Cuticular Properties and Postharvest Calcium Applications Influence Cracking of Sweet Cherries

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Abstract. The influence of stomata, the stylar scar, cuticular fractures, and Ca2+ on susceptibility of 'Bing' sweet cherry fruit (Prunus avium L.) to water injury was studied. Water injury was first detected as an increase in cell turgor. Water penetration caused separation of the cuticle from the epidermal cell wall. Swelling in the epidermal cell wall region resulted in cuticular fracturing that generally preceded fruit cracking. Uncracked fruit that had cuticular fractures softened rapidly. Stomata were sparsely distributed on the fruit surface and were often fixed in an open or partially open position. Water injury was not visible at stomata even when injury occurred adjacent to the stomatal region. Initial signs of injury were commonly visible near the stylar scar. Histochemical studies revealed that the surface of the stylar scar was devoid of a cuticle covering and was rich in insoluble carbohydrates. Greater penetration of solute containing 45Ca2+ occurred at the stylar scar. Fine fractures in the cuticle surface were observed in fruit at harvest time in 1985 and 1986. Cherry fruit with cuticular fractures had a higher water absorption rate than unfractured fruit. In immersion tests, Ca2+ reduced cherry cracking. EGTA increased fruit cracking; this increase was negated by adding Ca2+. Neither Ca2+ nor EGTA affected the water absorption rate of the fruit. EGTA decreased the cracking threshold of the fruit, while Ca2+ increased it. Soluble pectin content of the immersion solution rose with increasing incubation times. EGTA increased while Ca2+ markedly decreased soluble pectin concentration in the immersion solution. Histochemical studies indicated a breakdown of the cell wall structure in the epidermal region of water-injured fruit. Autoradiographs of fruit immersed in a solution containing 45Ca2+ showed the epidermal region to be the site of Ca2+ action in altering fruit cracking. Chemical name used: Ethyleneglycol-bis-(β-aminoethyl ether) N,N,N,N-tetraacetic acid (EGTA)

Materials and Methods

Commercially ripe 'Bing' cherries were harvested from orchards located in four districts of Washington State each season between 1984 and 1987. The fruit was immediately transported in plastic-lined cartons to Washington State Univ., Pullman, and placed in cold (4°C) storage for later use. Light microscopy. Water injury in cherry was studied on fruit immersed in aerated distilled water as described by Verner and Blodgett (1931). The cherries received either no water treatment or were incubated in water for 1 to 4 hr and prepared for microscopic observation. Percipar tissue (2 mm2) with fruit skin was cut from water-treated and control fruit. Whole fresh cherries located in four districts of Washington State each season between 1984 and 1987. The fruit was immediately transported in plastic-lined cartons to Washington State Univ., Pullman, and placed in cold (4°C) storage for later use. Light microscopy. Water injury in cherry was studied on fruit immersed in aerated distilled water as described by Verner and Blodgett (1931). The cherries received either no water treatment or were incubated in water for 1 to 4 hr and prepared for microscopic observation. Percipar tissue (2 mm2) with fruit skin was cut from water-treated and control fruit. Whole fresh cherries were observed directly using a Wilder (model 334) compound photomicroscope. Pericarp tissue (2 mm2) with fruit skin was cut from water-treated and control fruit. Whole fresh cherries were observed directly using a Wilder (model 334) compound photomicroscope.

Scanning electron microscopy. Tissue prepared for scanning electron microscopy (SEM) was obtained from untreated (control) and water-treated (1 hr) fruit. Cherry segments were cut, fixed, and dehydrated as described above. The segments were critical-point-dried, gold-coated (10 nm), and viewed using an
ETEC Autoscan (Electron Beam Technology/Perkin-Elmer, Hayward, Calif., scanning electron microscope.

