Mechanical Properties of Skins of Sweet Cherry Fruit of Differing Susceptibilities to Cracking

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ADDITIONAL INDEX WORDS. *Prunus avium*, epidermis, hypodermis, modulus of elasticity, strain, tensile test

ABSTRACT. Rain cracking of sweet cherry fruit (*Prunus avium* L.) may be the result of excessive water uptake and/or of mechanically weak skins. The objectives were to compare mechanical properties of the skins of two cultivars of contrasting cracking susceptibility using biaxial tensile tests. We chose ‘Regina’ as the less-susceptible and ‘Burlat’ as the more-susceptible cultivar. Cracking assays confirmed that cracking was less rapid and occurred at higher water uptake in ‘Regina’ than in ‘Burlat’. Biaxial tensile tests revealed that ‘Regina’ skin was stiffer as indexed by a higher modulus of elasticity (E) and had a higher pressure at fracture (p_{fracture}) than ‘Burlat’. There was little difference in their fracture strains. Repeated loading, holding, and unloading cycles of the fruit skin resulted in corresponding changes in strains. Plotting total strains against the pressure applied for ascending, constant, and descending pressures yielded essentially linear relationships between strain and pressure. Again, ‘Regina’ skin was stiffer than ‘Burlat’ skin. Partitioning total strain into elastic strain and creep strain demonstrated that in both cultivars most strain was accounted for by the elastic component and the remaining small portion by creep strain. Differences in E and p_{fracture} between ‘Regina’ and ‘Burlat’ remained even after destroying their plasma membranes by a freeze/thaw cycle. This indicates that differences in skin mechanical properties must be accounted for by differences in the cell walls, not by properties related to cell turgor. Microscopy of skin cross-sections revealed no differences in cell size between ‘Regina’ and ‘Burlat’ skins. However, mass of cell walls per unit fresh weight was higher in ‘Regina’ than in ‘Burlat’. Also, the ratio of tangential/radial diameters of epidermal cells was lower in ‘Regina’ (1.86 ± 0.12) than in ‘Burlat’ (2.59 ± 0.15). The results suggest that cell wall physical (and possibly also chemical) properties account for the cultivar differences in skin mechanical properties, and hence in cracking susceptibility.

Wherever sweet cherries are grown, rain-induced fruit cracking imposes a major limitation to production (Christensen, 1996). Susceptibility to rain cracking differs among cultivars (Christensen, 1995, 1999, 2000; Measham et al., 2009), but the mechanistic basis of differential cracking susceptibility among cultivars is not clear. From the coincidence of rainfall and fruit cracking, it is inferred that cracking is related to water uptake into the fruit. Water uptake, in turn, leads to an increase in volume, causing the fruit surface area to increase. When the limits of extensibility are exceeded, the fruit is expected to crack.

Based on the above logic, differential cracking susceptibility among cultivars could result from either (or both) of two, mechanistically unrelated, factors. First, the net import of water into the fruit will affect cracking by causing fruit volume to increase, thereby straining the skin beyond some defined upper limit. Second, mechanical properties of the fruit skin will affect the fracture limits. Recent investigations (Brüggewirth et al., 2014) have established that it is the epidermal and hypodermal cell layers (not the cuticle) that represent the structural “backbone” of the skins of sweet cherry fruit. Thus, differences in cracking susceptibility among sweet cherry cultivars could be the result of different water-uptake characteristics and/or of

the mechanical properties of the epidermal and hypodermal cell layers.

Water uptake across the fruit surface has been studied in detail in the last decade. The pathways and mechanisms of transfer have been identified (Beyer et al., 2005; Knoche et al., 2000; Weichert and Knoche, 2006). However, among 29 cultivars, there was no close relationship between variation in cracking susceptibility and variation in the major characteristics of the fruit surface: water permeance, strain, mass of cuticle per unit area, stomatal density, etc. (Peschel and Knoche, 2012). Some information is available on vascular transport, but to our knowledge differences among cultivars have not been identified (Brüggewirth and Knoche, 2015; Hovland and Sekse, 2004a, 2004b; Measham et al., 2009, 2010, 2014).

