table 3). The lack of penetration of the fungus through the wood of the resistant clones suggests that Yennoh and NJ672017002 seedlings may be limiting the advance of the pathogen.

In conclusion, xylem dysfunction following L. persoonii infection of susceptible clones is associated with wilting and death of branches. The canker pathogen invaded the wood xylem in the susceptible peach seedlings and significantly reduced the water transport through the infection zone. In the resistant clones, the xylem remains functional following

branch inoculation and the vascular cambium is able to continue to differentiate new xylem and phloem to replace the damaged tissue, perhaps because of reduced penetration by the fungus. Therefore, the tolerant clones were able to maintain adequate water transport through the necrotic canker zone during the summer following inoculation.

A determination of the actual cause of branch death in susceptible clones still needs further study because both xylem and phloem are damaged by the pathogen and we know little of the role of phloem in the disease.

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CHAPTER FOUR

Quantification of lignin content in wounds of peach clones selected for their resistance to *Leucostoma persoonii*

Abstract

Two peach cultivars and three selected peach clones that vary in their resistance to Leucostoma persoonii from highly susceptible to resistant were examined for their response to wounding. Field grown trees undergoing cold temperature acclimation were wounded to a depth of 2mm and lignin deposition in the wounded bark and wood to a 4mm depth was quantified using a thioglycolic acid assay. The measured constitutive lignin quantity was correlated with resistance in the field.

Introduction

Perennial canker of peach (Prunus persica (L.) Batch) which is caused by Leucostoma persoonii (Nits.) Hohn. (imperfect stage Leucocytospora leucostoma (Pers.) Hohn) and L. cincta (Fr.) Hohn. (imperfect stage Leucocytospora cincta (Sacc.) Hohn.), is the most serious disease reducing peach tree life in the colder areas of peach production. The disease is characterized by perennial cankers that may kill large branches and weaken scaffold resulting in the eventual splitting of branch crotches under heavy crop loads. Successful invasion by the pathogen requires the presence of

wounds or dead tissues on the host plants (Willison, 1933). Various histopathological investigations of perennial canker classified Leucostoma as a facultative bark parasite (Wisniewski et al, 1984; Biggs, 1984, 1986). The mode of pathogenesis of Leucostoma involved necrosis of the bark tissues. Alternatively, Leucostoma was described as a sapwood parasite with necrosis first progressing through xylem tissues prior to invading the bark (Banko and Helton, 1974; Tekauz and Patrick, 1974). Recently, it has been shown that the pathogen invades xylem tissues significantly reducing water transport (Chang, 1989).

The formation of lignin and wound periderm has been recognized as an important mechanism of disease resistance in many plant species (Vance et al, 1980; Hammerschmidt, 1984). Wound-induced lignification in almond bark has been associated with slower expansion of cankers caused by Phytophthora species (Doster and Bostock, 1988). Wilson (1982) suggested that efficient xylem compartmentalization would increase peach longevity. Previous data further indicated that the ability to heal wounds may play a part in the ability of different peach cultivars and clones to resist Leucostoma (Willison, 1933; Wensley, 1966; Weaver, 1963). Biggs (1989) also demonstrated that increases in the rate of suberin accumulation following wounding were correlated with observations of resistance of peach

cultivars in the field to infection by Leucostoma.

After screening over 700 peach seedlings in a germplasm population, we identified several individuals which exhibited a higher tolerance to Leucostoma than had previously been reported in the United States (Chang, et al, 1989). It was found that L. persoonii invaded the wood tissue reducing the hydraulic conductance in susceptible genotypes; whereas, in the resistant genotypes the xylem was not invaded (Chang, 1989).

The purpose of this research was to determine relative lignin content following wounding in several peach clones identified as susceptible and resistant to Leucostoma. Lignin deposition was quantitatively assayed using thioglycolic acid. This assay was adapted by Doster and Bostock (1988) for investigating lignification in inner bark of almond trees.

