

Water on the Surface Aggravates Microscopic Cracking of the Sweet Cherry Fruit Cuticle

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ABSTRACT. The effect of surface water on the frequency of microcracks in the cuticular membrane (CM) of exocarp segments (ES) of developing sweet cherry fruit (*Prunus avium* L.) was studied. Strain of CM and ES on the fruit surface was preserved by mounting a stainless steel washer on the fruit surface in the cheek region using an ethyl-cyanacrylate adhesive. ES were excised by tangentially cutting underneath the washer. Frequency of microcracks in the CM of ES was determined following infiltration for 10 minutes with a 0.1% acridine orange solution by fluorescence microscopy before and after exposure to deionized water (generally 48 hours). Exposing the surface of ES of mature 'Burlat' sweet cherry fruit to water resulted in a rapid increase in microcracks in the CM that approached an asymptote at about 30 microcracks/cm² within 24 hours. There was no change in microcracks in the CM when the surface of the ES remained dry. Incubating ES in polyethylene glycol solution that was isotonic to fruit juice extracted from the same batch of fruit resulted in a greater increase in frequency of microcracks as compared to incubation in deionized water. The water-induced increase in microcracks was closely related to strain of the CM across different developmental stages within a cultivar [between 45 and 94 days after full bloom (DAFB); $r^2 = 0.96$, $P \leq 0.001$, $n = 9$] or across different cultivars at maturity ($r^2 = 0.92$, $P \leq 0.0022$, $n = 6$). Incubating ES of developing fruit in enzyme solution containing pectinase and cellulase such that the outer surface remained dry resulted in complete rupture and failure of the ES. Time to rupture and percentage of ruptured ES were closely related to the strain of the CM ($r^2 = 0.92$, $P \leq 0.001$, $n = 9$ and $r^2 = 0.68$, $P \leq 0.0063$, $n = 9$, respectively). Removal of epicuticular wax had no effect on frequency of water-induced microcracks. Also, temperature had no effect on frequency of water-induced microcracks, but frequency of microcracks increased exponentially when exposing the outer surface of ES to relative humidities above 75%. At 100% humidity the increase in frequency of microcracks did not differ from that induced by liquid water. Local wetting the surface of intact fruit in the pedicel cavity or styler end region resulted in formation of macroscopically visible cracks despite of a net water loss of fruit. Uniaxial tensile tests using dry and fully hydrated CM strips isolated from mature 'Sam' sweet cherry fruit established that hydration increased fracture strain, but decreased fracture stress and moduli of elasticity. Our data demonstrate that exposure of the fruit surface to liquid water or high concentrations of water vapor resulted in formation of microcracks in the CM.

Rain-cracking of fruit during and after precipitation is a limitation in production of sweet cherry fruit worldwide. Cracking is thought to result from increased turgor caused by rapid water uptake into the fruit (Andersen and Richardson, 1982; Considine and Kriedemann, 1972). Water uptake through the sweet cherry fruit surface occurs by different mechanisms along parallel pathways, including diffusion through the cuticular membrane (CM) (Beyer et al., 2005), viscous flow along the pedicel/fruit juncture (Beyer et al., 2002), through guard cells or stomatal pores (Beyer et al., 2005), and through cracks in the CM (Peschel and Knoche, 2005).

Microscopic cracks in the CM, subsequently referred to as microcracks, frequently are observed on immature and mature sweet cherry fruit (Glenn and Poovaiah, 1989; Knoche et al., 2001; Sekse, 1995). Microcracks are minute fractures in the cuticle that do not traverse epidermal and hypodermal cell layers and are only detected by microscopy (Peschel and Knoche, 2005). Recent evidence suggests that formation of microcracks is closely related to strain of the CM (Peschel and Knoche, 2005). Strain of the CM results from rapid fruit surface expansion during stage

III ("final swell") in the absence of CM deposition (Knoche et al., 2004). Microcracks predispose fruit to subsequent rain-cracking (Peschel and Knoche, 2005). Furthermore, they increase the incident of fruit rot (Borve et al., 2000).

Studies on CM rheology established that the CM is a viscoelastic polymer and that hydrated CM are more elastic than dry CM and have a decreased fracture force (Petracek and Bukovac, 1995; Wiedemann and Neinhuis, 1998). Little information is available on rheological properties of the sweet cherry fruit exocarp (e.g., Bargel et al., 2004) and, to our knowledge, none on those of the isolated sweet cherry fruit CM or the effect of hydration thereon. If the effect of hydration on mechanical properties of the sweet cherry CM was similar to that reported for CM of other species, the presence of water on the strained CM of an intact sweet cherry fruit could induce microcracks.

The objectives of the present study therefore were to 1) develop a system for studying the effect of water on the sweet cherry fruit surface on frequency of microcracks in the CM and selected factors thereon, 2) identify whether the fragile, isolated sweet cherry fruit CM is sufficiently stable to be subjected to uniaxial tensile tests, and, if so, 3) quantify the effect of hydration on mechanical properties of the CM.

Materials and Methods

PLANT MATERIAL. Fruit of sweet cherry (*Prunus avium* 'Burlat', 'Hedelfinger', 'Kordia', 'Lapins', 'Sam', 'Van', 'Summit');

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grafted on *P. avium* 'Alkavo' rootstocks except for 'Lapins' where the rootstock was unknown) and sour cherry (*P. cerasus* L., 'Vowi', grafted on *P. avium* 'Alkavo' rootstock) were collected from commercial orchards located near Eisleben, Germany (lat. 51°31'N, long. 11°44'E), an experimental orchard of the Federal Fruit Variety Office near Potsdam (lat. 52°31'N, long. 12°51'E), or purchased locally off-season ('Lapins'). Fruit were harvested at commercial maturity except for 'Hedelfinger' fruit that was collected at weekly intervals between 45 and 94 days after full bloom (DAFB). Fruit were selected for uniformity (based on color and size) and freedom from visual defects. Fruit were processed fresh on the day of sampling except for 'Summit' used in the study of the effect of polyethylene glycol (3 d) and 'Hedelfinger' used to establish the effect of humidity on microscopic cracking (1 d). Conditions during interim storage were 5 °C and ≈90% relative humidity.