Little information is available on the mechanical properties of the sweet cherry fruit skin (Bargel et al., 2004). Recently, Brüggewirth et al. (2014) modified the biaxial bulging test to quantify the mechanical properties of excised fruit skins under standardized laboratory conditions. Modifications included dimensional fixation of a skin segment in a washer before excision, to prevent any release of elastic strain [built up in the skin due to growth (Grimm et al., 2012)]. Silicone oil was used to give a hydrostatic pressure to the skin segment from the physiological inside to prevent uncontrolled water uptake from that side—this could have confounded the test results (Simon, 1977). In contrast to the more common, uniaxial tensile tests of engineering, biaxial tensile tests better mimic the growth stresses and strains occurring in vivo. In a spherical organ of nearly isotropic growth, stresses and strains are of course fairly uniformly biaxial, not uniaxial. Also, sweet cherry skin exhibits high Poisson ratio properties, so a uniaxial tensile test using

Received for publication 18 Nov. 2015. Accepted for publication 23 Dec. 2015. This research was funded in part by grants from the Deutsche Forschungsgemeinschaft.

We thank Dieter Reese and Christoph Knake for constructing, engineering, and programming the elastometer; Friederike Schroeder and Simon Sitzenstock for technical support; Bishnu P. Khanal and Sandy Lang for helpful discussion and useful comments on an earlier version of this manuscript.

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a skin strip is associated with severe narrowing, and hence a gross overestimation of skin extensibility (Brüggenwirth et al., 2014).

The purpose of this study was to 1) quantify the key mechanical properties of two sweet cherry cultivars of contrasting cracking susceptibility, using a biaxial tensile test and 2) investigate the mechanistic basis of any differences between the two cultivars. Because many fruit may vary diurnally in diameter, and hence surface area (Lang, 1990; Measham et al., 2014; Montanaro et al., 2012; Ohta et al., 1997) and because this may cause the skin to fatigue, we also investigated the effects of repeated loading and unloading cycles on the mechanical properties of the fruit skin of the two cultivars.

Materials and Methods

PLANT MATERIAL. Fruit of sweet cherry ‘Adriana’, ‘Burlat’, ‘Hedelfinger’, ‘Merchant’, ‘Regina’, ‘Sam’, and ‘Samba’ grafted on ‘Gisela5’ (*Prunus cerasus* L. × *Prunus canescens* Bois) were collected from greenhouse-grown trees in the 2013 and 2014 seasons. All fruit were obtained from the experimental station of the Leibniz University, Hannover, Germany (lat. 52°14’N, long. 9°49’E). Water was supplied via drip irrigation. The drip irrigation was operated for 2-h intervals (corresponding to ≈15 mm of precipitation) as needed based on visual inspection of the moisture of the top soil (<20 cm). Fruit were harvested at maturity and selected for uniformity (based on color and size) and were free of visual defects. All fruit for mechanical testing were processed within 12 h of harvest. When held for more than 2 h, fruit was placed in a cold room at 5 °C, for shorter periods of time fruit was held at ambient temperature and covered with a damp paper towel. The ‘Regina’ and ‘Burlat’ fruit used for microscopy of the cross-sections of the fruit surface were fixed in Karnovsky solution (Karnovsky, 1965).

THE ELASTOMETER. For a detailed description of the elastometer, the reader is referred to Brüggenwirth et al. (2014). Briefly, an excised exocarp segment [ES (synonym fruit skin segment)] is mounted on a polymethylmethacrylate (Lucite®; Mitsubishi Rayon Lucite Group, Southampton, UK) chamber filled with silicone oil (AK10; Wacker Chemie, Munich, Germany). The chamber is pressurized by driving a motorized piston into it, causing the volume of the chamber to decrease at a rate of ≈153 μL·min⁻¹. The volume displacement causes the chamber pressure to increase and the ES to bulge outward. The pressure inside the chamber and the extent of bulging are monitored using a pressure transducer (Typ 40PC100G; Honeywell, Morristown, NJ) and a displacement transducer (KAP-S/5N; AST Angewandte System Technik, Wolnzach, Germany) from a material testing machine (BXC-FR2.5TN; Zwick Roell, Ulm, Germany). To preserve the in vivo strain of the fruit skin following excision, a brass washer (aperture diameter 12 mm) was glued to the shoulder of an intact sweet cherry fruit using cyanoacrylate adhesive (Loctite 406; Henkel/Loctite Deutschland, Munich, Germany) (Knoche and Peschel, 2006). By cutting underneath the washer with a razor blade, an ES was excised having an average thickness of ≈2.4 ± 0.02 mm. The ES remained fixed to the washer after excision, which prevented relaxation, thereby maintaining the in vivo strain that existed before excision (Brüggenwirth et al., 2014; Knoche and Peschel, 2006). The ES so-obtained comprise