Materials and Methods

Plant materials:

In spring 1984, open-pollinated peach seedlings from 15 peach clones,two peach * P. kansuensis hybrids, and one peach * almond (P. dulcis Webb.) hybrid were planted in a completely randomized design at the Horticultural Research Center, East Lansing, Michigan. Open-pollinated seedlings were used to maximize the genetic diversity in the

population to be screened. These seedlings , representing half-sib families with progeny number ranging from three to 73, were evaluated twice for canker necrotic length following inoculation with L. persoonii. Two 2-year-old branches were inoculated using the techniques developed by Scorza and Pusey (1984). Branches for the two experiments were inoculated in October 1985 and 1986 and evaluated in May 1986 and 1987, respectively. Two branches per seedling were each inoculated with 20 ul of a suspension of 10^7 L. persoonii conidia/ml derived from a mixture of two isolates collected in Hartford and Clarksville, MI. Canker necrotic length was measured as the length of necrotic area distal to the point of inoculation (canker necrotic rating). Additionally, inoculated branches were visually rated for canker using a scale where 1=dead, 2=severe wilting of expanding leaves, 3=weak growth, 4=healthy (Chang et a., 1989).

In 1987, eight peach seedlings that were identified as being resistant (Yennoh 1-39, NJ672017002 1-8, B8-20-171 17-45), intermediate (Babygold 8 16-11, NJ257 2-15, Reliance 3-1), or susceptible (Loring 18-30, Elberta 10-40) were selected for the lignin analysis. The open-pollinated seedlings are identified by the maternal parent, i.e. Yennoh, and the seedling clone number, i.e. 1-39.

In 1986, two resistant and one intermediate seedling

plus the cultivars Yennoh and Loring were grafted on Lovell rootstock and then planted in 1988 at Horticultural Research Center adjacent to the seedling block.

Wounding and Lignin analysis:

Two 2-year-old healthy branches per seedling which were chosen for wounding, were wounded by insertion of a 4 mm diameter cork borer through the bark to the xylem. Two days after wounding, the bark and 2 mm depth of wood was removed by a 10 mm diameter cork borer. This procedure was repeated four times on each branch from Sept. 6 to Sept. 12, 1987. The dead outer bark and browned tissue were carefully peeled or cut away from the periderm of each 10 mm disk prior to lignin extraction. The tissue sample were then extracted in methanol and the lignin was quantified as ligninthioglycolic acid (LTGA) (Doster and Bostock,1988).

Additionally, four trees of Loring and Yennoh, plus the seedlings Yennoh (1-39) and NJ672017002 (1-8) were selected for wounding on September 20, 1989. Two 2-year-old branches, approximately 10 mm in diameter were wounded by the triggered impact with the impeller of an empty staple gun. Samples were removed by a 10 mm cork borer to the 3 to 4 mm depth of wood then analyzed for lignin content (LTGA). Comparisons were made with samples taken from unwounded controls at day 0 and 2, 4, 8, 14, and 21 days after

wounding.

Result and Discussion

Since LTGA production can be viewed as an accurate representation of the relative lignin content in the tissue (Freudenberg, 1968), the LTGA data will be discussed as such.

The methanol-extracted tissues from eight peach seedlings yielded significantly different relative values of lignin (Table 1). The resistant Yennoh 1-39, NJ672017002 1-8, and B8-20-171 17-45 produced twice as much lignin as the susceptible seedlings Loring 18-30, Reliance 3-1, and Elberta 10-40. Babygold 8 16-11 which had intermediate resistance to L. persoonii, also had intermediate lignin levels. For the resistant clones (Yennoh 1-39, NJ672017002 1-8), lignin was significantly higher than in the susceptible cultivar Loring and intermediate clone NJ257 2-15 at all sampling times (Table 2). For the resistant clones, both the unwounded and wounded tissues had significant amounts of lignin content. Despite the increase in lignin in Yennoh, NJ257 (2-15) and Loring in response to wounding, the lignin content of these selections was still less than that produced by the resistant clones. Resistance of peach to Leucostoma persoonii in our selected clones was previously characterized by the apparent failure

Table 1. Relative lignification[W] detected in wounded bark and wood two days after wounding compared with mean disease symptom and canker necrotic length rating.

