

Association of Sweet Cherry Epidermal Characters with Resistance to *Monilinia fructicola*

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Abstract. Fruit from seven sweet cherry (*Prunus avium* L.) cultivars were harvested at full ripeness and inoculated under controlled conditions with conidia of the brown rot fungus *Monilinia fructicola* (Wint.). Cherries were assessed for incidence of brown rot, length of the incubation period, and number of conidia produced per fruit. Non-inoculated fruit were examined microscopically and the thicknesses of the cuticle and the external epidermal cell wall were determined. Percent infection and numbers of conidia produced per fruit were correlated negatively, and incubation period was correlated positively, with the thickness of the outer epidermal cell wall of the fruit.

Brown rot, incited by *Monilinia fructicola* (Wint.) Honey, is one of the most important diseases of sweet cherry in the temperate fruit-growing regions of North America (1). The disease is controlled using sanitation practices to reduce fungal inoculum in combination with protective fungicide programs (8). Host resistance is not a major component of disease management programs for sweet cherry, although some cultivars exhibit less brown rot in the field than others (1). Traits such as susceptibility to rain-induced splitting and heavy fruit clustering (7) often are associated with increased susceptibility to brown rot. However, these traits do not explain the range of relative susceptibility that currently exists in commercial cultivars. When specific characteristics of a cultivar have been suggested as an important factor in resistance, their significance is often unproven, and they rarely have been defined in precise terms (1). This research investigated cultivar differences in susceptibility to brown rot and examined the association of percentage infection, incubation period, and number of conidia produced per fruit with two epidermal characters—cuticle thickness and outer epidermal cell wall thickness.

Sweet cherries of seven cultivars (see Table 1) at harvest maturity (G. Tehrani, personal communication), collected with stems attached, were taken to the laboratory for inoculation under controlled conditions. A

minimum of 125 fruit, divided into five subsamples (four inoculated and one non-inoculated control), were used for each of seven cultivars. Fruit were washed in tap water for 2 min to remove most of the captan residue (5), dried, and the stems trimmed to ≈ 10 mm in length. Fruit were placed suture side up on 13-mm mesh autoclaved screens within $23^{\circ} \times 30^{\circ} \times 8^{\circ}$ -mm aluminum baking trays and were inoculated with a 30- μ l droplet of a conidial suspension containing 1×10^5 *M. fructicola* macroconidia/ml in Miller's solution (4). Conidial suspensions were tested routinely for percent germination on agar media to verify high (>95%) germinability. Noninoculated fruits were treated with Miller's solution only and served as controls to monitor the level of background infection.

Fruits were incubated in the dark for 22 hr at 20°C in a stainless steel inoculation room with a relative humidity >95% (monitored with an aspirated psychrometer), then moved to a controlled environment room (20°, 60% RH), where the inoculum droplets were allowed to dry over a 2-hr period. Fruits were incubated at 20° and >95% RH and observed daily for 6 days for the presence of necrosis and sporulation and were assessed individually on a scale from 0 to 3 (0 = no infection; 1 = necrosis equal in diameter to the inoculum droplet, no sporulation; 2 = necrosis larger in diameter than the inoculum droplet, no sporulation; and 3 = sporulating, necrotic lesion). Percentage infection was calculated as the percentage of fruit rated 2 or 3 on the 6th day after inoculation and corrected for background infections. Incubation period was calculated as the time required for 50% of the infected fruit to exhibit sporulation. At the end of the experiment, a representative subsample of 10 sporulating fruit was rinsed in water and the spores dislodged with a camel's hair brush. Conidial concentrations were determined with a haemocytometer and the data were used to calculate the mean number of macroconidia produced per fruit.

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Subsamples of 10 fruits per cultivar were prepared and fixed in formalin : acetic acid : ethanol on the day of harvest. Tissue pieces, $\approx 7 \times 7 \times 5$ mm, were cut from the fruit, fixed, and embedded in paraffin using standard histological procedures (3). Sections 8 μ m thick were mounted in 90% glycerine and examined under ultraviolet illumination (340–380-nm excitation, 430-nm barrier filters) to induce cuticle autofluorescence. The thickness of the cuticle and the outer epidermal cell wall, which was observed under brightfield illumination, was determined from three sections per fruit, 10 fruit per cultivar, with an ocular micrometer. A subsample (≈ 20 to 30) of washed fruit was collected for analysis of captan residues (performed by the Pesticide Residue Section of Agricultural Laboratory Services of Guelph). Data on percentage infection, length of the incubation period, and number of conidia per fruit were examined for correlations with cuticle thickness, epidermal cell wall thickness, skin thickness (the sum of the cuticle and epidermal cell wall thickness), and captan residue. Also, data were analyzed with one-way analysis of variance and a completely randomized design. Means for cultivars were separated with Duncan's multiple range test (6).