**Cracking study.** ‘Bing’ sweet cherries were harvested between 8:00 and 10:00 AM from the horticulture orchard located near the campus of Washington State Univ., Pullman, and immediately transported to the laboratory. The fruit pedicels were excised near the base to prevent them from puncturing other fruit during the treatment. The experimental design was completely randomized with three replicates of 50 fruit per treatment. The fruit were immersed in aerated solutions of distilled water (control) or 2.5-mM solutions of CaCl$_2$, MgCl$_2$, EGTA, EGTA + CaCl$_2$, or EGTA + MgCl$_2$. Fruit cracking was defined as any skin break 1.6 mm or longer (Verner and Blodgett, 1931). The increase in the number of cracked fruit at 2-hr intervals was obtained and a cherry cracking index was calculated according to Verner and Blodgett (1931).

Immersed fruit that were not cracked but had extensive cuticular fracturing were removed from the water treatment and set out at room temperature at 30% RH for 12 hr. Firmness readings on each fruit were recorded at the fractured site (treatment) as well as at a site lacking fractures (control). Firmness was measured by use of an Ametek spring force gauge following the method of Patten (1984).

**Water absorption study.** Cherries obtained from the Wenatchee district in 1985 and 1986 had cuticular fractures. Individual fruit having no cuticular fractures were paired with fruit of similar size and color having cuticular fractures. The fruit was weighed before and after 90 min of water immersion to determine the water absorption rate. Differences in the rate of water absorption were analyzed using a paired $t$ test.

The effect of EGTA and CaCl$_2$ on the rate of solution uptake and cracking threshold was performed on fruit harvested the morning of the experiment. The experimental design was completely randomized with 50 replicates of single fruit. Each fruit was weighed before treatment and again after cracking had occurred. The values obtained were used to calculate the rate of solution uptake and the cracking threshold (percent weight increase due to absorption that was necessary to induce cracking).

**Pectin estimation.** To test the effect of Ca$^{2+}$ and EGTA on pectin solubilization, fruit were treated for 2 or 10 hr and the pectin concentration of the immersion solution was determined. The 2- and 10-hr values were chosen because they represent initial and late stages of cracking. Pectin release into the incubation medium was measured using 30-ml aliquots following the method of Blumenkranz and Asboe-Hansen (1973).

**Autoradiography.** Cherries were immersed in a 10-mM CaCl$_2$ solution containing $^{45}$Ca$^{2+}$ [10 $\mu$Ci/ml $\times$ (1 Ci = 37 GBq)] for 60 min. The fruit was rinsed three times in 50 mM of CaCl$_2$ and immediately frozen in ethanol ($-60^\circ$C). Thick sections (200 $\mu$m) containing the stylar scar or fruit cross-sections were cut using a cryostat and exposed to XAR-5 diagnostic film for Ca$^{2+}$ localization.

**Results**

The surface of the cherry fruit is covered by a thin (1-$\mu$m) cuticular membrane that is largely devoid of surface microstructure. Minor amounts of a reticulate pattern of microstructure were observed near the fruit apex (Fig. 1A). The lack of abundant microstructure coupled with flat surface topography (Fig. 1 A and B) contribute to the glossy appearance of cherry fruit.
Sudan black B stained the cuticle black and intracellular contents blue. This stain was particularly useful because the cell wall region remained unstained and contrasted well with the cuticle (Fig. 1B).

The first morphological change of the fruit surface observed during water immersion treatments appeared as an increase in cell turgor followed by fracture formation in the cuticle (Fig. 2A and B). This change first occurred near the fruit apex or at the site of cuticular breaks. Cross-sectional views of water-damaged fruit revealed that water penetrated the outer epidermal cell wall and resulted in cuticle detachment (Fig. 3A and B). As swelling continued, fractures developed in the cuticle (Figs. 2A and B and 4A and B). These fractures were structurally distinguishable from cracks that penetrated the pericarp tissue. Cuticular fractures were minute and difficult to detect without the aid of a microscope. Scanning electron micrographs confirmed that the fractures traversed only the cuticle, thereby exposing the outer epidermal cell wall (Fig. 2B). Some degree of cuticular fracturing was nearly always observed and, in some case, severe fracturing occurred before fruit cracking began (Fig. 5A). Fruit cracking generally began at a point along a cuticular fracture and progressed along its length. However, many of the cuticular fractures did not develop into cracks and often both were evident on the fruit surface following extended immersion treatments.

