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Fruit Cracking of a Susceptible, an Intermediate, and a Resistant Sweet Cherry Cultivar

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Abstract. **Fruit were studied to determine if anatomical and physiological features explain the difference in susceptibility to rain-induced cracking of the sweet cherry (*Prunus avium* L.) cultivars Sue (resistant), Lapins (moderately resistant), and Van (susceptible). Water uptake as a percentage of fruit weight at cracking tended to be high in 'Sue', medium in 'Lapins', and low in 'Van' and was related to the percentage of cherries remaining sound after 4 hours of immersion, suggesting that this trait is a factor in determining resistance. Mesocarp cells of 'Sue' were more rectangular in section than those of the other cultivars. Skin elasticity and thickness of the cuticle did not explain resistance of 'Sue' to cracking. Magnesium, copper, and phosphorus mineral contents were not related to cracking susceptibility, but the content of calcium, which influences cell wall integrity, in the epidermis of 'Sue' was lower than in 'Van'. Calcium content was not different in the hypodermal cells of the two cultivars. None of the anatomical features examined in this study explain the resistance to fruit cracking of 'Sue'.**

Fruit cracking can cause severe losses of sweet cherry when rain persists before and during harvest. Cracking also occurs during periods of high atmospheric humidity, when a large difference in water potential between tree and fruit can cause movement of water from the branches and leaves into the fruit (Yamamoto, 1973). Water uptake by 'Bing' fruit first causes separation of the cuticle from the epidermal wall, followed by further swelling, which ruptures the cuticle (Glenn and Poovaiah, 1989). Resistance to cracking was associated with reduced stress factors in the outer cell layers of the stylar area of the fruit, according to measurements made by Yamamoto et al. (1990, 1996). Roser (1996) reported the resistance to cracking of 82 cultivars over 4 years. Splitting and cracking in fruits was reviewed by Opara et al. (1997) and cherry cracking was reviewed by Seske (1987, 1995b) and Christensen (1976).

Christensen (1972b) showed that a susceptible cultivar with high fruit sugar levels was more predisposed to cracking than was the same cultivar with low fruit sugar, but this sugar effect was not found in a resistant cultivar. Christensen (1976) concluded that susceptibility of cultivars to cracking was only slightly influenced by fruit osmotic concentration and fruit size, and that size of stomata and quantity and quality of colloids in the cells were more important. Cracking of the cultivar 'Napoleon' was not predicted consistently by percentage of soluble solids, osmotic or turgor pressure, or fruit water potential (Andersen and Richardson, 1982).

The question of why susceptible cultivars are more predisposed to cracking than resistant ones has not been completely explained. We undertook this study to survey anatomical and physiological characteristics of three cultivars to determine if they were correlated with resistance to cracking. Water uptake dynamics; cell shape of epidermal, hypodermal, and mesocarp cells; and cell mineral content of epidermal and hypodermal cells were examined in the present study. Electron and light microscopy were used to survey and compare the morphological features of 'Sue', 'Lapins', and 'Van' sweet cherries, cultivars with low, moderate, and high susceptibility to cracking, respectively.

Materials and Methods

Two mature trees each of 'Sue', 'Lapins', and 'Van' located at the Pacific Agri-Food Research Centre, Summerland, B.C., were used as fruit sources. Samples of 50 sound fruit per tree were used for studying the imbibition of water from distilled water baths held at 20 °C. Fruit were picked from representative branches and standardized for quality. The mean fruit weight was 9.0, 11.2, and 9.4 g for 'Sue', 'Lapins', and 'Van', respectively. Throughout the study period, percent cracking of unsorted fruit samples at harvest was 10% or less for 'Sue', 25% or less for 'Lapins', and 50% to 100% for 'Van'. Fruit were picked at full maturity based upon size, color, and commercial picking dates in the area, except in Expts. 1 and 6. Fruit with the stems attached were weighed before and after immersion in distilled water. Immersion periods varied from 1 to 4 h depending on the experiment. A cracked fruit was defined as one having a skin break >2 mm.