Percentage fruit infection, incubation period, and number of conidia per fruit varied significantly among cultivars (Table 1) and all were correlated significantly with both cuticle thickness and epidermal cell wall thickness in 1984 (Table 2). Cultivars with thicker cuticles and epidermal cell walls tended to exhibit relatively lower incidence of infection, longer incubation periods, and fewer conidia per fruit than their opposites. In the 2nd year of the experiment (1986), the cell walls were significantly thinner than those measured in 1984. However, the cultivar ranks for cell wall thickness for both years were correlated significantly (Spearman's $r = 0.90$, $P < 0.05$), and disease incidence and cell wall thickness were correlated significantly in the 2nd year (Table 2). There were no significant differences in cuticle thickness among the six cultivars tested in 1986.

Captan residues in the fruit were below that considered effective for brown rot control ($\bar{x} = 0.81$ mg·kg⁻¹) (5). Residue levels were not associated with the relative susceptibility of the various cultivars with respect to percent infection, incubation period, and the number of conidia per fruit ($r = -0.31$, 0.68, and -0.49 , respectively) (Table 2).

Cultivars varied in relative susceptibility to brown rot from year to year and, because their relative susceptibility to the fungus was correlated in both years with cell wall thickness, this relationship warrants further investigation. If thicker epidermal cell walls were associated with delayed infection, then the selection of cultivars for thicker cell walls could result in increased host resistance to *Monilinia* spp. Increased cell wall thickness also was associated with increased incubation periods and fewer conidia per fruit. Thicker epidermal cell walls could, there-

Table 1. Percentage infection, incubation period, number of conidia per fruit, cuticle thickness, and epidermal cell wall thickness of seven sweet cherry cultivars inoculated with 1×10^5 conidia/ml of *M. fructicola*.^{a,y}

Cultivar	Percentage infection		Incubation* period (hr) 1984	No. conidia/ fruit ($\times 10^6$) 1984	Cuticle thickness (μm)		Outer epidermal cell wall thickness (μm)	
	1984	1986			1984	1986	1984	1986
Valera	55 b	55 d	136 a	1.1 d	1.6 a	1.3 a	38 a	27 a
Viva	86 a	82 c	87 c	4.8 a	1.3 c	1.3 a	35 b	23 c
Venus ^w	---	100 a	---	---	---	1.2 a	---	24 bc
Bing	38 c	68 cd	119 ab	1.3 cd	1.6 ab	1.2 a	38 ab	25 b
Vega	74 a	94 ab	110 b	2.9 b	1.3 c	1.3 a	37 ab	24 bc
V690620	72 a	88 ab	92 c	3.0 b	1.4 bc	1.3 a	35 b	24 bc
Hedelfingen ^w	49 bc	---	129 ab	1.3 cd	1.6 ab	---	39 a	---

^aMeans for percentage infection were from 100 inoculated fruit per cultivar, means for incubation period were from four replicates with 25 fruit per replicate for cultivar, means for number of conidia per fruit, cuticle thickness, and epidermal cell wall thickness were from three replicates with 10 fruit per replicate per cultivar.

^yDifferent letters in columns denote significant differences according to Duncan's multiple range test ($P \leq 0.05$).

^xTime required for 50% of the fruit to exhibit sporulation.

^wData not collected for 'Venus' in 1984 and 'Hedelfingen' in 1986.

Table 2. Matrix of Pearson correlation coefficients for sweet cherry cultivars inoculated with 1×10^5 conidia/ml of *M. fructicola*.^z

Parameter	Percentage infection 1984	Incubation period 1984	Conidia/ fruit 1984	Captan residue 1984	Percentage infection 1986
Captan residue 1984	-0.309	0.683	-0.486		
Cuticle thickness 1984	-0.898**	0.871**	-0.906**	0.502	
Cell wall thickness 1984	-0.764*	0.951***	-0.813**	0.558	
Total skin thickness 1984	-0.784*	0.958***	-0.831**	0.502	
Cuticle thickness 1986	---	---	---	---	0.232
Cell wall thickness 1986	---	---	---	---	-0.831**
Total skin thickness 1986	---	---	---	---	-0.831**

^z***Significance levels ≤ 0.10 , 0.05, and 0.01, respectively.

fore, contribute to a reduction in the rate of disease increase. Preliminary scanning electron microscope observations of mature sweet cherry fruit showed that 90% of germinated *M. fructicola* conidia appeared to form appresoria and penetrate the epidermal surface directly (our unpublished data). Only a small percentage of penetrations occurred through the stomata. Curtis (2) reported similar results for cherry. The role of the cuticle in this pathosystem should be explored further.

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