The effect of the cuticular fractures on water loss and fruit softening was clearly evident in fruit maintained at room temperature (30% RH) for 12 hr (Fig. 5B). Data from firmness measurements showed that the fractured regions of the fruit were significantly softer than the surface areas that were not fractured (nonfractured surface = 1.70 N, fractured surface = 1.46 N; paired t test, \(a_{0.01} = 2.86\)).

Fruit cracking occurred unpredictably following the formation of cuticular fractures. Fracturing was most severe in fruit that did not readily crack. Evidence of internal cellular damage became apparent in some fruit as cellular contents exuded through stomatal apertures (Fig. 2A). Cross sectional examination of fruit sections from water-damaged tissue revealed regions where cell membranes separated from the cell wall and appeared disrupted (Fig. 4B). Lysis of epidermal cells became apparent in some fruit after several hours of immersion (Fig. 5A). The lysed cells appeared flaccid and discolored compared to unlysed cells.
Fig. 5. (A) Surface of a 'Bing' cherry fruit after several hours of water immersion treatment. The presence of lysed epidermal cells that have become flaccid is evident in comparison to viable cells that remain turgid (arrows). Cuticular fractures are dispersed throughout the fruit surface. (B) Photograph of 'Bing' sweet cherry fruit 12 hr following immersion treatment. The fruit on the left sustained no cuticular fractures and did not soften appreciably. The fruit on the right sustained severe cuticular fracturing, which resulted in desiccation and softening.

Staining of fruit sections with Sudan black B was positive for the cuticle and cellular contents (Fig. 1A). The cuticle covered the guard cells of the stomata and partially covered the inner walls of the stomatal chamber. Stomata were often fixed in an open or partially open position (Fig. 6A and B). No water damage was observed in the stomatal region even when it occurred adjacent to the stomata (Fig. 6B).

A probable site of water penetration exists at the stylar scar near the fruit apex. The surface of the stylar scar was covered by one to three layers of cellular debris. The cuticular membrane, which is found over most areas of the fruit surface, did not cover the stylar scar (Fig. 7A). PAS staining for carbohydrate polymers revealed that the stylar scar had much more PAS-positive substances than the surrounding tissue (Fig. 7B). The outer epidermal cell wall also stained more intensely with PAS reagent than the cell wall of the underlying mesocarp. Autoradiographs prepared from fruit that had received a 1-hr immersion treatment in 10 mM CaCl$_2$ containing $^{45}$Ca$_{2+}$ revealed a greater accumulation of Ca$_{2+}$ at the stylar scar than at other areas of the fruit surface (Fig. 7B inset).

A detailed study of the surface of untreated freshly harvested fruit revealed the presence of fine fractures (Fig. 8). Relatively few fractures were observed in fruit harvested from districts in Yakima and the horticulture farm in Pullman during the 1985 and 1986 harvest seasons. However, up to 40% of the fruit harvested from the Wenatchee district during the same period exhibited some degree of fracturing. The fractures were most commonly observed on the cheek opposite the ventral suture. Scanning electron micrographs revealed that the fractures traversed only the cuticle (Fig. 9A and C).

The water absorption rate of fruit with cuticular fractures was significantly higher than the absorption rate of unfractured fruit (paired $t$ test $t = 6.36$). Scanning electron micrographs of fruit with cuticular fractures before (Fig. 9A and B) and after (Fig. 9B and D) 60 min of water immersion showed that considerable water injury occurred throughout the cuticular fractures. Solubilization of wall materials resulted in cell separation in the water-damaged tissue.