Expt. 1. In 1990 and 1993, fruit of the three cultivars were picked on 5, 12, and 22 July. The amount of water taken up by cracked fruit was measured by weighing each fruit at the beginning of the experiment, examining fruit after 1 to 4 h of immersion, removing those which had cracked and weighing them, and calculating the percent weight gain of the cracked cherries. The fruit that remained sound after 4 h immersion were then weighed and their percent weight gain calculated. Data obtained for the three cultivars included: fruit weight increase due to water uptake that resulted in cracking; effect of picking date; and, a comparison of percent weight gain at failure vs. percent fruit remaining sound after 4 h immersion as measures of resistance to cracking.

Expt. 2. In 1992, water absorption via the stem cavity was investigated. Fruit of 'Lapins' were immersed in water with the stem intact; with the stem cut, but leaving a 2-mm stub attached to the fruit; or with the stem cut to a 2-mm stub, but sealed by filling the stem cavity with petroleum jelly. Twenty fruit were included in each treatment.

Expt. 3. In 1992, the aspect ratio (length : width ratio) of several cell types, sectioned perpendicularly to the fruit surface, was measured with a model JEM-100 CX II (JEOL Ltd., Tokyo) transmission electron microscopy (TEM). Twenty-five paired measurements were made per sample of epidermal, hypodermal, and mesocarp cells.

Expt. 4. A model JSM-840 (JEOL Ltd.) scanning electron microscope (SEM) with a Tracor Northern (Middleton, Wis.) energy dispersement spectrophotometer was used to determine the ratios of calcium, magnesium, copper, and phosphorus to potassium in epidermal and hypodermal cells of the three cultivars. Ten to 20 measurements of each cell type were made per cultivar.

Expt. 5. In 1992 and 1993, the thicknesses of the wax layer, cuticle, and epidermal cell wall were measured on samples, sectioned perpendicularly to the fruit surface, and prepared for light microscopy. Twenty-five measurements were made per cultivar.

Expt. 6. The force that resulted in skin rupture was measured with an Instron machine (Instron Corp., Canton, Mass.) using a 1.6-mm spherical tip. Ten fruit of each of the three cultivars from each of three harvests, 5, 12, and 20 July, were used. Fresh samples were prepared by bisecting the fruit longitudinally. They were mounted and the force was measured at the time of skin failure as the tip pushed through the flesh, then the skin.

Expt. 7. Lenticel (stomatal) imprints were made by using a suspension of gelatin that solidified on the fruit, removing the gelatin film, then measuring replicas of the lenticels using a light microscope. Seven samples were prepared per cultivar, and lenticel length, width, area, and number per unit area were recorded.

Microscopy. For electron microscopy, tis-

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Table 1. Effects of cherry cultivar and date of harvest on mean water uptake, as a percentage of fruit weight at cracking ("Cracked"), of sweet cherry after 4 h immersion in water ("Sound"). All 'Van' fruit had cracked after 4 h immersion. Data are from three harvest dates in each of 2 years. N = 50. (Expt. 1).

	Harvest					
	5 July		12 July		22 July	
Cultivar	Cracked	Sound	Cracked	Sound	Cracked	Sound
Sue	1.95 (0.08) a[y]	1.57 a[z]	1.20 (0.09) a[y]	1.12 a[z]	1.13 (0.11) a[y]	1.07 a[z]
Lapins	1.08 (0.06) b	1.26 b	0.98 (0.07) a	1.09 a	1.05 (0.07) b	0.92 a
Van	1.03 (0.06) b	---	0.74 (0.09) b	---	0.87 (0.05) b	---

[z]Mean separation within columns by Fisher's exact test for differences between proportion, $P \leq 0.05$.
[y]Mean separation within columns by the log rank test, $P \leq 0.05$.

