Hydraulic Conductance in Susceptible versus Tolerant Peach Seedlings Infected with *Leucostoma persoonii*

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Abstract. Eight open-pollinated peach families [*Prunus persica* (L.) Batsch] were selected from a germplasm collection that was screened for tolerance to *Leucostoma persoonii* (Nits.) Höhn. [imperfect state, *Leucocytospora leucostoma* (Pers.) Höhn] following field inoculation. The eight peach families were either susceptible or tolerant to *L. persoonii* infection based on canker length measurements. Three open-pollinated seedlings per family were chosen for evaluation. Following artificial inoculation, measurements of hydraulic conductance per pressure gradient (K_h) were made on 2-year-old branch segments from the 24 seedlings, and safranin dye was used to mark the conductive xylem pathways. For the peach families tolerant to *L. persoonii*, the specific K_h of the canker branch segments was greater than that for the most susceptible peach families. The inoculated branch segments from the tolerant peach families maintained $\approx 15\%$ to 30% of the water transport of control segments. Safranin dye movement indicated that the sapwood in inoculated branch segments of seedlings from the susceptible peach families was almost completely blocked. Isolation experiments indicated deeper penetration of the fungus into the xylem of seedlings of susceptible than tolerant families. Xylem dysfunction appears to be correlated with a reduction in K_h , and the seedlings in the tolerant peach families are better able to maintain water transport through the stem segment invaded by the fungus.

In the 1950s, peach 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 (Mich. Orchard and Vineyard Survey, 1986). *Cytospora* canker, caused by *Leucostoma persoonii* and *L. cincta* (Fr.) Höhn. [imperfect state, *Leucocytospora cincta* (Sacc.) Höhn.], is one of the most serious diseases reducing peach tree life in the central and northern United States (Gairola and Powell, 1970; Hildebrand, 1947; Jones and Leupschen, 1971; Luepschen, 1981; Luepschen et al., 1975) and in the eastern peach growing regions of Canada (Cline, 1982; Layne, 1976; Wensley, 1964).

Histopathological investigations of *L. persoonii* classified the canker pathogen as a facultative bark parasite (Biggs, 1984, 1986a, 1986b; Biggs and Stobbs, 1986; Wisniewski et al., 1984) or as a sapwood parasite (Banko and Helton, 1974; Tekauz and Patrick, 1974). These histopathological investigations concentrated on fungal growth in the bark. However, if the pathogen grew beyond the bark and into the xylem, there could be a noticeable reduction in water transport measured as K_h . K_h is the measured flow rate of a fluid divided by the pressure gradient. Reductions in K_h 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 *L. persoonii* and reported that pathogen infection reduced the xylem function in all inoculated cultivars in comparisons with uninoculated healthy plants. However, there were no significant differences among these peach cultivars for xylem dysfunction.

Recently, peach families tolerant to *L. persoonii* following

field inoculation were identified (Chang et al., 1989). The objective of our work was to determine if xylem dysfunction was associated with susceptibility to *L. persoonii*. In addition to K_h measurements, branch segments were sectioned and *L. persoonii* isolations were made to determine the depth of invasion of the fungus into the wood.

Materials and Methods

In Spring 1984, 653 open-pollinated peach seedlings from 15 peach clones, two peach $\times$ *P. kansuensis* hybrids, and one peach $\times$ almond (*P. dulcis* Webb) hybrid were planted in a completely randomized design at the Horticultural Research Center, East Lansing, Mich. These seedlings, representing half-sib families, were evaluated twice for length of canker necrotic tissue following inoculation of 2-year-old branches with *L. persoonii*. Branches were inoculated, using the technique developed by Scorza and Pusey (1984), for two experiments in Oct. 1986 and 1987 that then were evaluated in May of the following years. Two branches per seedling were each inoculated with $20\mu\text{l}$ of a suspension of 10^7 *L. persoonii* conidia/ml. The conidia were derived from a mixture of two isolates, one (MI R-1) collected in Hartford, Mich., and the other (MI 11.13) in Clarksville, Mich. (Adams et al., 1989). The following May, during bud-break, canker necrotic length was measured as the length of the necrotic area distal to the point of inoculation. Additionally, inoculated branches were rated visually for canker infection using a scale of 0 = dead, 1 = severe wilting of expanding leaves, 2 = weak growth and slight wilting of expanding leaves, and 3 = healthy. Since the canker ratings are nonparametric data, the Kruskal and Wallis test based on ranking observations from largest to smallest (Steel and Torrie, 1960) was used to test for significant differences among families. In the Kruskal and Wallis test, the null hypothesis is that the populations have the same mean.