Values obtained on the effect of CaCl$_2$, MgCl$_2$, EGTA, and distilled water on the cherry cracking index confirmed the role
Fig. 7. Sections prepared from mature ‘Bing’ cherry fruit and stained with Sudan black B (A) or PAS reagent (B). (A) the cuticle (arrow) of the fruit did not extend over the stylar scar (S). (B) PAS-positive material indicated that the stylar scar (S) was rich in carbohydrate material. The epidermal region of the fruit also had a greater abundance of carbohydrates. Autoradiographs were prepared from frozen thick sections of fruit containing $^{45}$Ca$^{2+}$ (inset). The fruit was previously immersed (1 hr) in CaCl$_2$ (10 mM) containing $^{45}$Ca$^{2+}$ and washed three times (50 mM CaCl$_2$). Note the greater radioactivity detected at the stylar scar (arrow) than in the adjacent tissue.

Fig. 8. Stereophotomicrograph of the surface of a mature ‘Bing’ cherry fruit. The presence of preharvest cuticular fractures is visible on the fruit surface.

of Ca$^{2+}$ in delaying fruit cracking (Table 1). At the concentrations tested, Mg did not reduce cracking below the level of the control fruit. EGTA resulted in cracking levels that were much higher than those in the CaCl$_2$ or water control treatments. EGTA induced small cracks that enlarged rapidly in the fruit. Small cracks that enlarged slowly were observed in fruit immersed in CaCl$_2$-containing solution. When CaCl$_2$ was included with the EGTA treatment, cracking levels were similar to levels obtained in the water control. When MgCl$_2$ was combined with EGTA, the cracking index was not significantly reduced below the cracking index for EGTA alone.

The effect of CaCl$_2$ and EGTA on the water absorption rate and cracking threshold of the fruit was studied to determine the relative importance of Ca$^{2+}$ on hydraulic permeability and tissue strength. Neither CaCl$_2$ nor EGTA affected the rate of water absorption (0.012% to 0.013%/min) when compared to the control. The cracking threshold for individual fruit within a treatment was variable, yet significant treatment differences between the CaCl$_2$ and EGTA treatments were observed. CaCl$_2$ increased the fruit cracking threshold compared to EGTA.

Chelating compounds are commonly used to extract pectin from cell wall preparations. Therefore, it appeared likely that EGTA might accelerate the solubilization of pectic materials from the cherry fruit cell wall structure. Soluble pectin content of the immersion solution increased with time in the control treatment and was accelerated by EGTA (Table 1). Significant levels of soluble pectin were detected after 2 hr of EGTA treatment, which coincided with the initiation of cracking. The immersion solution containing CaCl$_2$ had very low soluble pectin levels even after some cracking had occurred.

An increase in soluble pectin content of the immersion solution may not indicate a cause-effect relationship between pectin solubilization and cherry cracking. However, cuticular fracturing in water-damaged fruit did appear to involve solubilization of carbohydrate materials in the outer epidermal wall region (Fig. 4B). To confirm this observation, PAS staining for carbohydrate materials was performed on water-damaged cherry fruit (Fig. 10A) There clearly was less intense staining in the region of the cuticular fracture. Furthermore, it was evident that dissolution of wall carbohydrate material occurred at a very early stage of water damage. The region of cuticle-wall separation shown in Fig. 10A stained less intensely than adjacent uninjured tissue. Similar results were obtained with sections stained with ruthenium red. Autoradiographs of frozen cherry sections prepared from fruit that was immersed for 1 hr in a solution containing $^{45}$Ca$^{2+}$ show that Ca$^{2+}$ was localized in the epidermal region of the fruit (Fig. 10B).