CUTICLE ISOLATION. Exocarp segments (ES) comprising cuticle, epidermal and hypodermal cell layers and adhering mesocarp tissue were excised from the cheek region using a cork borer (i.d. 8.9 mm). Since the exocarp of mature sweet cherry fruit may be strained, only one ES per fruit was taken. CM were isolated by incubating ES at 25 °C in a 50 mM citric acid buffer solution (pH 4.0) containing pectinase (90 mL·L⁻¹; Panzym Super E flüssig; Novozymes AG, Dittingen, Switzerland), cellulase (5 mL·L⁻¹; Celluclast; Novozymes, Bagsvaerd, Denmark) and 30 mM NaN₃ to prevent microbial activity. After separation and isolation CM were desorbed at 25 °C as described earlier (Knoche et al., 2001). Following drying, CM mass was determined gravimetrically on a minimum of 10 CM disks per replicate with a total of five replications. CM mass per unit fruit surface area was derived by dividing the mass of individual CM disks by the area covered by the CM on the fruit (*A*) prior to excision and isolation. The *A* was calculated as the surface area of a cap of a sphere having a mean diameter equivalent to that of fruit from the same batch. Mean fruit diameter, in turn, was estimated from mean fruit mass assuming a spherical shape of the fruit and a density of 1 kg·dm⁻³ as first approximations. For quantifying strain of the CM, digitized calibrated images of a subsample of 10 individual CM discs were prepared at ×4 (MZ6 microscope; Leica Mikrosysteme GmbH, Bensheim, Germany) using a video camera (Hitachi Denshi Europa GmbH, Rodgau, Germany), the perimeter of the CM disks traced and the area quantified using image analysis (analySIS 3.0 software package; Soft Imaging System GmbH, Münster, Germany; Knoche et al., 2004). Strain [ϵ (in percent)] was calculated according to Eq. [1], where *A* represents the surface area that the CM covered on the fruit prior to excision and *A*₀ the area of the relaxed CM following isolation:

$$\epsilon = \frac{A - A_0}{A_0} \times 100 \quad [1]$$

Effect of surface moisture on microcracking of the CM

GENERAL EXPERIMENTAL PROCEDURE. Stainless steel washers (6.4 mm i.d., 17.8 mm o.d.; except for 'Hedelfinger' at 45 DAFB with 4.3 mm i.d. and 11.6 mm o.d.) were glued to the fruit surface in the cheek region using an ethyl-cyanacrylate adhesive (Loctite 406; Henkel Loctite Deutschland GmbH, München, Germany). Following curing overnight at ambient temperature and humidity ES were excised by cutting tangentially through the fruit underneath the washer using a razor blade such that an ES consisting of CM plus some adhering tissue remained attached to the washer. The cut surface was blotted using tissue paper. Preliminary ex-

periments demonstrated that the washer preserved the strain of the CM on the ES (S. Peschel, unpublished data). To establish the frequency of microcracks already present in the CM before experimental exposure to water, microcracks were infiltrated by placing ≈100 μL of an 0.1% acridine orange solution on the surface of the ES exposed in the washer. After 10 min the dye solution was removed by careful blotting, the ES transferred to the stage of a fluorescence microscope (model BX-60; Olympus, Hamburg, Germany) and viewed at ×100 (filter module U-MWU, 330–385 nm excitation wave length, 420 nm emission wave length; Olympus). Frequency of microcracks was determined on an individual ES basis. Thereafter, ES with washers attached were incubated in petri dishes filled with deionized water containing 30 mM NaN₃ unless specified otherwise. ES were completely submerged thereby exposing the inner and outer side of the ES to the incubation solution. ES with outer surface exposed to ambient atmosphere were used for control. Here, a glass or plastic cylinder (≈10 mm height, 10 mm i.d.) was mounted on the washer using a high-vacuum grease (Baysilone—Paste hochviskos; GE Bayer Silicones, Leverkusen, Germany). This cylinder effectively served as a "snorkel" thereby preventing contact of incubation solution with the outer surface. Following incubation, usually after 48 h at 22 °C, ES with washer attached were removed from incubation solutions, the dye solution re-applied and ES re-inspected for microcracks as described above. This procedure allowed the change in frequency of microcracks during the incubation period to be calculated on an individual ES basis. In some experiments, frequency of microcracks was established in two size categories. Short microcracks refer to cracks in the CM shorter than the longest dimension of the underlying epidermal cell, long microcracks to those that were longer. Experiments were carried out with a minimum of 10 replications per treatment, cultivar or sampling date with one replication representing an individual ES. This assay was then used in experiments focusing on 1) evaluating the system (time course, effects of polyethylene glycol and metabolic inhibitors) and 2) factors affecting formation of microcracks (effects of fruit development, cultivars, epicuticular wax, temperature and humidity).

EVALUATING THE SYSTEM. The time course of change in frequency of microcracks following exposure to water was monitored in 'Burlat' by inspecting ES at 0, 4, 8, 24, 48, and 144 h. ES with outer surface exposed to the atmosphere served as control.

To establish the effect of water on the CM surface in the absence of water uptake, ES from mature 'Burlat', 'Lapins', and 'Summit' fruit were incubated in isotonic polyethylene glycol solution (PEG-6000; Merck Eurolab GmbH, Darmstadt, Germany). Inner and outer side of ES were in contact with PEG solution. Osmolality of PEG solutions was adjusted to 1008 ± 2, 1446 ± 3, and 1048 ± 6 mmol·kg⁻¹ (model 5520 vapor pressure osmometer; Wescor, Logan, Utah), which approximately corresponded to the average osmolality of cell sap extracted from mesocarp tissue of the same batch of 'Burlat' (925 ± 12 mmol·kg⁻¹), 'Lapins' (1417 ± 92 mmol·kg⁻¹), and 'Summit' (1047 ± 47 mmol·kg⁻¹) fruit, respectively. ES incubated in deionized water served as control.

The effect of inhibitors of metabolism on microcracks in the CM was established by incubating ES excised from 'Hedelfinger' in solutions containing 30 mM NaN₃ or 0.1 mM carbonylcyanide *m*-chlorophenylhydrazone (CCCP; Sigma Taufkirchen, Germany). Deionized water served as control.

FACTORS AFFECTING FORMATION OF MICROCRACKS. The effect of water on frequency of microcracks was followed in developing

'Hedelfinger' fruit between 45 and 94 DAFB. Since the effect of surface moisture on microcracks in the CM of developing fruit is confounded by a simultaneous change in mechanical properties of the cellular tissue in the course of fruit softening (Kondo and Danjo, 2001), we attempted to separate the two effects by incubating ES with washer attached in enzyme solution used for isolating cuticles. The outer surface remained dry. However, as fruit matured an increasing percentage of CM ruptured before adhering cellular tissue was digested. Furthermore, it was technically impossible to quantify the frequency of microcracks on the curled CM fragments that remained attached to the washer. To avoid artifacts due to handling of the fragile and strained CM on the digesting ES, percentage of CM that ruptured by macroscopic inspection within a 40-d period and the time to rupture was recorded on an individual ES basis.

To establish whether sweet cherry cultivars respond in a similar manner to surface water, 'Burlat', 'Hedelfinger', 'Kordia', 'Summit', and 'Van' fruit were used as a source of ES. Data for 'Summit' were taken from Table 1, data for 'Burlat' and 'Hedelfinger' represented grand means of several experiments conducted under standard conditions. For comparison, the sour cherry cultivar Vowid was included in the experiment.

