Surface Moisture Induces Microcracks in the Cuticle of ‘Golden Delicious’ Apple

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Abstract. Formation of microcracks in the cuticular membrane (CM) of epidermal segments (ES) of apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf., ‘Golden Delicious’, ‘Braeburn’, ‘Idared’, ‘Jonagold’, and ‘Topaz’; all grafted on ‘Malling.9’ rootstocks] fruit was studied after exposure of the surface of the ES to water. Potential strain of the CM on the ES was preserved by mounting a stainless steel washer on the fruit surface using an ethyl-cyanacrylate adhesive. Subsequently, ES were excised by tangentially cutting underneath the washer. The number of microcracks in the CM was established by light microscopy before and after a 48-h incubation period in deionized water. Within 48 h, the number of microcracks rapidly increased when the outer surface of ES of ‘Golden Delicious’ apple was exposed to water, but there was essentially no increase in microcracks when exposed to the ambient atmosphere. The occurrence of microcracks depended on the region of the fruit surface and increased from the rim of the pedicel cavity to the calyx. Increasing the relative humidity (greater than 75% relative humidity at 22 °C) above the outer surface of the ES exponentially increased the number of microcracks. Water-induced microcracking was not limited to ‘Golden Delicious’, but also occurred in ‘Braeburn’, ‘Jonagold’, ‘Topaz’, and, to a markedly smaller extent, in ‘Idared’ apple. The mechanism of formation of microcracks in the CM of apple fruit and their role in fruit russetting are discussed.

Peel russetting is a commonly encountered, economically important surface defect in a number of horticultural crops, including apple (Faust and Shear, 1972a), tomato (Bakker, 1988), banana (Williams et al., 1990), and plum (Michailides, 1991). In most apple cultivars, russeted fruit is downgraded or rejected and used for processing. Anatomically, russetting is caused by a periderm formed in response to damage of epidermal cells, particularly during early fruit development (for review, see Faust and Shear, 1972a). Such damage may result from application of crop protection agents (Creasy and Swartz, 1981; Goffinet and Pearson, 1991), fungal infections (Gildemacher et al., 2004), breaking of epidermal hairs, mechanical injury, frost, or low temperature (Faust and Shear, 1972a). Also, extended periods of surface wetness or high humidity are conducive to russetting (Creasy, 1980; Tukey, 1969).

In our recent study on sweet cherry fruit, we observed formation of microscopic cracks

in the cuticular membrane (CM) when fruit surfaces were exposed to water or high humidity (Knoche and Peschel, 2006). These cracks were limited to the CM, did not traverse epidermal or hypodermal cell layers, and were only detectable by microscopy. Formation of microcracks was not related to water uptake, because microcracking occurred also under isotonic conditions where a net water uptake can be excluded as a factor. Also, hydration altered the rheological properties of the isolated sweet cherry CM *in vivo*, i.e., decreased fracture force and modulus of elasticity, and increased fracture strain, which is consistent with the water-induced microcracking of the CM. Based on these observations and arguments, it may be hypothesized that water also induced microcracking of the apple fruit CM, which, in turn, is followed by formation of a periderm that becomes visible as fruit russetting (Faust and Shear, 1972a). The objective of this study was to establish the effect of surface moisture, i.e., liquid water and water vapor, on formation of microcracks in the CM of apple fruit. We focused on ‘Golden Delicious’ apple because it is an important apple cultivar worldwide, is susceptible to russetting, and is often subject to reduced value resulting from surface defects.

Materials and Methods

Mature ‘Golden Delicious’, ‘Braeburn’, ‘Idared’, ‘Jonagold’, and ‘Topaz’ fruit (all

from trees grafted on ‘M.9’) were obtained from experimental orchards of the University of Hannover at Ruthe, Germany, the Horticultural Research Station Kühnfeld at Halle, Germany, and commercial orchards near Halle, Germany. Fruit were selected for uniformity and freedom from visual defects and processed fresh on the day of sampling, except for ‘Golden Delicious’ and ‘Topaz’ that were stored at 2 ± 0.5 °C and $92 \pm 2\%$ relative humidity (RH) for a maximum of 113 and 4 d, respectively.