**Discussion**

Direct absorption of water through the cherry cuticle is known to be an important mode of water penetration (Kertesz and Nebel, 1935; Verner, 1938; Westwood and Bjornstad, 1970). The absence of trichomes and abundant epicuticular wax ultrastructure may facilitate wetting of the cherry fruit surface, which is a prerequisite to absorption (Schonherr and Bukovac, 1972). Cu-
Fig. 9. Scanning electron micrographs of preharvest cuticular fractures both before (A and C) and after (B and D) 90 min of immersion in water. The fractures were 20 to 30 μm wide and 1 to 3 mm long (A) and only penetrated the cuticle (Cu). The water treatment solubilized cell wall materials, which led to cell separation (arrows).

Cuticular penetration is also facilitated by stomata, lenticels, and other surface breaks that are present in the cuticular structure (Martin and Juniper, 1970; Glenn et al., 1985; Glenn and Poo-vaiah, 1985).

Stomata have long been considered important sites for penetration of aqueous solutions (Schonherr and Buckovac, 1972; Sands and Bachelard, 1973). Penetration occurs primarily at the surface of guard and accessory cells (Greene and Bukovac, 1974). Aqueous solutions having a high surface contact angle do not penetrate stomata by mass flow (Schonherr and Buckovac, 1972). It is therefore unlikely that stomata provide a conduit for water penetration during rainfall. When stomatal density is considered (85 to 229 cm² for fruit vs. 5000 to 10,000 cm² for leaves), the relative importance of stomata in water absorption in cherry fruit as compared to leaves is diminished further (Christensen, 1972). We found no water damaged tissue near stomata even when water damage occurred in regions adjacent to stomata.

Cherry fruit do not form a periderm in response to wounding.
Tab 1. Effect of immersion treatments in water, CaCl₂, EGTA, MgCl₂, EGTA + CaCl₂, and EGTA + MgCl₂ on cracking index, pectin solubilization, rate of solution uptake, and cracking threshold in 'Bing' sweet cherries.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Control</th>
<th>CaCl₂ (2.5 mM)</th>
<th>EGTA (2.5 mM)</th>
<th>MgCl₂ (2.5 mM)</th>
<th>EGTA + CaCl₂ (2.5 mM)</th>
<th>EGTA + MgCl₂ (2.5 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracking index</td>
<td>31.0 ± 9.3</td>
<td>9.3 ± 5.4</td>
<td>59.3 ± 2.3</td>
<td>28.3 ± 2.3</td>
<td>28.3 ± 2.3</td>
<td>52.3 ± 2.3</td>
</tr>
<tr>
<td>Cracking threshold</td>
<td>3.6 ± 0.6</td>
<td>5.4 ± 0.6</td>
<td>73 ± 0.6</td>
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</tr>
<tr>
<td>Soluble pectin (2 hr)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>73 ± 0.6</td>
<td>---</td>
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</tr>
<tr>
<td>Soluble pectin (10 hr)</td>
<td>284 ± 3.4</td>
<td>8 ± 0.6</td>
<td>408 ± 6.3</td>
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*Cracking index calculated according to Verner and Blodgett (1931). Lower value indicates less cracking.

*Mean separation (in rows) by Duncan's multiple range test, 5% level.

*Cracking threshold expressed as percent weight increase required for crack development.

Fig. 10. (A) Photomicrograph of a section prepared from a water-injured cherry fruit. The section is stained for carbohydrates using Periodic acid-Schiff's reagent. Very little staining occurred in the region of the cuticular fracture (F). Carbohydrate materials appear solubilized in areas where the initial stages of injury are apparent (arrow). (B) Autoradiograph showing ⁴⁵Ca²⁺ localization in frozen sections of cherry fruit.

or for lenticel formation (Tukey and Young, 1939). Hence, it is not surprising that the stylar scar lacked formation of a hydrophobic periderm layer. The lack of cuticle or periderm barriers and the relative abundance of carbohydrate material found at the stylar scar may contribute to the susceptibility of the fruit apex to water damage. Greater ⁴⁵Ca²⁺ penetration occurred at the stylar scar, which suggested that water penetration may also be greater in that region. This is consistent with the observation that fruit cracking often occurs first at the apical region of the fruit (Verner and Blodgett, 1931). The importance of the stylar scar as a site of water penetration may be greater under field conditions, since water drops normally form at the fruit apex during rainfall and persist for some time after the rain has stopped (Verner and Blodgett, 1931).