The role of epicuticular wax (ECW) in water-induced microcracks in the CM was studied using the cellulose acetate stripping technique (Silcox and Holloway, 1986). Briefly, the cheek region of mature 'Hedelfinger' fruit was dipped in a viscous cellulose acetate solution in acetone. Following evaporation of the solvent, the hardened cellulose acetate film with ECW attached was carefully removed from the fruit surface. Subsequently, a washer was mounted on the stripped surface and the ES excised. ES from fruit not subjected to cellulose acetate stripping served as control.

Temperature response of water-induced microcracks in the CM was studied in 'Hedelfinger' ES at 5, 15, 25, 35, and 45 °C. The effect of relative humidity on frequency of microcracks was established at 25 °C by exposing the outer surface of ES ('Hedelfinger') to defined humidities. Holes (9 mm diameter) were drilled in bottoms of petri dishes and ES with washers mounted upside down in the petri dish such that the holes in the bottom of the dish aligned with the hole in the washer. Using this procedure, the surface of the ES was exposed to the atmosphere outside the petri dish. Subsequently, petri dishes were filled with deionized water, the lid positioned and sealed using parafilm and the entire dish with mounted ES incubated above saturated salt solutions, dry silica [0% relative humidity (RH)], or water (100% RH). Salt solutions and respective humidities at 25 °C were CaCl₂, 28% RH; NaCl, 75% RH; KCl, 84% RH; KNO₃, 92% RH (Wexler, 1995).

Cracking following localized exposure of fruit surface to water

To assess the role of localized surface wetness on water uptake and development of macroscopically visible cracks mature 'Burlat' sweet cherry fruit were placed on test tubes (20.6 mm i.d.) filled with water up to the rim such that stylar end or cheek region were in contact with water, but the remaining fruit surface was exposed to the ambient atmosphere. In a further treatment uptake was restricted to the pedicel cavity by placing fruit in an upright position in a holder and filling the pedicel cavity with water. Fruit completely submerged in water served as control. To minimize transpiration via the pedicel, pedicels were cut at the receptacle for all treatments except for that allowing water uptake in the pedicel cavity. Here the pedicel remained at full

length. The change in fruit mass with time (0, 2, 4, and 8 h) and development of macroscopic cracks that traversed the exocarp (0, 4, 8, and 25 h) was monitored on an individual fruit basis. The number of single fruit observations was 10. A second experiment was conducted using the same experimental plan, but water was replaced by an isotonic polyethylene glycol solution as the incubation medium.

Tensile tests

Sweet cherry fruit CM are very fragile and we found it impossible to preserve the strain and isolate an intact CM free of microcracks or to prepare CM strips free of wrinkles for mechanical testing. Relatively smooth specimens were obtained when spreading disks of 'Sam' sweet cherry fruit CM on Teflon sheets using a camel's hair brush. Following air drying CM disks were trimmed to strips (dimensions 6 × 10 mm²), mounted in cardboard frames using an ethyl-cyanacrylate adhesive (Loctite 406), and allowed to cure overnight. The frames were used to prevent unintentional stress of the fragile CM during handling. Frames with mounted CM strips were equilibrated either at 22 °C and 50% RH or hydrated by incubating in water at 22 °C for 24 h. Following equilibration, frames were mounted in a Zwick universal material testing machine (Universalprüfstand 1146, Zwick, Ulm, Germany) using Grilon clamps (Zwick, Ulm, Germany) and cut open before testing. Specimens were subjected to classical uniaxial tensile tests at a test velocity and grip distance of 3 mm·min⁻¹ and 5 mm, respectively, until failure occurred. Formation of cracks was always associated with maximum stress. We refer to formation of the first detectable crack as failure. Strain until failure (percent), stress at failure (N/mm²) and moduli of elasticity (MPa) were determined for dry and hydrated CM. Moduli of elasticity were estimated by fitting a linear regression line through the phase of maximum slope of stress strain diagrams. Coefficients of determination averaged 0.99 for hydrated and dry CM. Occasionally, cracks in the CM occurred in or adjacent to clamps. Since such strips may have been damaged during clamping, these observations were excluded from data analysis. Numbers of replications used in the final analysis were 16 and 24 for dry and hydrated CM, respectively.

Terminology and data analysis

Microscopic cracks in the CM are referred to as microcracks (Peschel and Knoche, 2005). These cracks are limited to the CM, do not extend into epidermal and hypodermal cell layers or the mesocarp of the fruit and are only detected by microscopy (Glenn and Poovaiah, 1989). Thus, fruit having microcracks would be classified as intact based on visual inspection.

Data were subjected to analyses of variance (ANOVA). ANOVA (PROC GLM), comparison of means, and regression analysis (PROC REG) were carried out using SAS (version 8.02; SAS Institute, Cary, N.C.). Data in figures and tables are presented as mean and SE. Regression analysis was performed on treatment means.

Results

Exposing the CM on ES excised from mature 'Burlat' sweet cherry fruit to water resulted in a rapid increase in microcracks in the CM (Fig. 1A). Within 24 h of incubation frequency of microcracks approached an asymptote at ≈30 microcracks/cm². About 80% of these microcracks were shorter than the longest

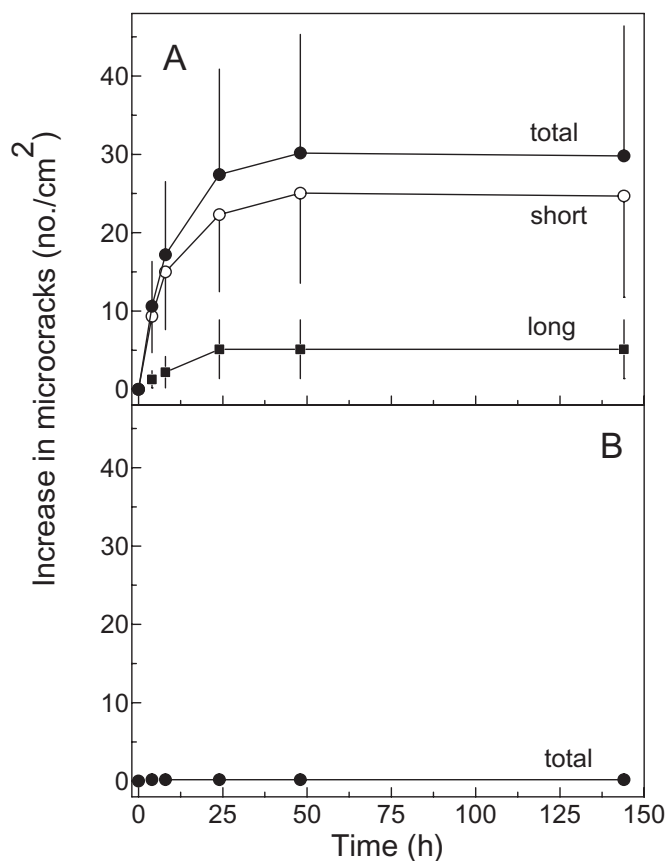


Fig. 1. Time course of change in frequency of microcracks in the cuticle (CM) of exocarp segments of 'Burlat' sweet cherry fruit during exposure of (A) outer and inner surface to the water or (B) outer surface to ambient atmosphere and inner surface to water. The increase in frequency of microcracks was calculated relative to the frequency at initiation of the experiment (0 h). Microcracks shorter than the longest dimension of underlying epidermal cells are referred to as "short," those longer than longest dimension of underlying cell as "long." "Total" refers to the sum of long and short microcracks.