sue samples of 1 mm^3 were used. Two samples were prepared, one from each of two fruits of each cultivar. For TEM (Expt. 3), the tissue was excised and preserved in 5% glutaraldehyde in Sorenson's buffer with caffeine, at pH 6.8, then vacuum infiltrated with a change of clean buffer. Tissue samples were washed several times in Sorenson's buffer with caffeine, then fixed in buffered 2% osmium tetroxide. After fixation, the tissue was dehydrated in an ethanol gradient, vacuum infiltrated with propylene oxide, then embedded in JEMBED-812 epoxy resin for sectioning. Sections were cut with an ultramicrotome (Reichert-Jung, Ultracut E, Vienna), mounted on copper grids, and stained with uranyl acetate in 75% ethanol, followed by Reynold's lead citrate. The stained sections were examined with the TEM operated at 80 kV.

For SEM (Expt. 4), the tissue sample was excised and preserved in the buffered glutaraldehyde solution (5%, pH 6.8) and stored at 4 °C in the same buffer for several days. The aldehyde-preserved samples were quench-frozen in liquid nitrogen, cryofractured, warmed to 4 °C overnight, then fixed in buffered 2% osmium tetroxide. The fixed samples were dehydrated in ethanol, critical-point dried using CO_2 with a CPD 020 critical-point dryer (Balzer's Furstentum, Liechtenstein), mounted on aluminum stubs, and coated with gold/platinum using a sputter coater (Desk II, Denton Vacuum Inc., Cherry Hill, N.J.). Samples were examined with the SEM operated at 15–25 kV.

For light microscopy (Expt. 5), excised tissue blocks (2 mm^3) were fixed in a solution of formalin, acetic acid, 95% ethanol, and distilled water (2:1:10:7, v/v). The fixed tissue was dehydrated in a series of t-butyl alcohol, embedded in Paraplast® (Sherwood Medical, St. Louis) and cut, in a plane perpendicular to the fruit surface, into sections 10 m thick. These were stained with safranin and fast green (Sass, 1958). Photomicrographs were made using phase contrast illumination and measurements were made from the photos.

Table 2. Effects of cultivar and date of harvest on percentage of cherries remaining sound, after 4 h immersion in water. Data from three harvest dates in each of 2 years. N = 50. (Expt. 2).

	Harvest		
Cultivar	5 July	12 July	22 July
Sue	68 a[z]	40 a	52 a
Lapins	34 b	10 a	34 a
Van	0 c	0 b	0 b

[z]Mean separation within columns by Fisher's exact test for differences between proportions. $P \leq 0.05$.

Statistical analysis. For statistical analysis of Expt. 1, the distributions of water uptake of the three cultivars at the three harvest dates were compared using the log rank test (Savage's exponential scores test) and mean percent water uptake and standard error were estimated using the SAS procedure LIFETEST (SAS Institute, 1989), where percent water uptake was measured and uncracked cherries were the censured observations. The cultivars were tested in pairs using a significance level of $P \leq 0.05$. Other experiments were analyzed using analysis of variance procedures.

Results

Expt. 1. The mean water uptake as a percentage of fruit weight at time of cracking reflected the cultivars' resistance classification (Table 1) with 1.49%, 1.04%, and 0.88% for 'Sue', 'Lapins', and 'Van', respectively. The mean percent water uptake of the fruit that did not crack after 4 h immersion was lower than that of those that cracked in 'Sue' but slightly more in 'Lapins', which is explained by the variation in the uptake resulting in splitting. Similar percentages of water uptake suggested that the rates of water uptake in the cultivars were similar. The percentage of fruit that remained sound after 4 h of immersion also reflected the cultivars' resistance class (Table 2). Means over the three harvest dates were 54%, 30%, and 0% for 'Sue', 'Lapins', and 'Van', respectively. The relationship between the two measures of cracking resistance is illustrated in Figure 1. Percent water uptake of fruit that cracked was correlated with percentage of sound fruit after 4 h in a curvilinear relationship. The response of the cultivars at the three harvest dates was similar.