Cuticular permeability is also affected by breaks occurring in the cuticle (Faust and Shear, 1972; Glenn et al. 1985). The development of preharvest cuticular fractures has been reported in apple fruit and is believed to be induced by environmental conditions that promote division and expansion of cells in the epidermal region (Faust and Shear, 1972). An important finding in this study was the occurrence of preharvest cuticular fractures that markedly increased the water absorption rate in cherry fruit. Preharvest cuticular fractures may also facilitate desiccation and pathogen invasion. The cause of preharvest fracture formation in fruit that has not been rain-damaged has not yet been determined. The inconsistent occurrence of preharvest fractures from year to year suggested that cultural or environmental factors were involved. The cheek of the fruit (side opposite the ventral suture) underwent more swelling and growth and consistently showed greater cuticular fracturing than other portions. This difference indicated that cell expansion may be responsible for cuticular fracturing, as reported for apple (Tetley, 1929; Faust and Shear, 1972).

Verner and Blodgett (1931) divided water (rain) injury in sweet cherry into three distinct types of cracking based on location on the fruit surface. They also described the occurrence of very fine, concentric rings of broken tissue that were little more than skin deep. Considine (1982) described development of cuticular fractures in 'Sultana' grape (Vitis vinifera) due to water absorption. This study documents the occurrence of fine, cuticular fractures that form early during the development of water injury and can become very extensive before fruit cracking begins. Cuticular fracturing accelerated softening due to water loss and may account for rapid quality deterioration of various lots of commercial fruit that have been water-damaged. While no practical means yet exists for commercial sorting of fruit having cuticular fractures, representative samples of fruit could be inspected to assess the extent of injury. This information would provide a better means of quality control during...
years when rains occurred and would be valuable for growers who must decide whether to harvest the crop.

The mechanisms involved in cherry fruit cracking have been studied for many years (Verner, 1939; Andersen and Richardson 1982). The present study suggests an important role for cell wall breakdown in cherry cracking. Calcium appeared to reduce cracking susceptibility of 'Bing' sweet cherries by delaying cell wall breakdown. An estimated 60% of the total Ca\(^{2+}\) found in plant tissues is localized in the cell wall, where it is believed to interact with pectic polymers in conferring tissue rigidity (Dey and Brinson, 1984; Fry, 1986). The use of chelating agents to extract Ca\(^{2+}\) from the cell wall of cell suspension cultures is reported to induce wall loosening and cell separation (El Hinnaway, 1974). In this study, treatments with a Ca\(^{2+}\) chelator promoted cherry cracking and pectin solubilization. The effect of the chelator could be completely negated by adding back an equal concentration of CaCl\(_2\). Further reduction in the level of fruit cracking occurred in the presence of solutions containing CaCl\(_2\) alone. These results indicate that Ca maintains or strengthens the cell wall structure and must be present in a free (Ca\(^{2+}\)) form to actively reduce cherry cracking. Various formulations of foliar-applied fertilizers contain chelating materials. These materials may increase cracking susceptibility in cherries, and should be used with caution.

In summary, this study showed that Ca\(^{2+}\) and cuticular structure influenced that susceptibility of 'Bing' sweet cherry fruit to water injury. Ca\(^{2+}\) was effective in protecting the epidermal region of the fruit from processes that lead to cracking. Stomata were relatively unimportant in determining the susceptibility of 'Bing' cherry fruit to water damage as compared to the styal scar and preharvest cuticular fractures. Efforts directed at controlling the development of cuticular fractures through cultural practices may prove beneficial in reducing cracking susceptibility in 'Bing' sweet cherry.

Literature Cited