cuticle, epidermis, hypodermis, and some adhering mesocarp tissue.

The surface area (A) of the bulging ES is calculated from Eq. [1], where r is the radius of the washer aperture and h is the height of the bulge ES (Brüggenwirth et al., 2014).

$$A = (r^2 + h^2) \times \pi \quad [1]$$

An estimate of the total strain (ϵ) is obtained from the change in surface area (ΔA) relative to the initial surface area (A_0) of the bulging ES and is given in Eq. [2].

$$\epsilon = \frac{\Delta A}{A_0} \quad [2]$$

In some experiments, the modulus of elasticity (E) was estimated from the height (h) of the bulging ES, the radius (r) of the aperture, the pressure (p), and the thickness (t) of the bearing layer ($t = 0.1$ mm) using Eq. [3] (Brüggenwirth et al., 2014).

$$E = \frac{p \times r^2 \times (r^2 + h^2)}{h^3 \times t \times 2} \quad [3]$$

All mechanical tests were performed at 22 °C.

EXPERIMENTS. The modulus of elasticity [E (megapascals)], the pressure at fracture [p_{fracture} (kilopascals)], and strain at fracture [$\epsilon_{\text{fracture}}$ (square millimeters per square millimeter)] was quantified for a range of cultivars. Biaxial tensile tests were carried out as described above.

Total, elastic, and creep strains and the effects of repeated loading, holding, and unloading cycles were measured for ‘Regina’ and ‘Burlat’. These cultivars were selected because of their contrasting cracking susceptibilities (Christensen, 1999). ES were excised and mounted as described above. Experiments were initiated by increasing the pressure during the loading phase. During the subsequent holding phase, pressure was maintained constant and then decreased in the unloading phase. The time period for a complete loading/holding/unloading cycle was ≈5 minutes. Preliminary experiments established that the test protocol had to be adapted to the cultivar tested because fracture limits differed between ‘Regina’ and ‘Burlat’. First, the pressure applied to ‘Burlat’ skin was about half of that in ‘Regina’. Second, the number of loading and unloading cycles was reduced by half for ‘Burlat’ skin, compared with that for ‘Regina’. Higher numbers of cycles resulted in fracture of the skins (M. Brüggenwirth, unpublished data). Three types of experiments were conducted. In the first experiment, the effect of increasing loading was studied by increasing the pressure stepwise in 5-kPa increments (0 to 40 kPa for ‘Regina’ and 0 to 20 kPa for ‘Burlat’). In the second experiment, pressure was held constant during each cycle (25 kPa for ‘Regina’ and 10 kPa for ‘Burlat’). The last experiment comprised pressures that decreased stepwise by 5-kPa increments (40 to 0 kPa for ‘Regina’ and 20 to 0 kPa for ‘Burlat’). Data were analyzed by partitioning the strain during each cycle into a total strain, an elastic strain, and a creep strain. The elastic strain was defined as the strain during the loading phase, and the creep strain was defined as the deformation occurring during the holding phase. Total strain represented the sum of the elastic strain and the creep strain.

The effect of eliminating cell turgor by destroying plasma membrane integrity was investigated in ‘Regina’ and ‘Burlat’.

To do this, the ES were frozen for 30 minutes at -18°C , then thawed for 30 minutes at 22°C , and then tested in the elastometer.