Seedling	Mean[X] disease symptom	mean canker necrotic length	Relative[Y] lignification
NJ672017002 1-8	4.0	5.0	16.18a[Z]
Yennoh 1-39	4.0	4.8	15.09a
B8-20-171 17-45	4.0	4.9	15.01a
Babtgold 8 16-11	2.5	8.0	11.45ab
NJ 257 2-15	2.0	7.2	9.00b
Loring 18-30	1.0	12.0	8.89b
Reliance 3-1	2.5	8.6	8.83b
Elberta 10-40	1.0	13.2	7.79b

[W] Relative lignification was measured in bark and wood tissue with 2mm depth.

[X] 1986 and 1987 data combined. Canker rating: 1=dead, 2=severe wilting, 3=weak growth, 4=healthy.

[Y] LTGA yield expressed as A_{280} nm per gram tissues in 5 ml of 0.5N NaOH.

z Different letters are significantly different according to L.S.D. at 0.05 level.

Table 2. Relative lignification of unwounded and wounded tissues[w] sampled 2, 4, 8, 14, and 21 days postwounding (DAW) for five peach families.

	Relative lignin content[x]				
Treatment	Yennoh (1-39)	NJ672017002 (1-8)	Yennoh	NJ257 (2-15)	Loring
Unwound	20.07a[y]	19.37a	8.47bc	11.48b	6.09c
2 DAW	18.76a	20.06a	9.54b	11.89b	5.46c
4 DAW	20.13a	20.13a	7.93b	7.30b	4.97b
8 DAW	17.25b	20.92a	13.05c	9.17d	6.07e
14 DAW	20.99a	20.42a	19.11a	9.13b	8.08b
21 DAW	22.21a	20.16a	19.76b	14.18c	13.47c
Mean	20.50a	19.90a	12.98b	10.19b	7.34b

w Tissues included bark and wood from 0 to 4 mm deep into the wood.

x Relative lignification was measured as LTGA yield and expressed as spectrometer absorption at A^{280} nm per gram tissue in 5ml of 0.5N NaOH.

y Different letters are significantly different according to L.S.D. at 0.05 level.

of the fungus to penetrate into wood even though there was some growth on the surface of the wood (Chang, 1989). This observation may be attributed to the resistant peach clones containing significant amounts of lignin; the lignin preventing further penetration by the fungus. Biggs (1984) found that Leucostoma could penetrate wound periderm of the Loring cultivar of peach. If Leucostoma became established in a wound in a susceptible host such as Loring, the small amount increase in lignin deposition might represent an insufficient defense mechanism. In contrast, in our resistant peach seedlings, the lignin may be able to prevent damage the xylem vessels so they remained functional seven months after inoculation and the vascular cambium continued to differentiate new xylem and phloem during the growing season to replace the damaged tissues (Chang, 1989).

Although a good correlation between constitutive lignin quantification and resistant performance in the field has been demonstrated, the ability of lignin to wall-off the pathogen has not been tested because the wounding was not followed by inoculation. However, Doster and Bostock (1988) found that lignin deposition in almond inner bark appeared to be a response to wounding and infection by Phytophthora.

During the first eight days after wounding, the cultivar Yennoh did not have a significantly increased amount of lignin, and the major accumulation occurred two

weeks after wounding. It is interesting that its open-pollinated progeny Yennoh 1-39 has higher lignin production.

Previous literature associating lignin with disease resistance discusses the rate of lignin or suberin accumulation in response to wounding and/or inoculation (Doster and Bostock, 1988; Biggs, 1989). However, our time-course data indicated that our resistant selections, Yennoh 1-39 and NJ672017002 1-8, initially contained high lignin amounts that did not change in response to wounding. Even the unwounded resistant controls have significantly higher amounts of constitutive lignin than that found to accumulate in susceptible clones 21 days postwounding. If lignification is to play a major role in restricting pathogen development, it must occur early.