dimension of the underlying epidermal cell, the remaining 20% were longer. When ES were incubated with outer surfaces exposed to the ambient atmosphere there was no increase in frequency of microcracks within 144 h (Fig. 1B).

Incubating ES excised from mature 'Burlat', 'Lapins', and 'Summit' fruit in isotonic PEG solutions resulted in a greater increase in frequency of microcracks as compared to incubation in water (Table 1).

There was no significant difference in the increase in frequency of microcracks when incubating ES in 30 mM NaN_3 (13.1 ± 1.7 microcracks/cm²), 0.1 mM CCCP (10.9 ± 4.5 microcracks/cm²), or in water (12.4 ± 2.5 microcracks/cm²).

The change in fruit mass with time of developing sweet cherry fruit followed a sigmoidal pattern characteristic of stage III development. The increase in fruit mass was accompanied by a continuous decrease in CM mass per unit fruit surface area (Fig. 2A). Estimating the mass of CM on a per fruit basis revealed that CM mass on a whole-fruit basis increased by only 13% between 45 and 94 DAFB, while fruit mass and hence, surface area increased by 369% and 180%, respectively. The absence of CM deposition during stage III of fruit development resulted in marked strain of the CM (Fig. 2B) and an increasing frequency of short and long microcracks in the CM present already on the fruit before the experimental exposure to water (Fig. 2C).

Table 1. Increase in frequency of microcracks in the cuticle of sweet cherry fruit exocarp segments (ES) during a 48 h exposure to water or polyethylene glycol (PEG). The ES were excised from the cheek region. Outer and inner side of ES were exposed to water or PEG solution that was isotonic to juice extracted from the same batch of fruit.

Cultivar	Microcracks in 48 h (no./cm ²)	
	H ₂ O	PEG
Burlat	9.9 (± 1.5)	40.4 (± 11.3)
Lapins	20.8 (± 3.5)	42.6 (± 11.4)
Summit	33.0 (± 10.0)	34.2 (± 6.0)
Grand mean ^a	21.2 (± 4.6) a	39.1 (± 4.5) b

^aMain effect incubation solution significant. Mean separation by Tukey's Studentized range test, $P \leq 0.05$.

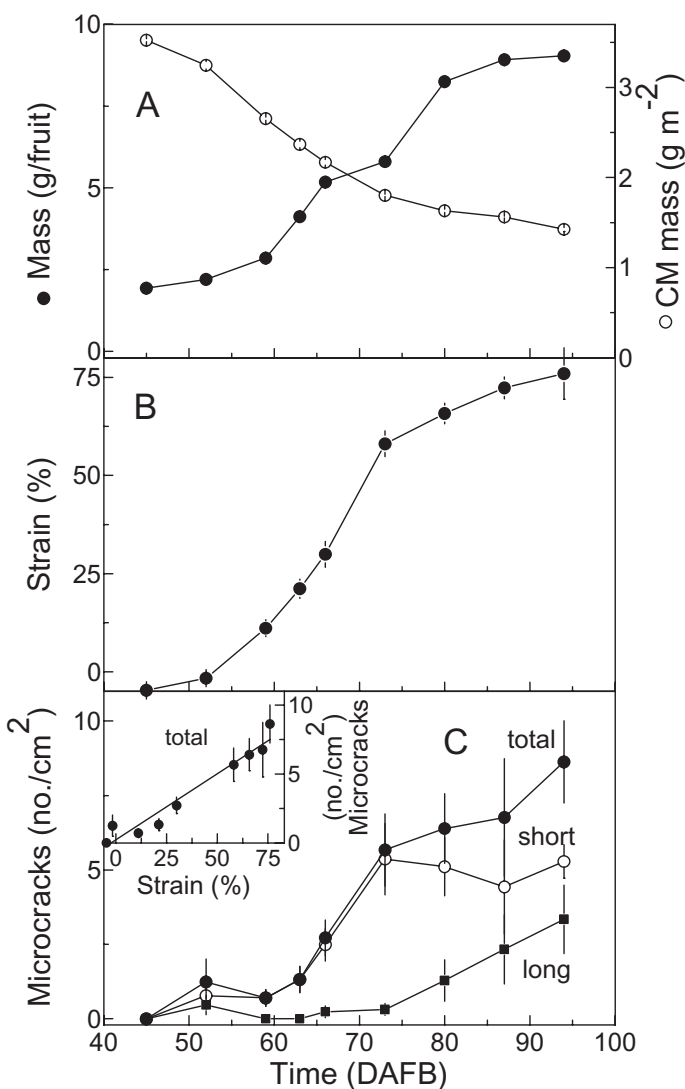


Fig. 2. Change of mass of fruit and of cuticle (CM) per unit fruit surface area (A), of strain of the CM (B) and of frequency of microcracks in the CM (C) of exocarp segments of developing 'Hedelfinger' sweet cherry fruit before exposure of the outer surface to water. Microcracks shorter than the longest dimension of underlying epidermal cells are referred to as "short," those longer than longest dimension of underlying cell as "long." "Total" refers to the sum of long and short microcracks. Scale of time axis in days after full bloom (DAFB). Inset: Frequency of microcracks as a function of strain of the CM. The regression equation is: microcracks (no./cm²) = $0.10 (\pm 0.01) \times \text{strain} (\%)$; $r^2 = 0.98$, $P \leq 0.001$, $n = 9$.

Exposing these ES for 48 h to water on the outer surface had little effect on frequency of microcracks up to ≈ 59 DAFB (Fig. 3A). Thereafter, however, frequency of microcracks increased rapidly. Up to 80 DAFB short microcracks accounted for the increase in frequency of microcracks. From 80 DAFB onwards frequency of short microcracks decreased, but that of long microcracks increased. For ES having a dry surface there was no change in microcracks regardless of developmental stage (data not shown).