CRACKING AND WATER UPTAKE. Cracking and water uptake relationships were established for ‘Regina’ and ‘Burlat’ sweet cherry fruit by incubation in deionized water at 22°C . Water uptake was restricted to the fruit surface by previously sealing the stem cavity with silicone rubber (SE9186 Coating; Dow Corning, Midland, MI). Cracking was quantified in two batches of 25 fruit each, by visual inspection of fruit at 2, 4, 6, 8, 10, and 24 h for macroscopically visible cracks. The time to 50% fruit cracking (T_{50}) was calculated using a four-parameter sigmoidal regression model, fitted to a plot of the percentage of fruit cracking vs. time (Winkler et al., 2015). Fruit that had cracked was removed from the solution, and noncracked fruit was returned for further incubation. Water uptake was quantified gravimetrically after 0.75 and 1.5 h ($n = 15$). The water uptake required for 50% fruit cracking (WU_{50}) was calculated by multiplying the T_{50} by the rate of water uptake.

MICROSCOPY. Dimensions of epidermal and hypodermal and parenchyma cells were quantified in fruit of ‘Regina’ and ‘Burlat’ fixed in Karnovsky solution (Karnovsky, 1965). Fruit were removed from the fixation solution and thoroughly washed in deionized water. Cross-sections were prepared from the shoulder region of the fruit, using parallel razor blades (spacing 3 mm), and the sections were incubated for 7 min in 0.1% (w/w) calcofluor white (fluorescent brightener 28; Sigma-Aldrich Chemie, Munich, Germany). Thereafter, cross-sections were rinsed with deionized water, positioned on a microscope slide, transferred to the stage of a fluorescence microscope (model BX-60; Olympus Europa, Hamburg, Germany), and viewed under ultraviolet light at $\times 400$ (model BX-60, filter U-MWB 330–385 nm excitation wavelength, ≥ 420 nm emission wavelength; Olympus Europa). Calibrated micrographs were taken using a digital camera (DP71, Olympus Europa), and the digital images were analyzed (software CellP, Olympus Europa). For the cells of the epidermis, hypodermis, and outer mesocarp, the cell sizes were measured (one radial and two tangential diameters) and the distances of the cell centers from the cuticle were quantified along a line drawn perpendicular to the fruit surface.

MASS OF CELL WALLS. Tissue cylinders (8 mm diameter) were punched using a biopsy punch (Kai Europe, Solingen, Germany) and cut to discs of 2.5 mm height using parallel razor blades. The discs comprised cuticle, epidermis and hypodermis, and the outer mesocarp. Discs were frozen in liquid N_2 and held at -20°C . For analysis, discs were transferred into 3 mL of 80% ethanol, homogenized for 2 minutes, and thereafter heated to 80°C for 15 minutes. Samples were then transferred into Eppendorf tubes and centrifuged at $20,800 g_n$. The supernatant was removed, the pellet washed with 2 mL of 80% ethanol, and centrifuged again. This procedure was repeated twice using pure acetone. Thereafter, the pellet was dried at 70°C and weighed. Dry mass of cell walls per unit fresh weight was

Table 1. Modulus of elasticity (E), pressure fracture (p_{fracture}), and strain fracture ($\epsilon_{\text{fracture}}$) of exocarp segments (ES) excised from cheeks of selected, greenhouse-grown, sweet cherry fruit at maturity ($n = 25$).

Cultivar	E	p_{fracture}	$\epsilon_{\text{fracture}}$
	[mean $\pm$ SE (MPa)]	[mean $\pm$ SE (kPa)]	[mean $\pm$ SE ($\text{mm}^2 \cdot \text{mm}^{-2}$)]
Adriana	17.7 ± 1.4^z bc	40.3 ± 1.3^z de	0.17 ± 0.01^z b
Burlat	8.7 ± 0.5 e	24.9 ± 1.2 f	0.20 ± 0.01 ab
Hedelfinger	25.3 ± 1.1 a	67.8 ± 1.9 a	0.20 ± 0.01 ab
Merchant	15.2 ± 0.8 cd	38.8 ± 1.3 e	0.18 ± 0.01 b
Regina	18.9 ± 1.0 bc	59.1 ± 2.0 b	0.22 ± 0.01 a
Sam	14.9 ± 0.7 d	48.1 ± 1.5 c	0.22 ± 0.01 a
Samba	19.2 ± 1.0 b	46.0 ± 1.3 cd	0.17 ± 0.01 b

^zMean separation by Tukey’s studentized range test at $P < 0.05$.