Expt. 2. When 'Lapins' cherries were immersed with 1) stem left intact, 2) stem cut leaving a 2-mm stub, or 3) cut but both cut stem and stem cavity filled with petroleum jelly, percentage of cracking after 4 h in distilled water was 60%, 80%, and 70%, respectively. Thus, preventing water uptake through the stem : fruit interface did not affect cracking. When only the stylar half of the cherry was immersed in distilled water, no weight gain, or even a slight loss, was noted. Transpiration via the exposed surface and intact stem explains this observation.

Expt. 3. No differences were observed in the aspect ratio of epidermal and hypodermal cells among the three cultivars (Table 3). Mesocarp cell aspect ratio was higher in 'Sue' than in 'Van' or 'Lapins', indicating a more rectangular cell shape.

Expt. 4. Electron probe analysis of the epidermal cells showed a lower Ca : K ratio in 'Sue' than in 'Lapins' or 'Van', while 'Lapins' hypodermal cells had a greater ratio than those of 'Sue' and 'Van' (Table 4). The ratios for Mg, Cu, and P did not differ consistently among cultivars or cell types and were not correlated with cracking resistance. Copper was present in the hypodermal cells but not in the epidermal cells.

Expt. 5. Thickness of the wax layer did not differ among the cultivars in 1992 and 1993 (Table 5). Thickness of the cuticle was greater

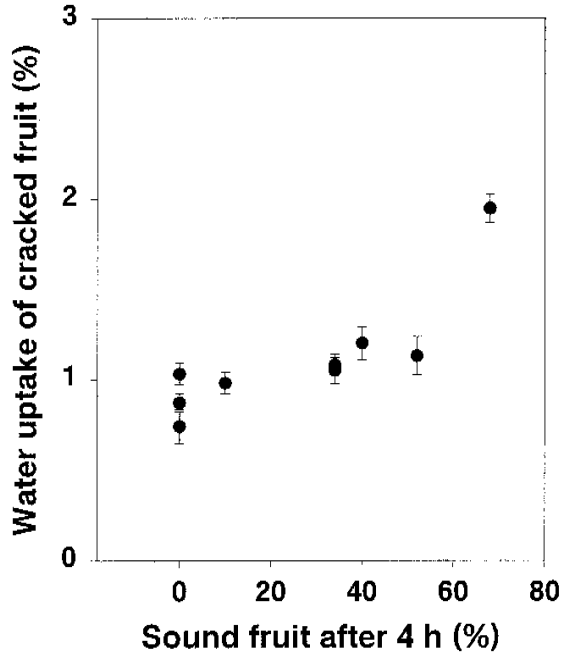

Fig. 1. Relationship between water uptake as a percentage of total fruit weight at the time of cracking and percentage of sound fruit after 4 h immersion in water. Derived from Tables 1 and 2. N = 50.

Table 3. Aspect ratio of epidermal, hypodermal, and mesocarp cells of three sweet cherry cultivars. (Expt. 3).

Tissue orientation[z]	Cultivar	Aspect ratio (L : W) Epidermis	Hypodermis	Mesocarp
Longitudinal	Sue	1.97 a[y]	2.60 a	1.70 a
	Lapins	1.92 a	2.11 a	1.27 c
	Van	1.76 a	2.29 a	1.45 b
Radial	Sue	1.62 a	2.60 a	1.45 b
	Lapins	1.78 a	3.38 a	1.45 b
	Van	1.69 a	2.70 a	1.34 c

[z]The microtome blade moved perpendicular (longitudinal), or parallel to the fruit surface (radial).
[y]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$, n = 25.

Table 4. Ratio of Ca, Mg, Cu, and P to K in epidermal and hypodermal cells in fruit of three sweet cherry cultivars as measured by SEM X-ray energy dispersement spectrophotometer. (Expt. 4).