Previous studies have shown that host resistance to Leucostoma differs, and these differences might be due to time of defoliation (Weaver, 1963), rate of wound healing (Wensley, 1966), and rate of suberization (Biggs, 1989). These host resistant mechanisms might be expected during the rapid growing season. However, these mechanisms might be of little value during the dormant period when little formation of wound periderm occurs (Layne, 1984). Our studies of resistance to Leucostoma in peach and lignin deposition differ fundamentally from similar studies on suberin deposition by other researchers because of differences in

physiology of peach during the dormant season. In initial field trials, a diverse peach population was inoculated in spring and fall with L. persoonii (Chang et al, 1989). Spring inoculation lead to callus formation in susceptible and resistant genotypes and no statistically significant difference were measurable. In contrast, fall inoculations caused rapid necrosis of functional xylem in early spring followed by rapid death of inoculated branches in susceptible genotypes and survival of inoculated branches in resistant genotypes (Chang et al, 1989).

While other workers have studied suberin formation in peach that were wounded during the growing season (Biggs and Miles, 1988), or rate of suberin accumulation in wounded and inoculated seedlings growing in the greenhouse (Biggs, 1989), here we purposely conducted wounding experiments during the dormancy in the field. The constitutive lignin quantity measured in our study of resistant genotypes apparently is present year-round and continues to be expressed during the dormant period when other disease defense mechanisms are likely non-functional. By inoculating the seedling populations following fall acclimation, we were imposing a severe disease pressure on the trees, and may have been sufficiently severe to have inadventantly selected for those genotypes which accumulate large amounts of lignin during acclimation.

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CHAPTER FIVE

Heritability of *Leucostoma persoonii* canker resistance among diverse peach genotypes

Abstract

Heritability of resistance to the canker causing pathogen, Leucostoma persoonii, was estimated in a population of diverse peach genotypes. Disease resistance was measured as the amount of necrotic tissue, i.e. canker length, following artificial inoculation in the field. The heritability was estimated by (1) partitioning the variance components in the least statistical model, and (2) parent-offspring regression by regressing the average performance of the seedlings on the mean performance of its female parent. The two estimates of heritability for canker necrotic length were very similar, 0.65, 0.72, respectively.

Introduction

Perennial canker, incited by Leucostoma persoonii or L. cincta, is recognized as a severe disease limiting peach longevity and productivity in the colder areas of peach production (Cline, 1982; Gairola and Powell, 1970; Hildebrand, 1947; Jones and Luepschen, 1971; Layne, 1976. 1984; Luepschen, 1981; Wensley, 1964). Since both cultural practices and chemical treatments do not adequately control

the disease, the ultimate approach is through host plant resistance. Studies of peach susceptibility to Leucostoma infection based on either natural infection or artificial inoculation have been conducted. However, almost all commercial peach cultivars failed to have resistance to the canker pathogen. (Dhanvantari and Dirk, 1981; Dhanvantari, 1978; Gairola and Powell, 1970, Hildebrand, 1947; Layne, 1984; Luepshen, 1981, Palmiter and Hickey,1970). Where some level of resistance was found, the reaction tended to be inconsistent among experiments. Possibly this lack of resistance to Leucostoma can be attributed to the narrow genetic base of peach cultivars in North America (Layne, 1976, 1984). Following the inoculation of a diverse peach population and subsequent evaluation of disease resistance and cold hardiness, we reported that some peach genotypes may have a higher tolerance to Leucostoma canker than has been previously found in U.S. germplasm (Chang et al. 1989[a]; Chang et al. 1989[b]).

Studies of various tree crops including sweet cherry, peach, nectarine, walnut, and plum have shown considerable additive genetic variability for commercially important traits (Hansche, Beres and Brooks, 1966; Hansche, Hess and Beres, 1972; Hansche, Beres, and Forde, 1972; Hansche, Hesse, and Beres, 1975; Hester, Hanche, Beres, and Asay, 1977; Hansche, 1986, 1986). A knowledge of heritability

estimates is useful as a guide for breeders in improving tree crops and in maximizing the breeding efficiency.