When ES were incubated in enzyme solution containing pectinase and cellulase and the outer surface remained dry, an increasing percentage of ES ruptured in the course of fruit development (Fig. 3B). The cracks formed in these enzyme treatments differed from the microcracks in the CM. They were markedly larger and easily detectable by visual inspection. Furthermore,

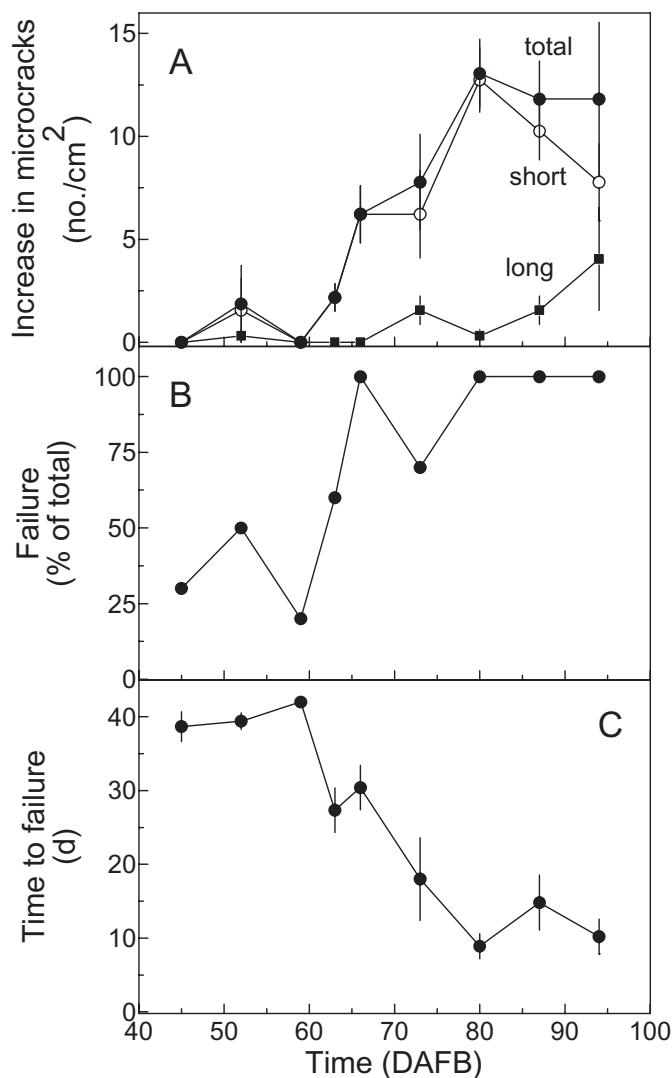


Fig. 3. (A) Increase in microcracks in the cuticle (CM) of exocarp segments (ES) of developing ‘Hedelfinger’ sweet cherry fruit after a 48 h exposure of outer and inner side of ES to water. Microcracks shorter than the longest dimension of underlying epidermal cells are referred to as “short,” those longer than longest dimension of underlying cell as “long.” “Total” refers to the sum of long and short microcracks. (B) Percentage of ES that ruptured and failed during a 48 h incubation period in enzyme solution containing pectinase and cellulase and (C) the time until failure of ES when incubated in enzyme solution. Outer sides of ES (B, C) were exposed to ambient atmosphere, inner sides to enzyme solution. Scale of time axis in days after full bloom (DAFB).

they often traversed the entire ES extending deep into epidermal cell layers and adhering mesocarp tissue. The time to failure of the ES decreased from ≈ 42 d at 59 DAFB to ≈ 10 d at 80 DAFB (Fig. 3C). The water-induced increase in microcracks in the CM, the percentage of ruptured ES and the time until failure when incubated in enzyme solution were linearly related to strain of the CM (Fig. 4).

Comparing frequency of microcracks in different sweet cherry cultivars at maturity revealed positive relationships between microcracking and strain of the CM (Fig. 5). First, frequency of microcracks in the cheek region prior to experimental exposure to water was a linear function of the strain of the CM. Second, the increase in frequency of microcracks following a 48 h exposure of the outer surface to water was linearly related to strain of the CM (Fig. 5, inset). Third, there was no change in frequency of microcracks when ES were incubated with outer surfaces exposed to the ambient atmosphere (data not shown). Interestingly, there was no difference between the sour cherry cultivar Vowii and the sweet cherry cultivars investigated. Across all cultivars, the increase in frequency of microcracks upon the 48-h exposure to water was positively related to the number of microcracks already present on the ES before the experiment [Increase in microcracks (no./cm²) = $6.5 (\pm 2.5) + 1.2 (\pm 0.2) \times$ microcracks – before (no./cm²), $r^2 = 0.32$, $P \leq 0.001$, $n = 127$].

Removal of ECW had no significant effect on frequency of water-induced microcracks (‘Sam’, 12.1 ± 5.8 vs. 7.1 ± 1.1

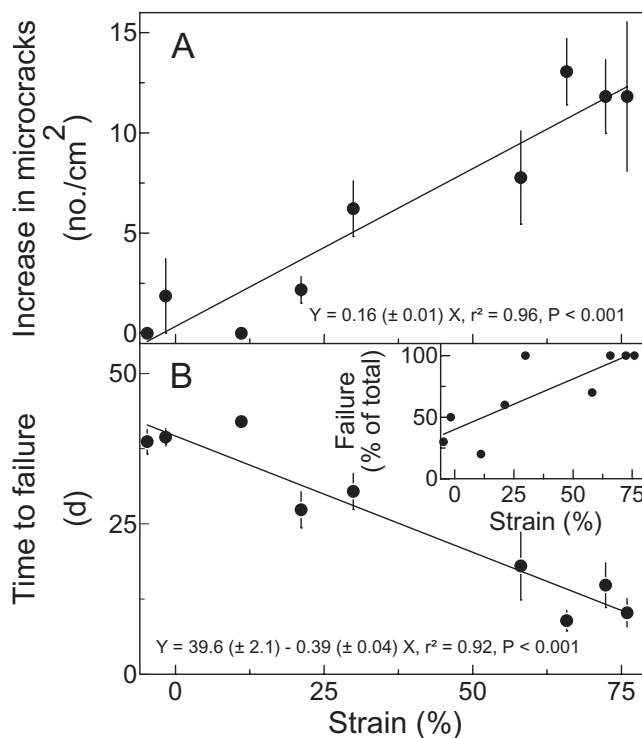


Fig. 4. (A) Relationship between the increase in microcracks in the cuticular membrane (CM) of exocarp segments (ES) of developing ‘Hedelfinger’ sweet cherry fruit and strain of the CM after a 48 h exposure of outer and inner side of ES to water. (B) Relationship between time until failure of ES incubated in enzyme solution containing pectinase and cellulase and strain of the CM. Here, outer side of ES was exposed to ambient atmosphere, inner side to enzyme solution. Inset: Percentage of ES that ruptured and failed within 40 d when incubated in enzyme solution vs. strain of the CM. The regression equation for the latter relationship was: failure (%) = $40.1 (\pm 10.1) + 0.82 (\pm 0.21) \times$ strain (%); $r^2 = 0.68$, $P \leq 0.0063$, $n = 9$.