Fig. 1. (A) Time course of cracking and of water uptake (inset) of ‘Regina’ and ‘Burlat’ sweet cherry fruit incubated in deionized water. (B) Relationship between cracked fruit and water uptake. For further details, see Table 2.

calculated. The experiment was conducted with seven replicates comprising 10 discs per replicate.

DATA ANALYSIS. Data analysis was limited to segments that fractured in the center of the washer aperture. Segments fracturing at the edge of the aperture were excluded because artifacts resulting from the mounting procedure could not be excluded. Data are presented as means $\pm$ SES of means. Mean comparisons were made using Tukey’s studentized range test at $P < 0.05$ (packet multcomp 1.2–12, procedure glht, R 2.13.1; R Foundation for Statistical Computing, Vienna, Austria). Significance of coefficients of correlation (r) and of determination

(r^2) at the probability levels 0.05, 0.01, and 0.001 are indicated by *, **, and ***, respectively.

Results

The mechanical properties of the fruit skins differed significantly among cultivars (Table 1). ‘Hedelfinger’ and ‘Regina’ had the stiffest fruit skins as indexed by high values for E and $p_{fracture}$, whereas ‘Burlat’ had the lowest values for E and $p_{fracture}$. There was little difference among the various cultivars for the value of $\epsilon_{fracture}$, which ranged from 0.17 to 0.22 (Table 1).

Incubating fruit in deionized water resulted in cracking of both ‘Regina’ and ‘Burlat’ (Fig. 1). Cracking was markedly more rapid in ‘Burlat’ than in ‘Regina’ as indexed by a lower T_{50} (Table 2). Cumulative water uptake increased linearly with time (i.e., a constant uptake rate) in ‘Regina’ and ‘Burlat’ and occurred at similar rates in the two cultivars. Calculating cumulative water uptake at 50% cracking revealed that ‘Regina’ fruit required, on average, about three times more water to crack than ‘Burlat’ fruit (Table 2).

Repeated loading, holding, and unloading of the fruit skin imposed cyclic changes in the skin strains in both cultivars (Fig. 2). The maximum number of strain cycles sustained by ‘Regina’ segments was markedly higher than that by ‘Burlat’ segments (M. Brüggewirth, unpublished data). Increasing pressure during any one loading cycle resulted in an instantaneous and proportional increase in strain. During the holding phase, the strain of the skins of both cultivars continued to increase. Upon release of the pressure, the skins of both cultivars returned to their initial dimensions, i.e., before the application of pressure.

Plotting total strains during any one cycle against the pressure applied for ascending, constant, or descending pressures yielded the relationships depicted in Fig. 3. In both cultivars, strains were essentially linearly related to the applied pressure with little difference between the ascending and descending paths [i.e., minimal hysteresis (Fig. 3)]. However, the slopes of the strain/pressure

relationships were markedly higher for the skins of ‘Burlat’ than for those of ‘Regina’ (Table 3). Thus, a given pressure caused markedly higher strain in the less stiff ‘Burlat’ skin than in the stiffer ‘Regina’ skin. Partitioning total strain into its elastic and creep components demonstrated that 1) most of the total strain in both cultivars was accounted for by the elastic component, with the creep strain being relatively small and 2) the slopes of both the elastic strain vs. pressure and of the creep strain vs. pressure relationships

Table 2. Water uptake and cracking in ‘Burlat’ and ‘Regina’ sweet cherry. Cracking was indexed by the amount of water uptake for 50% of fruit to crack (WU_{50}) or by time to 50% fruit cracking (T_{50}).