This study was undertaken to estimate the genetic and environmental parameters and to examine the heritability of Leucostoma resistance traits in a diverse peach population.

Materials and Methods

Heritability estimates were obtained by two methods. The first method used the linear statistical model. Least squares estimates were calculated for the variance among the peach families, year, year and peach family interaction, progeny within peach family, year and progeny within peach family interaction, sampling branches within progeny within peach family, and year and sampling branches within progeny within peach family interaction. The second method involved a linear regression of offspring performance on the average performance of its female parent.

(1) Linear Statistical Model

In Spring 1984, open-pollinated peach seedlings from diverse background were planted in a completely randomized design at the Horticultural Research Center, East Lasing, MI (Chang et al., 1989). Open-pollinated peach progeny, instead of clones, were used to increase the genetic diversity of the population to be screened. From this population, 14 peach families with at least 6 progeny were chosen to

Table 1. **Parents of the 18 clones used in the heritability estimates of *Leucostoma* resistance**

Clone	Parent	Parents used in the model	
Babygold 8	PI35201 x Ambergen		LS[y]
Canadian Harmony	Redskin x Sunhaven	POR[x]	
Elberta	Chinese Cling (open-pollinated)	POR	
Glohaven	(J.H. Hale open-pollinated) x Kalhaven		
Harken	Redskin x Sunhaven	POR	LS
Loring	Frank x Halehaven	POR	LS
Reliance	(Minn. PH04559 x Meredith) open-pollinated		
Red Hale	Unknown		LS
Yennoh	Plant Introduction from Russia		
B8-11-147	(K82xSunrise)x[(Red CxNJ191)xOkinawa]	POR	LS
B8-20-171	(5110417 x Ta Tao 3) x C2R31T45		LS
B8-21-20	Orange Cling x RR65-1		LS
C2-28-89	Kasna Dupnishka open-pollinated	POR	LS
C4-11-97	peach x almond	POR	LS
NJ257	Honeydew Hall x Jefferson		LS
NJN69	(NJN55 x NJC68) x Marzochella	POR	LS
NJ672017002	(PI35321 x Cherryred) x Prunuskansuensis		LS
RR37-15	NJ174 x Prunuskansuensis		LS

[x] Parent-offspring regression

[y] Least square model

estimate the heritability of L. persoonii resistance (Table 1).

Two 2-year-old branches of about 17 mm in diameter on each seedling were inoculated with 20ul of a suspension of 10^7 Leucostoma persoonii conidia per milliliter derived from isolates collected from cankers on peach at Clarksville and Hartford, Mich. A wound-freezing inoculation technique developed by Scorza and Pusey (1984) was followed. The trees were inoculated in early October of 1985, 1986, and 1987, then evaluated in the spring when the trees began to leaf out. All the inoculated branches were measured for length of canker necrosis, distal to the point of inoculation. Additionally, inoculated branches were rated visually for disease symptoms using a scale: 1=dead, 2=severe wilting of expanding leaves, 3=weak growth and 4=healthy (disease symptom rating). Fourteen peach families with 6 progeny were inoculated and evaluated for canker infection over three years. They were chosen to estimate the component variance and used to estimate the heritability in the seedling population of resistance to Leucostoma infection. The seedlings in this study represented open-pollinated half-sib families.

Phenotypic variability of all observations of canker necrotic length on this diverse collection of peach progeny over all 3 years is described by the following model:

$$Y_{ijkl}=U + F_i + P_{ij} + S_{ijk} + Y_e + (FY)_{il} + (PY)_{ijl} + E_{ijkl}$$

where Y_{ijkl} represents k sample branch with jth progeny within ith peach family in the lth year. The effects in this model stand for the overall population mean (u); a random effect contributed to each family (F_i); the effect of progeny within family (P_{ij}); the effect of sampling branches within progeny within peach family (S_{ijk}); the year effect (Y_e); the interaction of family * year ($(FY)_{il}$); the error due to the year * S_{ijk} (E_{ijkl}).