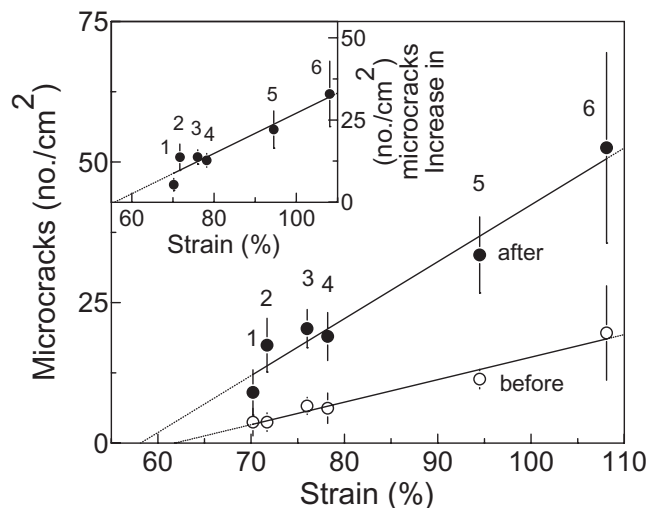


Fig. 5. Relationship between frequency of microcracks in the cuticular membrane (CM) of exocarp segments (ES) and strain of CM isolated from the cheek of mature fruit of selected sweet (1 = 'Kordia', 2 = 'Van', 3 = 'Hedelfinger', 5 = 'Burlat', 6 = 'Summit') and sour cherry cultivars (4 = 'Vowi') before and after a 48 h exposure of the inner and outer side of ES to water. Inset: Increase in microcracks during the 48 h exposure. The increase in microcracks was calculated by difference. Data for 'Summit' were taken from Table 1, data for 'Burlat' and 'Hedelfinger' represent grand means of several experiments conducted under standard conditions. Regression equations were: microcracks – before (no./cm²) = -24.7 (± 2.9) + 0.40 (± 0.03) × strain (%), $r^2 = 0.97$, $P \leq 0.003$, $n = 6$; microcracks – after (no./cm²) = -58.9 (± 8.5) + 1.01 (± 0.10) × strain (%), $r^2 = 0.96$, $P \leq 0.006$, $n = 6$; increase in microcracks (no./cm²) = -34.2 (± 7.4) + 0.61 (± 0.09) × strain (%), $r^2 = 0.92$, $P \leq 0.0022$, $n = 6$.

microcracks/cm² for control fruit vs. fruit without ECW, respectively; 'Hedelfinger', 11.8 ± 1.8 vs. 22.7 ± 8.1 microcracks/cm² for control fruit vs. fruit without ECW, respectively).

Temperature (range: 5 to 45 °C) had no effect on frequency of water-induced microcracks (Fig. 6A). However, frequency of microcracks increased exponentially as relative humidity above the ES increased (Fig. 6B). At 100% humidity the frequency of microcracks did not differ from that of ES exposed to liquid water.

Locally wetting of the fruit surface in pedicel cavity, cheek, or stylar end region resulted in a net water loss due to transpiration (Table 2). Rates of water loss were constant with time and larger for fruit wetted in pedicel cavity or cheek region than for fruit wetted in the stylar scar region. Interestingly, macroscopically visible cracks were observed in some treatments despite a net water loss of the fruit. Percentage of fruit with macroscopic cracks was highest in fruit wetted in the stylar end region followed by fruit wetted in the pedicel cavity region (Table 2). These cracks were always restricted to the area exposed to water. There were no macroscopic cracks in fruit wetted in the cheek region. When the same experiment was repeated using an isotonic PEG 6000 donor solution, rates of net loss of water were -32.4 ± 2.9, -26.9 ± 1.1, and -25.9 ± 1.4 mg·h⁻¹ for exposure of pedicel cavity, cheek, or stylar end region to PEG solution, respectively, and -1.6 ± 0.2 mg·h⁻¹ for whole fruit incubated in PEG solution. There was no macroscopic cracking in localized or whole-fruit exposure to PEG (data not shown).

Stress strain diagrams obtained for CM strips of 'Sam' sweet cherry fruit were somewhat irregular (Fig. 7). Despite our effort in preparing smooth specimens for testing, some wrinkles, however, remained and these most likely accounted for the irregularities in the diagrams (S. Peschel, unpublished data). Hydration increased

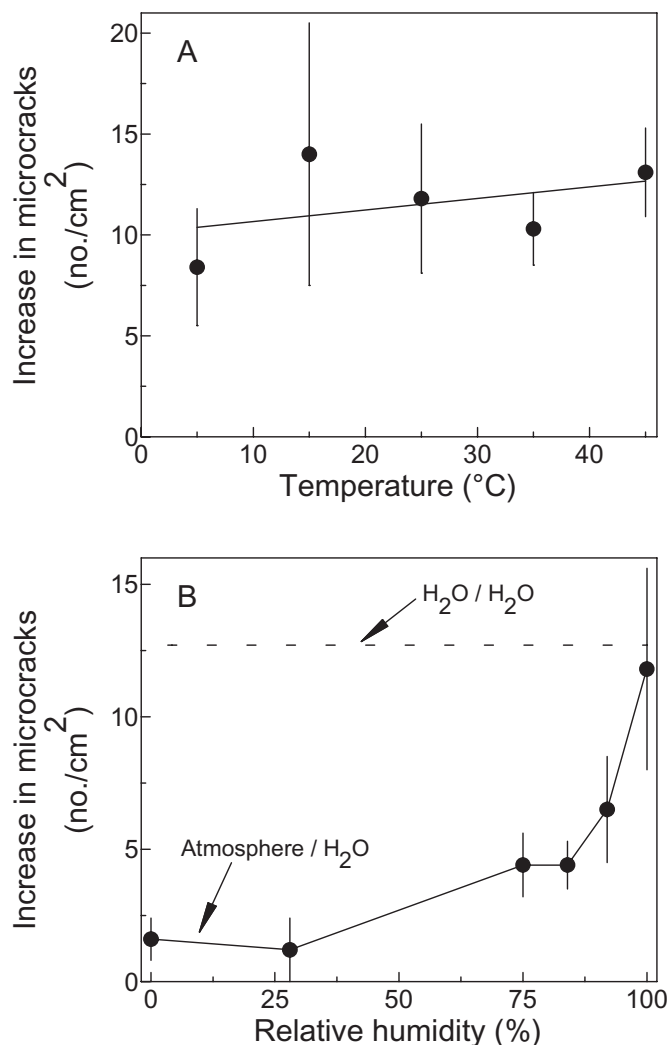


Fig. 6. (A) Effect of temperature on the increase in microcracks in the cuticular membrane (CM) of exocarp segments (ES) excised from mature 'Hedelfinger' sweet cherry fruit after a 48-h exposure of outer and inner side of ES to water. (B) Effect of relative humidity on the increase in microcracks in the CM of ES of 'Hedelfinger' sweet cherry fruit. The outer side of ES was exposed to the atmosphere at the respective humidity, the inner side to water. Horizontal dashed line indicates increase in microcracks in the CM when outer and inner side of ES was exposed to liquid water.