The effect of surface moisture on formation of microcracks in the CM was studied using epidermal segments (ES) according to the procedure of Knoche and Peschel (2006). Briefly, stainless steel washers (6.4 mm i.d., 17.8 mm o.d.) were mounted in the equatorial region of the apple fruit surface using an ethyl-cyanacrylate adhesive (Loctite 406; Henkel Loctite Deutschland GmbH, München, Germany). After curing overnight, ES were excised by cutting tangentially underneath the washer using a razor blade such that an ES consisting of CM plus some adhering tissue remained attached to the washer. This procedure preserved any strain of the CM on the ES. To establish the frequency of microcracks in the CM already present before experimental exposure to water, microcracks were infiltrated by placing ≈ 100 μ L 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 $\times 100$ (filter module U-MWU: 330 to 385 nm excitation wavelength; 420 nm emission wavelength; Olympus). Frequency of microcracks was determined on an individual ES basis. Thereafter, ES with washers attached were incubated at 22 ± 0.5 °C in petri dishes filled with deionized water containing 30 mM Na₃N unless specified otherwise. ES were completely submerged, thereby exposing inner and outer sides of the ES to water. ES

Fig. 1. Effect of surface water on formation of microcracks in the cuticle of epidermal segments (ES) of ‘Golden Delicious’ apple (H₂O/H₂O). ES with the surface exposed to the ambient atmosphere served as control (Atmosphere/H₂O). Regression equations were: H₂O/H₂O: increase in microcracks (no./cm²) = $175.40 (\pm 6.25) \times (1 - e^{-0.09 (\pm 0.01) \times \text{time(h)}})$, $r^2 = 0.99$, $P \leq 0.0001$; Atmosphere/H₂O: increase in microcracks (no./cm²) = $7.34 (\pm 0.10) \times (1 - e^{-0.07 (\pm 0.01) \times \text{time(h)}})$, $r^2 = 0.99$, $P \leq 0.0004$.

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with the outer surface in contact with the ambient atmosphere ($50 \pm 10\%$ RH) were used for control. Here, a glass or plastic cylinder (≈ 10 mm height, 10 mm i.d.) was mounted on the washer using high-vacuum grease (Baysilone–Paste hochviskos; GE Bayer Silicones, Leverkusen, Germany). This cylinder effectively served as a “snorkel”, thereby preventing wetting of the outer surface. After incubation, usually after 48 h, ES with washers attached were removed from solution, the dye solution reapplied, and ES reinspected for microcracks as described previously. This procedure allowed the change in frequency of microcracks during the incubation period to be calculated on an individual ES basis. The minimum number of replications was 10. Initial experiments focused on the time course of formation of microcracks by inspecting ES obtained from the equatorial region of mature ‘Golden Delicious’ fruit at 4, 8, 24, 48, and 144 h. To establish potential positional effects on microcracking, washers were mounted in a subsequent experiment on the rim of the pedicel cavity (“pedicel”), halfway between pedicel cavity and the equatorial region (“pedicel center”), in the equatorial region (“center”), halfway between the calyx and the equatorial region (“calyx center”), or on the rim of the calyx cavity (“calyx”). As a result of the concave geometry of pedicel and calyx cavities, it was technically impossible to mount the washers in either cavity. The effect of RH on formation of microcracks was established by exposing the outer surface of the ES to an atmosphere ranging from 0% to 100% RH; the inner surface was in contact with deionized water (Knoche and Peschel, 2006). Humidities were established using solutions of saturated salts, dry silica (0% RH), or liquid water (100% RH). Salt solutions and respective humidities at 22 °C were: CaCl_2 , 30% RH; NaCl , 75% RH; KCl , 85% RH; and KNO_3 , 93% RH (Wexler, 1995).

The effect of storage duration was investigated using ‘Golden Delicious’ fruit held in storage at 2 ± 0.5 °C and $92 \pm 2\%$ RH. Fruit were removed at 0, 6, 11, and 113 d and the number of microcracks determined.