Following the field inoculation experiments with *L. persoonii*, eight open-pollinated peach families were selected that

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Abbreviations: K_h , hydraulic conductance per pressure gradient.

were previously rated as susceptible or tolerant to *L. personii* (Chang et al., 1989). K_h measurements were made on branches from three seedlings chosen at random from each of the eight families. Two branches per seedling were each inoculated with 20 μ l of a suspension of 10^6 *L. personii* conidia/ml in Oct. 1986 as described by Chang et al., (1989). Two healthy branches within each tree were used as the noninoculated controls. Branches were collected between 0700 and 0800 hr the following May, and the basal ends were immediately immersed in water and recut under water to minimize the introduction of embolisms. To reduce lateral transport of water, lateral twigs were removed from the branches and wounds were immediately sealed with fingernail polish. The branches were trimmed to ≈ 0.25 m in the laboratory and stored 12 h under water at room temperature. A separate series of experiments showed that storage had no effect on K_h for 24 h. Both ends of each branch segment were shaved with a new razor blade until smooth. Vinyl tubing was then firmly clamped to the distal end. The stem surface was vacuum infiltrated with filtered 0.1 M oxalic acid, pH 2.0 at -87 kPa for 5 min before the conductivity measurement. Filtered oxalic acid was used to minimize possible artifacts caused by microbial growth in the fluid (Sperry et al., 1988). Pressure of 5 kPa was applied with a distilled water column and the rate of flow measured with a stopwatch and pipet (Zimmerman, 1978). K_h = (the rate of flow in $\text{m}^3 \cdot \text{s}^{-1}$) / (applied pressure gradient in $\text{MPa} \cdot \text{m}^{-1}$). All K_h measurements were done within 12 h of the initial collection time. Three readings were used to calculate a mean K_h of each stem segment. Specific conductivity (specific K_h) = K_h / cross-sectional area. Percent specific K_h = (specific K_h of the inoculated branch segments) / (specific K_h of the control stem segments) 100.

Canker necrotic length, specific K_h , and percent specific K_h were analyzed in a nested design with the following sources of variation: family, seedling within family, and branch within seedling.

Safranin-O dye (0.5%) was poured into the vinyl tube immediately after finishing K_h measurements. The height of the dye in the tubes was kept constant (0.5 m) for each branch segment for 1.5 h. The dye marked the xylem vessels capable of water transport. The hydroconductivity experiment was repeated the following year.

L. personii in the wood was isolated to determine the depth and degree of the fungal invasion. The branch segments containing the inoculated wound were trimmed to 6-mm lengths, debarked, and surface sterilized with 95% ethanol. Longitudinal sections of wood (1 mm deep) were excised beginning at the surface margins of necrosis and proceeding inward to the pith and beyond, through the stem, in 1-mm increments. The 1-mm sections of tissue layers were placed on Leonian's agar medium (Leonian, 1921). The culture plates were incubated at 25C under cool-white fluorescent light for 2 weeks and scored for the presence of *L. personii*.