Table 2. Effect of localized surface wetness on water uptake and cracking of 'Burlat' sweet cherry fruit. Water uptake was restricted to cheek, stylar end and pedicel cavity regions by placing fruit on a test tube ("cheek," stylar end") filled with water or by filling the pedicel cavity with water ("pedicel cavity"). Fruit submerged in water served as control ("whole fruit"). Negative rates of water uptake refer to transpiration.

Region exposed	Rate of water uptake (mg·h ⁻¹ per fruit)	Coefficient of determination (r^2)	Fruit with cracks (% of total)		
			4 h	8 h	25 h
Pedicel cavity	-17.9 ± 1.0 c ^z	0.99	0	0	30
Cheek	-19.0 ± 1.0 c	1.00	0	0	0
Stylar end	-12.4 ± 1.2 b	0.99	10	20	40
Whole fruit	26.7 ± 2.1 a	1.00	50	90	100

^zMean separation by Tukey's Studentized range test, $P \leq 0.05$.

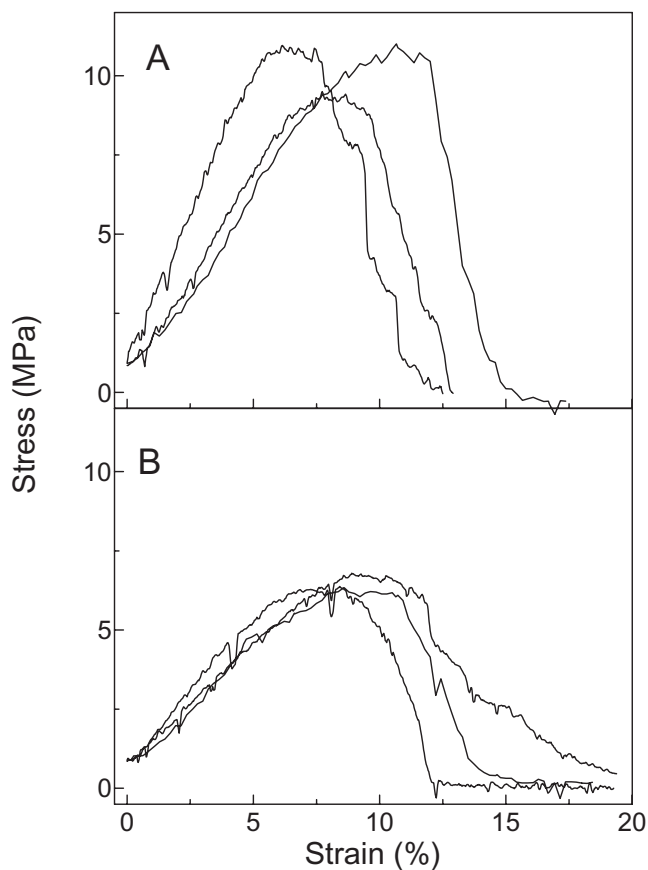


Fig. 7. Representative strain stress diagrams obtained in uniaxial tensile tests using dry (A) or fully hydrated strips (B) of enzymatically isolated 'Sam' sweet cherry fruit cuticles.

fracture strain of the sweet cherry fruit CM, but fracture stress and moduli of elasticity were significantly decreased in hydrated as compared to dry CM (Table 3).

Discussion

The experimental system established allowed to monitor formation of microcracks in the sweet cherry fruit CM under controlled, laboratory conditions. This system offers several advantages over intact fruit assays and orchard conditions in that 1) it provides a high level of control also of factors that are difficult to control in the field, 2) factors may be varied beyond the ranges encountered under field conditions, and 3) treatments may be applied that are impossible to impose on intact fruit. Clearly, the high variability of frequency of microcracks on field-grown fruit could limit a critical analysis. However, by monitoring frequency of microcracks before and after experimental exposure to water, the increase in frequency of microcracks may be calculated on an individual ES basis. We found this parameter to be markedly

Table 3. Effect of hydration on selected rheological properties of enzymatically isolated 'Sam' sweet cherry fruit cuticles as determined in uniaxial tensile tests.

Treatment	Fracture stress (MPa)	Fracture strain (%)	Modulus of elasticity (MPa)
Dry	9.2 ± 0.5 a ²	7.3 ± 0.5 b	156 ± 9 a
Hydrated	6.5 ± 0.5 b	9.2 ± 0.6 a	88 ± 5 b

²Mean separation within columns by Tukey's Studentized range test, $P \leq 0.05$.

less variable than either frequency of microcracks before or after experimental exposure to water. Numerous factors may be responsible for the high variability of microcracks on field-grown fruit, including the wettability of the fruit surface that results in formation of discrete, randomly distributed droplets on the fruit surface (Peschel et al., 2003), mechanical damage resulting from contact with neighboring leaves, fruit, or branches during spray application or wind. Furthermore, duration of surface wetness will vary depending on fruiting habit (i.e., single vs. clustered fruit) and exposure of fruit within the tree canopy. Considering the random nature of many of these factors the high variability in frequency of microcracks is not surprising.

The data obtained using this system provide three important observations. First, frequency of microcracks on the fruit surface of sweet cherry fruit increases upon exposure of the strained CM on the exocarp to high water activities (i.e., liquid water or water vapor). Second, strain of the CM represents the "driving force" in development of microcracks in this and our earlier study (Peschel and Knoche, 2005). This conclusion is based on several arguments. Frequency of microcracks in the CM of field-grown fruit was closely related to the strain of the CM before exposure to surface water in the laboratory assays. The water-induced increase in microcracks was a linear function of strain on fruit across cultivars and developmental stages. Third, the strain of the CM in vivo exceeds the maximum failure strain of an isolated CM not supported by underlying cell walls, since incubation of ES in enzyme solution used for CM isolation resulted in complete rupture and failure even in the absence of surface water.

Theoretically, different mechanisms may be responsible for formation of microcracks in response to surface moisture.