To establish whether microcracking in ‘Golden Delicious’ was representative of other apple cultivars, ES excised from ‘Braeburn’, ‘Idared’, ‘Jonagold’, and ‘Topaz’ were investigated.

Unless specified otherwise, data are presented as means $\pm$ SE of means. Analysis of variance, linear and nonlinear regression analysis (Proc REG, Proc NLIN), and comparison of means were carried out using the Statistical Analysis System software package (Version 8.02; SAS Institute, Cary, NC).

Results and Discussion

Exposure of the CM surface of ES of ‘Golden Delicious’ apple to water increased the number of microscopic cracks in the CM within 24 h (Fig. 1). In contrast, when the CM

Fig. 2. Relationship between the number of microcracks in the cuticle of epidermal segments (ES) of ‘Golden Delicious’ apple before and after 48-h exposure to liquid water and the water-induced increase in microcracks. The increase in microcracks was calculated on an individual ES basis as the difference between the number of microcracks after and before exposure to water. Regression equations were: microcracks—after (no./cm²) = $1.5 (\pm 0.1) \times$ microcracks—before (no./cm²) – 53.4 (± 16.9), $r^2 = 0.76$, $P \leq 0.0001$ and increase in microcracks (no./cm²) = $0.5 (\pm 0.1) \times$ microcracks—before (no./cm²) – 53.4 (± 16.9), $r^2 = 0.25$, $P \leq 0.0002$.

surface remained dry, there was no effect on microcracking.

The distribution of microcracks on the apple fruit was nonuniform. In general, the number of microcracks before and after exposure to water and the increase during the 48-h incubation in water increased from the pedicel cavity and toward the calyx end of the fruit (Table 1). Plotting the increase in microcracks during the 48-h exposure to water versus the number of microcracks present before the experiment revealed a positive significant relationship suggesting that segments having a larger number of cracks were more prone to water-induced microcracking of the CM (Fig. 2).

Increasing the RH above the ES from 0% to 100% exponentially increased the number of microcracks (Fig. 3). At 100% RH, the increase in microcracks was lower (105.4 ± 10.3 no./cm²) than that obtained when exposing the CM surface of ES to liquid water (157.0 ± 19.3 no./cm²). It is interesting to note that frequency of microcracks significantly increased during storage (Table 2). Within ≈ 4 months, the number of microcracks approximately doubled.

Water-induced microcracking of the CM was not limited to ‘Golden Delicious’ apple, but also occurred in ‘Braeburn’, ‘Jonagold’, ‘Topaz’, and, to a smaller extent, in ‘Idared’ apple (Table 3).

Fig. 3. Effect of relative humidity (RH) during 48-h exposure of epidermal segments of ‘Golden Delicious’ apple at 22 °C on formation of microcracks in the cuticle. The regression model was: increase in microcracks (no./cm²) = $e^{0.046RH(\%)}$, $r^2 = 0.98$, $P \leq 0.0001$.

Table 2. Effect of storage duration on microscopic cracks in the cuticle of ‘Golden Delicious’ apple.

Storage duration (d)	Microcracks (no./cm ²)
0	118.4 (± 13.9) b ²
6	114.4 (± 13.5) b
11	165.7 (± 19.1) b
113	236.1 (± 14.0) a

²Means separation by Tukey’s studentized range test ($P \leq 0.05$).

The data presented here clearly demonstrate that exposure of the surface of apple fruit to water, or high concentrations of water vapor, induces microcracking of the CM. Because microcracking of the CM is an early event in russetting (Faust and Shear, 1972a), this observation would account for the significant relationships between surface wetness or high humidity and russetting of apple fruit reported in the literature (Creasy, 1980; Faust and Shear, 1972b; Tukey, 1969). Furthermore, postharvest handling of apple usually involves high-humidity environments in storage rooms, wrappings, or packages, in which microcracking of the CM as a result of high humidity may provide entry ports for fruit rot pathogens (Borve et al., 2000) and pathways for excessive water loss (Maguire et al., 1999).