Results and Discussion

Field inoculations in Fall 1986 and 1987 revealed significant differences among the peach families both in canker necrotic length and canker rating in response to *L. personii* infection. The open-pollinated 'Yennoh' progeny (numbered 1-31, 1-39, and 4-11) and open-pollinated NJ672017002 progeny (numbered 1-8, 2-32, and 4-16) had the shortest longitudinal length of necrosis (cankers) following inoculation and the highest rating (Table 1). These families were considered the most tolerant. Dhanvantari and Dirks (1983) found that linear canker extension

Table 1. Mean values for *L. personii* canker length and canker ratings in May following artificial inoculations in Fall 1986 and 1987 of open-pollinated seedlings of eight peach selections.^{z,y}

Parent	Canker necrotic length (cm)		Canker ratings ^{x,w}	
	1986	1987	1986	1987
Loring (2-7, 11-27, 11-32)	15.7 a	19.0 a	0.0	1.7
Elberta (6-32, 11-34, 11-38)	17.0 a	11.8 ab	0.0	1.0
Canadian Harmony (3-17, 3-20, 5-9)	13.1 abc	8.7 cd	0.5	1.7
Harken (2-6, 2-29, 2-44)	11.6 abc	15.0 ab	0.5	1.7
Reliance (1-33, 1-41, 15-16)	13.8 ab	7.8 cde	0.3	0.8
Babygold 8 (2-13, 2-14, 3-10)	12.8 abc	11.2 bc	0.5	1.0
NJ672017002 (1-8, 2-32, 4-16)	7.0 c	5.4 de	2.3	3.0
Yennoh (1-31, 1-39, 4-11)	7.1 bc	3.2 e	2.5	3.0

^zData are the mean of two branches from each of three seedlings per selection.

^yMeans followed by different letters are significantly different according to LSD at 0.05 level.

^xCanker rating is 0 = dead, 1 = severe wilting of expanding leaves, 2 = weak growth and slight wilting of expanding leaves, and 3 = healthy.

^wAll populations do not have the same mean according to the Kruskal and Wallis test at the 1% level (Steel and Torrie, 1960).

following artificial inoculation with *Leucostoma* was related to visual ratings of the incidence of naturally induced cankers during 10 years.

Specific K_h and percent specific K_h differed significantly among the eight peach families (Table 2). The specific K_h and percent specific K_h tended to be higher for the more tolerant than for the more susceptible peach families. The percent specific K_h was significantly correlated with the canker necrotic length and canker rating ($r = 0.74$ and 0.75 , respectively). The longer the canker necrotic length, the less water was capable of being transported, and the more severe was the wilting. This result suggested that the tolerant plants were able to transport more water through the infected zone than the susceptible plants. This ability could explain why branches from the tolerant seedlings remained healthy 7 months after inoculation and infection.

The variation in specific K_h and percent specific K_h was quite large and probably could be attributed to experimental variation in the size, age, and vigor of the plants plus genetic variation among the three seedlings per family. For example, the 1988 percent specific K_h means for Yennoh 1-31, 1-39, and 4-11 were 30.0%, 37.5%, and 33.5%, respectively. Likewise, the 1988 percent specific K_h means for NJ672017002 1-8, 2-32, 4-16 were 32.0%, 16.0%, and 15.5%, respectively. Yennoh 1-39 and NJ672017002 1-8 had previously been identified in the germplasm population as the most tolerant seedlings.

Fewer vessel elements were stained with safranin-O in the inoculated branches than in the noninoculated controls (Fig. 1). The extent of xylem dysfunction following inoculation was less in the tolerant seedlings than in susceptible seedlings. In susceptible seedlings, the outer xylem ring was nonconductive, while in the more tolerant seedlings the outer and some of the inner xylem vessels were conductive. Since branches had been

Table 2. Specific K_h ^z and percent specific K_h ^y for *Leucostoma* canker infection following artificial inoculations in Fall 1986 and 1987 of open-pollinated seedlings of peach.^{*w}