(2) **Parent-offspring Regression**

Eight randomly selected female parents which had been grafted on Tennessee peach rootstock were planted in blocks adjacent to the orchard of diverse peach progeny in 1985. Ten grafted trees per clone were selected for artificial inoculation. The two randomly selected healthy branches of each grafted tree and each progeny were inoculated as described above the same day. Using data from 1986 and 1987 inoculations, the performance of the female parent was rated as the mean canker length over two years of 10 replicated clones. The performance of the progeny from the eight families was rates as the mean disease response over two years of inoculation.

These data were based on unadjusted data for the year-effect, because no significant variation doe to year effect has been evident in previous studies (Chang et al., 1989).

The heritability was based on the regression of the mean of the performance of peach offspring on the average performance of its female parents.

Results and discussion

(1) Variance Components

The expected and actual mean squares of the linear statistical model are present in Table 2. The year variation over three years data did not dramatically influence the peach genotypic performance rather performance was based on the canker necrotic length rating or on the disease symptom rating following artificial inoculation in the field. Since the year variability is relatively small, it does not significantly decrease the efficiency of selection of the more disease resistant genotypes among the diverse peach population. However, statistically estimating the variability due to year, and statistically removing the yearly environmental effect would increase the selection efficiency and the rate of genetic gain.

The mean squares of year * sampling branches within progeny within family (E_{ijkl}) and mean squares (S_{ijk}) of sampling branches within progeny within family are attributable to the environmental effect. The year * sampling branches within progeny within family is quite large, which might be due to environmental effects on cold

Table 2. The analysis of variance of the linear statistical model of heritability of canker resistance among half-sib families

Sources of Variation	d.f.	Mean Squares	Expected Mean Squares of
Among peach families (F_i)	13	179.03	$V^2+yV^2_b+bV^2_{rw}+byV^2_w+bnyV^2_f$
Progeny within family (P_{ij})	68	38.48	$V^2+yV^2_b+bV^2_{yw}+byV^2_w$
Year (Yl)	2	6.28	$V^2+bV^2_{Yw}+bnV^2_{Yf}+bnfV^2_y$
Year * peach family $(FY)_{il}$	26	32.96	$V^2+bV^2_{yw}+bnV^2_{yf}$
Year * progeny within family ($Y*P_{ij}$)	136	25.02	$V^2+bV^2_{yw}$
Sampled branch within progeny within family (S_{ijk})	82	8.54	$V^2+yV^2_b$
Y * Sijk (E_{ijkl})	163	13.55	V^2

V: Standard deviation

y: no. of years

b: no. of sample branches

n: no. of sibs/half-sib families

f: no. of half-sib families

hardiness in different years or on different sampling branches within each tree. The inoculated branches were removed from the trees after canker evaluation each year. The error variance probably is attributable to the variations among branches such as in size, age, year, and orientation as well as to branch response to different environmental effects in different years. Error due to sampling branches within progeny within family was negative and thus is ignored. If combining two error terms to one, the actual values for the error was 11.87. In the estimated variance components, variability due to year was rather small of negative value,if assuming 0 instead of negative values. However, the interaction between year and peach family was relatively low and was not significant. This indicated that performance was stable among the peach clone families over the tested 3 years. The estimated variance component, due to the progeny within family, was relatively large. Doubtless, this error term for peach families was a combined effect of genetic and environmental variability. Therefore, it was not appropriate to estimate how much variability due to the environmental effect without clonal propagation.

The component variance due to the family effect is the covariance of half-sib families and this is equal to one quarter of the additive genetic variance (Falconer, 1980).

The summation of all the variance components represents the phenotypic variance, V^{P2} in this diverse peach seedling population (see statistical model, Table 3). The narrow sense heritability was calculated as the additive variance over the phenotypic variance. The heritability of canker necrotic length was 0.65.