The data obtained on the effect of surface moisture on formation of microcracks in apple parallel those established earlier in sweet cherry. First, the time courses of microcracking on exposure of the CM surface to liquid water or to the ambient atmosphere were similar (Knoche and Peschel, 2006). Second, increasing humidity markedly

Table 1. Microcracks in the cuticle of different regions of the surface of ‘Golden Delicious’ apple before and after 48-h exposure of excised epidermal segments to water².

Position	Microcracks (no./cm ²)		
	Before	After	Increase
Pedicel cavity	117.8 $\pm$ 18.0 ab ²	184.6 $\pm$ 21.4 b	66.8 $\pm$ 6.4 b
Pedicel center	97.9 $\pm$ 13.3 b	211.4 $\pm$ 24.7 ab	113.5 $\pm$ 13.8 ab
Center	114.4 $\pm$ 13.5 ab	236.6 $\pm$ 31.6 ab	122.2 $\pm$ 21.6 ab
Calyx center	159.2 $\pm$ 16.6 ab	277.6 $\pm$ 29.8 ab	118.4 $\pm$ 14.3 ab
Calyx	165.7 $\pm$ 21.4 a	318.6 $\pm$ 30.1 a	152.9 $\pm$ 16.8 a

²The increase in microcracks was calculated as the difference between the number of microcracks after and before exposure to water.

³Means separation within columns by Tukey’s studentized range test ($P \leq 0.05$).

Table 3. Formation of microcracks in the fruit cuticle of epidermal segments of selected apple cultivars before and after 48-h exposure to water².

Cultivar	Microcracks (no./cm ²)		
	Before	After	Increase
Braeburn	154.2 ± 18.5 a ³	267.0 ± 27.2 a	112.8 ± 13.4 ab
Golden Delicious	123.9 ± 11.9 ab	294.9 ± 26.3 a	171.0 ± 15.6 a
Idared	70.9 ± 9.3 b	95.1 ± 11.4 b	24.2 ± 3.1 c
Jonagold	122.2 ± 13.7 ab	218.5 ± 29.3 a	96.4 ± 21.9 b
Topaz	107.8 ± 18.6 ab	207.8 ± 30.2 ab	100.0 ± 14.8 b

²Data for 'Golden Delicious' taken from Figure 1. The increase in microcracks was calculated as the difference between the number of microcracks after and before exposure to water.

³Means separation within columns by Tukey's studentized range test ($P \leq 0.05$).

increased the number of microcracks in both apple and sweet cherry. Whether the mechanistic basis for microcracking of the CM in apple is the same as in sweet cherry is currently unknown. Water uptake followed by increased turgor of the epidermal system and subsequent CM cracking is unlikely to be involved in microcracking in both species, because unrestricted water uptake also occurs through the cut surface of the ES in the control. Because there was essentially no increase in microcracking in apple or sweet cherry ES under these conditions, water uptake by the epidermal cell layer was not involved. In our earlier study with sweet cherry fruit, we obtained a highly significant, linear relationship between microcracking and percentage strain of the CM on mature fruit of selected sweet cherry cultivars. In this experiment, strain of the CM ranged from 70% to 108% (Knoche and Peschel, 2006). The strain in sweet cherry resulted from rapid surface expansion during final swell (Stage III of fruit development) in the absence of CM deposition (Knoche et al., 2004). Unfortunately, no published data for an analogous analysis of CM deposition in the developing apple fruit are available. However, because fruit surface expansion and CM deposition apparently occur simultaneously in apple (Meyer, 1944; Miller, 1982), strain of the

CM must be markedly smaller as compared with the sweet cherry fruit. In fact, preliminary data from our laboratory indicate that strain of the mature apple fruit CM may be even lower than 10%. Thus, the relationship among microcracking of the CM, fruit surface expansion, CM deposition, and water-induced cracking is likely to be more complex in apple than in sweet cherry. Because microcracking and russetting are economically important, this subject merits further studies.

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