Cultivar	Rate of water uptake [mean $\pm$ SE (mg·h ⁻¹)]	Cracking	
		WU_{50} [mean $\pm$ SE (mg)]	T_{50} [mean $\pm$ SE (h)]
Burlat	14.1 $\pm$ 1.1 ^a	50.5 $\pm$ 2.3	3.6 $\pm$ 0.2
Regina	17.7 $\pm$ 1.6 a	145.8 $\pm$ 0.7	8.2 $\pm$ 0.4

^aMean separation by t test at $P < 0.05$.

Fig. 2. Time course of change in strain of the fruit skin (ϵ) of (A, C, E) ‘Regina’ and (B, D, F) ‘Burlat’ sweet cherry when subjected to repeated loading, holding, and unloading cycles. (A, B) Pressures (p) during the holding cycles were increased in 5-kPa increments from 0 to 40 kPa for ‘Regina’ and from 0 to 20 kPa for ‘Burlat’. (C, D) Pressures during the holding cycles were held constant at 25 kPa for ‘Regina’ and 10 kPa for ‘Burlat’. (E, F) Pressures during the holding cycles were decreased stepwise in 5-kPa increments from 40 to 0 kPa for ‘Regina’ and from 20 to 0 kPa for ‘Burlat’. Insets (A–F): Time courses of change in pressure.

were markedly higher for ‘Burlat’ than for ‘Regina’ (Table 3).

Destroying the plasma membranes by a single freeze/thaw cycle decreased the values of both E and p_{fracture} . In ‘Burlat’, the value of $\varepsilon_{\text{fracture}}$ was increased by a single freeze/thaw cycle, but in ‘Regina’ there was no effect (Table 4). The E and p_{fracture} values of ‘Regina’ always exceeded those of ‘Burlat’.

Cell sizes in both ‘Regina’ and ‘Burlat’ skins increased with increasing distance below the surface, to a depth of $\approx 200\ \mu\text{m}$ but remained constant thereafter (Fig. 4A and B). Cell sizes were similar in the two cultivars. Dry mass of cell walls per unit fresh weight, however, was higher in ‘Regina’ ($21.5 \pm 0.6\ \text{mg}\cdot\text{g}^{-1}$ fresh weight) than in ‘Burlat’ ($15.8 \pm 0.6\ \text{mg}\cdot\text{g}^{-1}$ fresh weight). The shape of the cells as indexed by the anticlinal aspect ratio (i.e., tangential diameter/radial diameter) decreased with increasing distance from the surface. Cells at the surface had a lower aspect ratio in ‘Regina’ (1.86 ± 0.12 , $r^2 = 0.124^{**}$, $n = 85$, estimated as the y -axis intercept of a linear regression line of a plot of aspect ratio vs. distance from the surface) than in ‘Burlat’ (2.59 ± 0.15 , $r^2 = 0.231^{***}$, $n = 90$). As distance

from the cuticle increased, cells became more closely isodiametric (aspect ratio ≈ 1).

Discussion

Our data establish that ‘Regina’ and ‘Burlat’ differ in the mechanical properties in biaxial tensile tests of their fruit skins. The tensile tests show that 1) the skin of the less cracking-susceptible ‘Regina’ was stiffer and had a higher fracture pressure than that of the more cracking-susceptible ‘Burlat’, 2) the repeated loading and unloading cycles did not cause the skin to fatigue in either cultivar, and 3) the greater stiffness of ‘Regina’ is likely to result from differences in the physical and chemical properties of the cell walls of ‘Regina’ skin, compared with those of ‘Burlat’.