Tissue	Cultivar	Element : K ratio Ca	Mg	Cu	P
Epidermis	Sue	0.13 c[z]	0.08 b	---	0.10 a
	Lapins	0.24 a	0.08 b	---	0.05 c
	Van	0.20 b	0.11 a	---	0.08 b
Hypodermis	Sue	0.20 b	0.11 a	0.20 b	0.13 a
	Lapins	0.28 a	0.10 a	0.28 a	0.10 a
	Van	0.21 b	0.07 b	0.21 b	0.10 a

[z]Mean separation by Duncan's multiple range test, $P \leq 0.05$, n = 10 to 20.

Table 5. Thickness of the wax layer, cuticle, and epidermal cell wall in fruit of three sweet cherry cultivars. (Expt. 5).

Year	Cultivar	Thickness (μm) Wax layer	Cuticle	Epidermal wall
1992	Sue	1.29 a[z]	0.85 a	4.96 a
	Lapins	1.23 a	0.58 b	4.53 a
	Van	1.30 a	0.16 c	4.15 a
1993	Sue	1.19 a	0.71 b	3.58 ab
	Lapins	1.09 a	0.94 a	3.39 b
	Van	1.22 a	0.89 ab	4.85 a

[z]Mean separation by Duncan's multiple range test, $P \leq 0.05$, n = 25.

Table 6. Instron reading, measured with a 1.6-mm spherical tip, at time of skin rupture for fruit of three sweet cherry cultivars. (Expt. 6).

Cultivar	Instron reading (g) 5 July	12 July	20 July
Sue	49 a[z]	43 ab	52 a
Lapins	43 a	36 b	32 b
Van	46 a	51 a	42 ab

[z]Mean separation by Duncan's multiple range test, $P \leq 0.05$, n = 10.

in 'Sue' than in 'Lapins' or 'Van' in 1992, but it tended to be thinner in 'Sue' than in the other cultivars in 1993. Measurements of epidermal cell wall thickness showed little if any difference between cultivars.

Expt. 6. Instron readings of the skin rupture force showed that 'Sue' skin was no stronger than that of 'Van' (Table 6).

Expt. 7. Lenticel replicas on a gelatin film showed that 'Sue' fruit had more lenticels per cm^2 (60) than did 'Lapins' (28) or 'Van' (37).

Discussion

Measurement of mean percentage of fruit weight gained at cracking as a predictor of resistance was high for 'Sue', intermediate for 'Lapins', and low for 'Van', consistent with the resistance classification of the three cultivars. The percent sound fruit after immersion in water for 4 h followed a similar pattern. Christensen (1976) found that stomata size and the capacity of the fruit to take up water before cracking explained ≈72% of the variation in cultivar differences in rate of water absorption, but that the rate of absorption was only slightly correlated with cracking index (Christensen, 1972a). We were unable to calculate the rate of water uptake by the cultivars in these experiments but further information on this point would be useful. Christensen (1976) found that the rates of water uptake were linear and were not related to the cracking index of the cultivars.

Time at which cracking occurred and the percentage of water uptake at cracking differed among cultivars. Variation may have resulted from, for example, higher turgor pressure at the beginning of the experiment due to environmental factors related to fruit position on the tree; consequently, less water uptake was needed to exceed the failure threshold and cause cracking.

Differences in resistance to cracking among cultivars was explained in part by the differences in percent water uptake at the time of cracking, with fruit of the resistant 'Sue' taking up more water before cracking than 'Van', and in two of the three harvest dates, more than 'Lapins', the intermediate cultivar. The cracking threshold of 'Lapins' was similar to that of 'Van' at the time of the first harvest but was higher at the second. An understanding of why cultivars differ in their water uptake cracking thresholds may explain, in part, resistance to cracking. A possible explanation is cultivar differences in the volume of air-filled voids between cells, which would allow water uptake without increase in fruit volume. This could be determined by measuring the ratio of percent water uptake to volume increase of resistant and susceptible cultivars. If they prove to be similar, differences in cell adhesion may be an explanation. Cultivars with cells weakly adhering to each other may crack at lower turgor pressure than those with cells that strongly adhere. Cell adhesion appears to be determined by covalent linking of noncellulosic polysaccharides to cellulose microfibrils (Herdia et al., 1995), pectins (Carpita and Gibeaut, 1993), and cross-links involving cinnamic acids (Ralph et al., 1995).