Water uptake

Glenn and Poovaiah (1989) hypothesized that localized water uptake damaged epidermal cells and that this damage preceded development of microcracks in the CM. In our system the water potential of the ES would represent the driving force for water uptake and uptake, in turn, could result in increased turgor and possibly, microcracks in the CM. However, there are several arguments against this hypothesis. First, the permeability of the sweet cherry fruit CM for vapor uptake is markedly lower than for uptake from liquid water (Beyer et al., 2005), but there was no difference in frequency of microcracks between ES exposed to saturated water vapor or to liquid water. If water uptake was a factor, then the water-induced increase in frequency of microcracks should have been higher in ES exposed to liquid water than to water vapor. Second, incubating ES in PEG that was isotonic to juice extracted from fruit prevented water uptake, but increased frequency of microcracks in the CM even above that of the water control. At present, we do not have an explanation for the higher frequency of microcracks in the PEG than in the water treatment. It should be noted that Moing et al. (2004) reported lower osmolality of juice extracted from exocarp than from mesocarp tissue making water uptake into the ES in our isotonic PEG treatments even less likely. Based on these arguments water uptake is unlikely to be a factor in formation of microcracks in the CM.

Effect of water on mechanical properties of CM

The effect of hydration on mechanical properties of enzymatically isolated sweet cherry fruit CM as determined in uniaxial tensile tests was similar to that reported for tomato (*Lycopersicon esculentum* Mill.) fruit CM by Matas et al. (2005), Petracek and

Bukovac (1995), and Wiedemann and Neinhuis (1998). Hydration increased elasticity and susceptibility to fracture in tomato (Petracek and Bukovac, 1995) and sweet cherry fruit (this study). Both effects would account for the water-induced increase in microcracks of the CM provided that 1) water on the surface of an ES increased hydration of the non-isolated CM and 2) the strain of the nonisolated CM supported by underlying cell walls was larger than the fracture strain of the dry isolated CM. These conditions were likely met in our ES/washer system. First, the CM on the exocarp is subjected to a marked gradient in hydration from a fully hydrated stage at the cell wall/CM interface to a less hydrated stage on the outer surface facing the ambient atmosphere. When exposing the outer surface to high water activities (i.e., liquid water or water vapor at saturation), sorption of water in the CM would occur until the CM is fully hydrated at equilibrium (Chamel et al., 1991). Thus, surface wetness is likely to increase CM hydration. Second, the CM on the ES must be strained beyond its fracture strain, since isolation of strained CM by incubating ES with washers attached in enzyme solution yielded 100% rupture of CM. The observation that the same strain of the CM was survived without cracks while attached to the ES is not contradictory to the above observation. The exocarp forms the structural backbone of the sweet cherry fruit and the CM represents only the outer most layer of a complex composite comprising CM and cell walls of epidermis and hypodermis. The structural support of the CM by these cell walls, however, is expected to decrease during maturation when sweet cherry fruit soften. Softening results in a gradual transfer of tensional forces from epidermal and hypodermal cell layers to the CM beginning as early as 30 DAFB (Kondo and Danjo, 2001). These arguments support the hypothesis that hydration of the strained CM on the exocarp induced microcracks.

Clearly, a direct quantitative comparison of the stress/strain relationships of an isolated CM strip subjected to an uniaxial tensile test and a nonisolated CM on a developing fruit is difficult. In an uniaxial tensile test the isolated CM is subjected to constant strain rates at defined stages of hydration. In vivo, however, the CM is part of the exocarp composite and subjected to biaxial strain during fruit growth, where strain rates are markedly lower than in tensile tests and often subjected to diurnal oscillations (Ohta et al., 1997). Our data demonstrate that the structural support of the CM by underlying epidermal and hypodermal cell layers is indeed important (Figs. 2 and 3). Its contribution, however, will be difficult to quantify and likely to be variable on a temporal [i.e., cell wall degradation during maturation (Kondo and Danjo, 2001)] and spatial scale [i.e., uneven thickness of cell walls in anticlinal vs. periclinal regions (Peschel and Knoche, 2005)]. Finally, the fragile nature of the isolated sweet cherry CM and the presence of stomata and microcracks limit a critical analysis of rheological properties of the isolated CM polymer that would be possible with more stable CM using the biaxial tensile test system described by Bargel et al. (2004).

Are microcracks relevant for macroscopic fruit cracking?

Microcracks in the CM predispose sweet cherry fruit to subsequent rain cracking. First, microcracks focus stress in a particular region of the exocarp thereby promoting crack extension. Glenn and Poovaiah (1989) reported that larger cracks in the sweet cherry fruit exocarp often formed along microcracks in the CM. Also, we observed a decrease in water-induced short microcracks and a concomitant increase in longer microcracks (Fig. 3A), which

would be accounted for by extension of short to long microcracks. The driving force for crack extension in vivo could result from fruit growth, water uptake via pedicel, pedicel/fruit juncture or fruit surface, or cell wall degradation during maturation. Second, microcracks provide high flux pathways for rapid uptake by viscous flow that bypass the CM as the penetration barrier and increase permeability for osmotic water uptake (Peschel and Knoche, 2005). Water uptake, in turn, increases turgor of cells in the immediate vicinity of the microcrack which increases stress in this region. When the fracture stress of the epidermal and hypodermal system is exceeded, macroscopically visible cracks develop.

This hypothesis is supported by several arguments. First, when limiting water uptake to selected regions of the sweet cherry fruit surface, we observed macroscopically visible cracks in the wetted region despite a net water loss of the fruit (Table 2). Second, incubating ES in isotonic PEG increased microcracking of the CM (Table 1), eliminated water uptake and had no effect on macroscopically visible cracks, while water increased frequency of macroscopically visible cracks (Table 2). Third, our earlier study demonstrated that most microcracks in the CM form above periclinal cell walls, where the structural support by underlying cell walls may be limiting (Peschel and Knoche, 2005). Cracks that extend into the dermal system, however, usually do not traverse cell walls, but extend into the dermal system between cells (Glenn and Poovaiah, 1989). This fracture mode would be consistent with the above hypothesis where the initial microcrack forms at the point of limiting structural support of the CM, but subsequent crack extension into deeper cell layers takes place along the weakest part of the tissue (i.e., along cell walls of neighboring cells).

In summary, surface moisture and water vapor in addition to fruit growth (Peschel and Knoche, 2005) induce microcracks in the sweet cherry fruit CM that is strained by fruit growth and surface expansion in the absence of CM deposition. These microcracks represent the first detectable symptoms in a series of steps that ultimately result in the well known phenomenon of macroscopically visible fruit cracking. So far, efforts to minimize cracking have focused on protecting fruit late during development by rain shelters, Ca applications, etc. The evidence provided in this and our earlier study, however, suggests that initial steps toward preventing cracking should be taken earlier. Also, our data provide an explanation for fruit cracking occasionally observed under rain shelters or in greenhouses where wetting of the fruit surface is effectively prevented, but exposure to water vapor at high concentrations may still be a factor.

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