‘REGINA’ AND ‘BURLAT’ DIFFER IN MECHANICAL PROPERTIES OF THEIR FRUIT SKINS. The higher cracking susceptibility of ‘Burlat’ than of ‘Regina’ is consistent with published ratings (Christensen, 1999) and correlates with a weaker mechanical architecture of the fruit’s skin. First, the rates of water uptake did not differ, but cracking was faster (lower T_{50}) in ‘Burlat’ than in ‘Regina’. This resulted in a lower WU_{50} in ‘Burlat’. Thus, cracking in ‘Burlat’ required only one-third the amount of water uptake as in ‘Regina’. Second, direct evidence for a weaker skin of ‘Burlat’ than ‘Regina’ comes from the biaxial tensile tests. The cultivar comparison established a lower value of E , and hence a low resistance to extension for ‘Burlat’ than for ‘Regina’ (Table 1). Also, the value p_{fracture} for ‘Burlat’ was less than half that for ‘Regina’ (Table 1). These two cultivars fell one at the upper end and the other at the lower end of the range of mechanical properties of the seven cultivars investigated here. The number of loading/holding/unloading cycles that could be applied without fracture in ‘Regina’ was twice that in ‘Burlat’ (M. Brüggewirth, unpublished data). Finally, the slopes of the relationships between total strain and the elastic and the creep strain components vs. pressure were larger in ‘Burlat’ than in ‘Regina’. This indicates that a given strain was reached at a lower pressure in ‘Burlat’ than in ‘Regina’ (Fig. 3). While the relationship between the lower stiffness

Fig. 3. (A, B) Total strains and (C, D) elastic and creep strains of the skin of (A, C) ‘Regina’ and (B, D) ‘Burlat’ sweet cherry as affected by the pressure applied in the elastometer. (A, B) Data from experiments with cyclic application of increasing, constant, or decreasing pressures.

Table 3. Parameter estimates and ses of linear regression equations describing the relationships between the pressure (kilopascals) applied in the elastometer and total strain, elastic strain, and creep strain (all strains millimeter square per millimeter square) of ‘Regina’ and ‘Burlat’ sweet cherry fruit skin.

Cultivar	Strain component	Parameter estimates		Coefficient of determination
		$[\times 10^3 \text{ (slope } \pm \text{ SE)}]$	$[\times 10^3 \text{ (intercept } \pm \text{ SE)}]$	r^2
Regina	Total	4.2 ± 0.2	-1.5 ± 5.2	0.99***
	Elastic	2.9 ± 0.2	0.9 ± 1.1	0.98*
	Creep	0.7 ± 0.1	-0.6 ± 2.9	0.91
Burlat	Total	7.7 ± 0.7	9.1 ± 9.3	0.99***
	Elastic	5.3 ± 0.6	17.1 ± 7.8	0.98**
	Creep	2.1 ± 0.6	-3.9 ± 8.1	0.93

*Significance of coefficient of determination at $P < 0.05$, 0.01 , and 0.001 indicated by *, **, and ***, respectively.

Table 4. Effect of destroying plasma membranes of exocarp segments excised from the cheeks of mature ‘Burlat’ and ‘Regina’ sweet cherry fruit by a freeze/thaw cycle. The effects on the modulus of elasticity (E), pressure at fracture (p_{fracture}), and strain at fracture ($\epsilon_{\text{fracture}}$) were investigated ($n = 20$).

Cultivar	Treatment	E	p_{fracture}	$\epsilon_{\text{fracture}}$
		[mean $\pm$ SE (MPa)]	[mean $\pm$ SE (kPa)]	[mean $\pm$ SE (mm ² ·mm ⁻²)]
Burlat	Control	15.5 $\pm$ 2.0 ^z c	26.7 $\pm$ 1.6 ^z c	0.16 $\pm$ 0.01 ^z b
	Freeze/thaw	5.3 $\pm$ 0.4 d	12.5 $\pm$ 0.8 d	0.20 $\pm$ 0.01 a
Regina	Control	33.9 $\pm$ 1.9 a	64.5 $\pm$ 2.2 a	0.17 $\pm$ 0.01 b
	Freeze/thaw	25.7 $\pm$ 2.3 b	45.5 $\pm$ 2.5 b	0.16 $\pm$ 0.01 b

^zMean separation by Tukey’s studentized range test at $P < 0.05$.

Fig. 4. Properties of cells of cross-sections through the skin of (A, C) ‘Regina’ and (B, D) ‘Burlat’ sweet cherry fruit. (A, B) Relationship between the logarithm of cell volume and the distance of cell centers from the cuticle. (C, D) Relationship between the ratio of tangential to radial diameters of cells and the distance of cell centers from cuticle.

and lower fracture pressure of the cracking-susceptible ‘Burlat’ vs. the higher values of these of the less cracking-susceptible ‘Regina’ are merely correlative (i.e., they are not necessarily causal), nevertheless they are consistent with the general concept in material science that for a pressurized hollow vessel, a weaker shell increases the likelihood of failure.