The Ca content of cells, as indicated by the K : Ca ratio, was not related to cracking resistance of cultivars, but spray applications of Ca salts to fruit in the field are known to reduce fruit cracking (Christensen, 1976; Meheriuk et al., 1991). The reduced cracking was explained by the direct reduction in the rate of water absorption caused by the increase in the osmotic concentration of the water in contact with the treated fruit (Christensen, 1976).

Our second measure of resistance to cracking was the percentage of cherries remaining sound after immersion for 4 h. Using this predictor, the three cultivars differed at the first harvest; 'Van' continued as more susceptible at the second and third harvests, but 'Lapins' and 'Sue' did not differ significantly.

Cultivars differ in stem thickness, and in the force required to separate the stem from the fruit. During and after rains, water often pools in the stem cavity. Anatomical features associated with the stem's attachment to the fruit are not critical since preventing water contact with this part of the fruit did not influence cracking.

Kertesz and Nobel (1935) noted smaller subepidermal cells in cultivars susceptible to cracking. Results from our study showed no difference in cross-sectional area for epidermal, hypodermal, or mesocarp cells among resistant, intermediate, and susceptible cultivars (data not shown). Mesocarp cells in the resistant cultivar 'Sue' had a higher aspect ratio than did those of 'Van', the highly susceptible cultivar. Assigning significance to mesocarp cell shape is difficult. Glenn and Poovaiah (1989) observed cuticle cracking resulting from swelling of cells in the epidermal cell region, and water uptake rate was enhanced if cracks were present. Differences among cultivars in cuticle cracking in 1992 suggested the possibility that the cuticle had a role in determining cracking reaction, but these differences were not observed in 1993. The type of cuticular cracking we observed was described by Glenn and Poovaiah (1989) and also by Seske (1995a). We noted cracks in 'Van' in 1992, but not in 1993, even though considerable rain-induced cracking occurred in 1993. Our experience suggests that cuticular cracking may be determined by environmental conditions, and is not a consistent cultivar trait involved in determination of resistance or susceptibility.

Christensen (1976) reported that cracking susceptibility was related to lenticel size and the type and amount of colloids in the intracellular space. The size of lenticels of 'Van' and 'Sue' was similar (0.88 and 0.87 mm^2, respectively), so lenticel size did not explain the difference in resistance to cracking. The higher density of lenticels on 'Sue' fruit did not result in greater water uptake by noncracked fruit in

this cultivar than in 'Lapins'. Thickness of the wax layer, cuticle thickness, and cell shape or size also did not account for the resistance to cracking of 'Sue'. Similarly, skin rupture force did not appear to be a factor, since values for 'Van' and 'Sue' were similar.

The results of this series of experiments suggest that the water uptake threshold at which fruit cracked is a major factor explaining the difference in resistance of 'Sue', 'Lapins', and 'Van'. The resistant, intermediate, and susceptible cultivars were matched to high, intermediate, and low water uptake thresholds. We suggest that the cultivar thresholds may be due to differences in volume increase : water uptake ratios or differences in cell adhesion. Anatomical features do not appear to determine resistance or susceptibility to cracking. The amount of colloids, such as soluble pectin and other carbohydrate polymers, has been proposed as the most important factor contributing to the osmotic potential of the apoplast driving water uptake by cherry fruits (Christensen, 1976), and differences in polymerization of pectin side chains occur in cherries (Batisse et al., 1996). Further studies to investigate the role of pectins, intracellular colloids, and cell adhesion in cherry fruit cracking, along with experiments to investigate why cultivar differences in water uptake cracking thresholds exist, will be of value in understanding the susceptibility and resistance of cherry cultivars to rain-induced cracking.

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