(2) Parent-offspring Regression

The parent-offspring regression is commonly used to estimate the heritability of quantitative traits in different crop species. Progeny mean values are usually regressed on values from one or two parents, depending on pollination control. Peach presumably is highly self-pollinated (Hesse, 1976; Hansche 1986). The performance of the female parent has been used to determine the mid-parent performance (Hansche 1986a, 1986b). According to a report by Falconer (1981), use of the parent performance will cause the heritability estimate to be biased downward by about 5%. The linear regression coefficient gives the estimate of heritability as the ratio of additive genetic variance to phenotypic variance of the parents.

Generally, the mean canker necrotic length rating was lower in the seedling population compared to the parental performance over the 2 years of observations (Table 4). The apparent increase in resistance to Leucostoma infection seen in the progeny may have occurred by self-pollination

Table 3. Estimates of variance components of the canker necrotic length rating of a diverse peach seedling population inoculated during fall of 1985, 1986 and 1987 and evaluated during spring of 1986, 1987, and 1988.

Variance components	Values	Actual values in estimating heritability
Error (V^2)	13.55	11.87[x]
Sample branch (V^2_b)	-1.69	
Year * progeny within family (V^2_{yw})	5.73	5.73
Year * Family (V^2_{yf})	0.66	0.66
Year (V^2_y)	-0.16	0.00
Progeny within family (V^2_w)	2.48	2.48
Half-sib family (V^2_f)	4.00	4.00

V: Standard deviation

[x] Summation of error variance

Table 4. The mean values of the canker necrotic length on diverse peach genotypes.

Clonene	Parent canker crotic length[x] (cm)	Offspring canker necrotic length[y] (cm)
NJN69	13.48	11.13
Elberta	13.08	12.76
Harken	11.02	9.36
Loring	10.34	9.58
Can. Harmony	9.67	8.28
C2-28-89	9.26	9.43
B8-11-147	8.26	7.40
C4-11-97	5.06	5.68

[x] mean of 10 clones over 2 years, the performance of female parent was estimated by the mean of the necrotic length ratings from 10 replicated clones following inoculations in fall of 1986 and 1987.

[y] mean of 20 progeny per clone over 2 years.

increasing the additive gene effect, or alternatively a grafted propagation effect or age difference. The heritability estimate and the standard deviation of the estimate, based on parent-offspring regression of the canker necrotic length rating, are listed on Table 5.

Since the heritability estimate in this parent-offspring regression probably is biased by about 5%, the heritability of disease resistance in the diverse progeny probably is calculated to be 0.76 * 0.95 = 0.72. This adjusted estimate agrees well with the heritability estimate of 0.65 obtained from the variance components. It may well represent the true value of heritability for canker resistance in this peach population.

Several factors influence the breeder's selection of a breeding scheme to improve their genetic stocks. These include (1) quantity and types of genetic variance, (2) environmental effects, (3) the interaction between environment and genotype, and (4) linkage effects. Genetic and environmental effects, and interactions between genotype and environment were estimated in this population. The estimate of heritability for canker length were high. The year and year by peach family interactions were relatively small. This will assist the breeder in discriminating between genotypes for superior resistance to Leucostoma persoonii in this segregating population without a masking

Table 5. Narrow-sense heritability (h^2) by parent-offspring regression over 2 years data.

Female parent		Progeny			
mean of necrotic length	Standard deviation	mean of necrotic length	Standard deviation	h^2	Standard deviation of h^2
10.02	2.7	9.20	2.2	0.76	0.11

effect from year to year. The highly resistant progeny from the most resistant families could be readily identified and maintained in the breeding program. If the highly resistant trees were often associated with undesirable horticultural traits, resistant F_1 individuals could be intermated to produce a large F2 population. Selection could be practiced for desirable recombinant F2 plants having both disease resistance and horticulturally important commercial traits. Then the superior F2 plants could be backcrossed to elite commercial peach cultivars, then selfed 1 or 2 generations to fix the desirable traits. A logical breeding scheme for canker resistance will be a combined approach of recurrent selection with backcrossing, or mass selection with backcrossing.

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