MECHANICAL BEHAVIOR OF FRUIT SKIN IN LOADING/UNLOADING CYCLES. Increasing the number of loading/unloading cycles did not change skin stiffness or fracture threshold (Fig. 3). Also, it made little difference whether the loads increased, decreased, or were maintained constant. Unlike tomato (*Solanum lycopersicum* L.) skin (Matas et al., 2004), there was no strain hardening in sweet cherry. In both cherry cultivars, elastic strain accounted for most of the deformation, whereas creep strain during the holding phases was low. These findings suggest diurnal oscillations in the diameter of a developing fruit (Measham et al., 2014) are unlikely to weaken the fruit skin.

GREATER STIFFNESS OF ‘REGINA’ SKIN IS THE RESULT OF DIFFERENT PHYSICAL AND CHEMICAL PROPERTIES OF THE CELL WALLS. The tissue’s water relation affects its mechanical

properties. For example, the shape of a turgid cell resists change as a result of the tangential tension in the cell wall. However, when we destroyed the plasma membrane by imposing a freeze:thaw cycle, the value of E remained five times higher and that of p_{fracture} three times higher in ‘Regina’ than in ‘Burlat’. Hence, because the difference persists in spite of turgor loss, the different E and p_{fracture} values of these two cultivars must be related

to differences in the cell walls themselves—not to cell turgor differences. The reason for the different cell wall mechanical properties of ‘Burlat’ and ‘Regina’ is unknown, but a number of factors may be involved:

First, mass of cell walls per unit fruit mass was higher in ‘Regina’ than in ‘Burlat’. Also, microscopy indicates that the epidermis of ‘Regina’ has thicker anticlinal cell walls ($2.6 \pm 0.1 \mu\text{m}$) than ‘Burlat’ ($2.2 \pm 0.1 \mu\text{m}$) (C. Schumann, unpublished data). Cell wall thickness will affect the mechanical properties. Second, the epidermal cells of ‘Burlat’ had a higher mean anticlinal aspect ratio than those of ‘Regina’. This is consistent with greater strain during growth and/or with less strain relaxation upon preparation of skin sections for microscopy. Third, cell walls could differ in their compositions—differences in cell wall composition can result in differential mechanical behaviors (Chanliaud et al., 2002). For example, the mechanical properties, such as firmness, of a range of sweet cherry cultivars were related to the chemistry of their cell walls, in particular to the composition and solubility of pectin (Basanta et al., 2013; Batisse et al., 1996; Salato et al., 2013). At this stage we do not

know which of these factors is the most critical. Based on earlier data from our laboratory a role of the cuticle in mechanical properties of the sweet cherry fruit skin can be excluded (Brüggenwirth et al., 2014).

Conclusions

Biaxial tensile tests establish that the less cracking-susceptible fruit skin of ‘Regina’ was stiffer and fractured at a higher pressure than the more cracking-susceptible ‘Burlat’. These differences in mechanical properties probably result from different physical and/or chemical properties of the cell walls. Further studies are required to establish relationships between the physical and chemical properties of the cell walls and their mechanical characteristics.

As in earlier studies, the pressures at fracture were of a similar order of magnitude to those reported previously for both cell and fruit turgor (Brüggenwirth and Knoche, 2016; Knoche et al., 2014; Schumann et al., 2014). However, the strains at fracture, resulting from surface area increase following water uptake (0.3% to 1.1%), were markedly lower in the

cracking assay than in the biaxial tensile tests. The reason for this striking discrepancy is unknown and deserves further study. A possible explanation is that cracking at the whole-fruit level is a “local” phenomenon, where failure is triggered by a weak point somewhere in the skin of the whole fruit, whereas the much smaller area of skin employed in the biaxial tensile test will reduce the chances of including such a weak spot in the sample, so its result is proportionately more likely to be representative of a “perfect” fruit skin (Brüggenwirth and Knoche, 2016).

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