Parent	Specific K_h		Percent specific K_h	
	1986	1987	1986	1987
Loring (2-7, 11-27, 11-32)	0.58 c	1.02 b	5.9 cd	8.8 bc
Elberta (6-32, 11-34, 11-38)	0.92 bc	1.52 ab	6.0 cd	17.9 bc
Canadian Harmony (3-17, 3-20, 5-9)	1.81 abc	0.78 b	14.2 abcd	5.9 c
Harken (2-6, 2-29, 2-44)	0.63 c	0.82 b	5.4 d	11.4 bc
Reliance (1-33, 1-41, 15-16)	1.18 bc	0.93 b	10.9 bcd	11.8 bc
Babygold 8 (2-13, 2-14, 3-10)	1.81 abc	2.81 ab	20.3 a	20.7 ab
NJ672017002 (1-8, 2-32, 4-16)	3.10 a	3.44 a	15.2 abc	21.4 ab
Yennoh (1-31, 1-39, 4-11)	2.48 ab	3.16 a	18.5 ab	33.9 a

^zSpecific K_h = volume [(flow in $m^3 \cdot s^{-1}$)/applied pressure gradient in MPa per m of stem length]/cross-sectional area of the stem in m^2 .

^yPercent specific K_h = (specific K_h of inoculated branch)/(the control branch)100.

^{*}Data are the mean of two branches from each of three seedlings per selection.

^wMeans followed by the same letters are not significantly different according to LSD at 0.05 level.

Fig. 1. Transverse sections of a pair of peach branches used for hydraulic conductance measurements of Canadian Harmony (3-17). Left: branch was inoculated with *L. peroonii*; right: uninoculated control. Stained xylem (arrows) indicate safranin-O (0.570) movement through the functional xylem. The xylem in the inoculated branch was nonfunctional.

inoculated in October and evaluated in the following May, the presence of conductive xylem vessels would become critical during the growing period.

Above the necrotic zone of *L. peroonii* infection, K_h , specific K_h , and quantity of dyed xylem vessels were equivalent in control and in inoculated branches (data not presented). Vessel blockage was apparently localized around the infected canker area; therefore, wilting symptoms were not likely due to transport of fungal toxins.

To understand the involvement of the *L. peroonii* fungus in disrupting xylem flow, we determined the depth of *L. peroonii* invasion into the xylem. In susceptible families, it was possible to isolate the fungus from the margin of the surface necrosis down to the pith of the branch. For the tolerant families, the fungus was isolated only as deep as the secondary layer (≈ 2 mm) beneath the margin of the surface necrosis. Percent infection was calculated as the percentage of 1-mm sections per in-

Table 3. Percent of 1-mm sections per inoculated stem from which *L. peroonii* was reisolated for open-pollinated seedlings of peach.

Parent (open-pollinated seedlings)	Infection ^z (%)
Loring (2-7, 11-27-32)	90
Elberta (6-32, 11-34, 11-38)	71
Canadian Harmony (3-17, 3-20, 5-9)	97
Harken (2-6, 2-29, 2-44)	95
Reliance (1-33, 1-41, 15-16)	70
Babygold 8 (2-13, 2-14, 3-10)	20
NJ672017002 (1-8, 2-32, 4-16)	9
Yennoh (1-31, 1-39, 4-11)	5

^zData are the mean of three seedlings per selection.

oculated branch from which *L. peroonii* was reisolated. Tolerant seedlings had <10% infected branch sections; however, the susceptible ones ranged from 20% to 90% infected branch sections (Table 3). The lack of penetration of the fungus through the wood of seedlings from tolerant families suggests that xylem characteristics of Yennoh and NJ672017002 seedlings limit the advance of the pathogen.

In conclusion, xylem dysfunction following *L. peroonii* infection of susceptible seedlings was associated with wilting and death of branches. The canker pathogen invaded the xylem in the susceptible peach seedlings and significantly reduced K_h through the infection zone. In the tolerant seedlings, the xylem remained functional following branch inoculation, perhaps because of reduced penetration by the fungus. Therefore, the tolerant seedlings are able to maintain adequate water transport through the necrotic canker zone during the summer following inoculation. The actual cause of branch death in susceptible seedlings still needs further study because both xylem and phloem are damaged by the pathogen.

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