Russeting and Microcracking of 'Golden Delicious' Apple Fruit Concomitantly Decline Due to Gibberellin A_{4+7} Application

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ABSTRACT. The effect of four applications of gibberellin A_{4+7} [GA_{4+7} (10 mg·L^{-1} at 10-day intervals beginning with petal fall)] on water-induced russeting, formation of microcracks. and on fruit growth and deposition of the cuticular membrane (CM) was studied in developing 'Golden Delicious' fruit (*Malus ×domestica* Borkh.). Submerging developing apple fruit in deionized water for 48 h induced russeting in untreated control but not in GA_{4+7}-treated fruit. Immersing in water during early fruit development, 19 days after full bloom (19 DAFB), resulted in more russeting than immersions occurring later (139 DAFB). Water on the outer surface of epidermal segments increased the frequency of microscopic cracks in untreated controls but to a lesser degree in GA_{4+7}-treated fruit. The effect of GA_{4+7} on water-induced russeting and formation of microcracks was larger during early as compared with later stages of fruit development. Fruit treated with GA_{4+7} consistently had fewer microcracks as compared with non-treated control fruit. GA_{4+7} had no effect on amounts or rates of cutin or wax deposition, strain, or mechanical properties of the CM as compared with the non-treated control. Thus, the decrease in russeting and formation of microcracks in the cuticle of GA_{4+7}-treated fruit must be accounted for effects on underlying epi- and hypodermal tissues.

Russeting is an important peel defect in many apple cultivars, and russeted fruit is subject to reduced value (Faust and Shear, 1972a). Apple cultivars differ in their susceptibility to russeting, and fruit in early stages of development is more susceptible than that in later stages (Faust and Shear, 1972a; Simons and Chu, 1978; Skene, 1982). Russeting may be induced by a range of factors, including agrochemicals (Creasy and Swartz, 1981; Sanchez et al., 1992), environmental conditions such as freezing temperatures, extended periods of high humidity and surface wetness (Creasy, 1980; Faust and Shear, 1972b; Simons and Chu, 1978; Tukey, 1969), or microorganisms (Daines et al., 1984; Gildemacher et al., 2004; Heidenreich et al., 1997). Under orchard conditions, russeting is avoided and peel appearance improved in sensitive cultivars by applying gibberellins such as gibberellic acid (GA_3) or GA_{4+7} (Elfving and Allen, 1987; Meador and Taylor, 1987; Steenkamp and Westraad, 1988; Taylor, 1975; Wertheim, 1982). A typical application scheme comprises four treatments with GA_{4+7} at 10 mg·L^{-1} applied in 10-d intervals starting at the onset of petal fall (Greene, 1993; Looney et al., 1992).

Microscopic cracks in the cuticular membrane are the first detectable symptoms in russet development (Faust and Shear, 1972a; Hatch, 1975; Simons and Chu, 1978; Verner, 1938). A periderm is subsequently formed as a wound reaction that replaces the primary epidermal tissue on the fruit surface. This periderm tissue causes the brown and rough appearance of a russeted fruit surface (Verner, 1938).

Recent studies from our laboratory established that exposure of the fruit surface to liquid water and water vapor induced microscopic cracks in the CM of mature 'Golden Delicious' apple (Knoche and Grimm, 2008). Also, applications of GA_3 and GA_{4+7} enhanced CM deposition in developing tomato (*Solanum lycopersicum* L.) fruit (Knoche and Peschel, 2007). Therefore, it may be hypothesized that GA_{4+7} reduces russeting by decreasing the formation of microcracks in the cuticle and that GA_{4+7}-reduced microcracking is related to increased CM deposition and decreased strain of the CM in the course of development.

The objectives of the present study were to investigate whether 1) a decrease in fruit russeting by GA_{4+7} is paralleled by a decrease in the formation of microcracks in the CM; and 2) a decrease in microcracking by GA_{4+7} can be accounted for by effects of GA_{4+7} on fruit growth, cuticle deposition or strain. and the mechanical properties of cuticles isolated from developing apple fruit. We chose 'Golden Delicious' apple, because 'Golden Delicious' is grown worldwide and is susceptible to russeting.

Materials and Methods

PLANT MATERIAL. 'Golden Delicious' fruit were obtained from two experimental orchards at the Horticultural Research Station Kühnfeld at Halle, Germany (lat. 51°49' N, long.

12°00′ E), and at the Agricultural Institute of Slovenia at Ljubljana, Slovenia (lat. 46°10′ N, long. 14°42′ E). Trees, grafted on 'M.9' and 'Malling Merton 106' rootstocks at the Halle and Ljubljana site, respectively, were selected for average flower bud density. Treated trees were sprayed to runoff with 10 mg·L^{-1} GA$_{4+7}$ (Provide; Valent Biosciences, Libertyville, IL) at 6, 16, 26, and 38 DAFB and 4, 13, 23, and 33 DAFB at the Halle and Ljubljana sites, respectively. A minimum of 30 trees was used per treatment. Fruits were randomly harvested at 2-week intervals and processed fresh on the day of sampling.

FRUIT RUSSETING. The effect of immersing fruit in water on russeting was studied at the Ljubljana site at 19, 39, 48, and 139 DAFB. The mean equatorial diameters of the fruit at these dates were 9.7, 25.1, 33.0, and 62.7 mm, respectively. Attached fruit from trees treated with GA$_{4+7}$ and from non-treated control trees were immersed in deionized water for 48 h using polyethylene zip-lock bags that were secured around the fruits. After 48 h, the bags were removed and the fruit was tagged. At 141 DAFB, fruit were harvested and kept in cold storage at 3 °C. For russet analysis, the entire fruit was peeled, the peel positioned on a glass plate, and photographed. The total peel surface area and the area affected by russeting were quantified using image analysis (NIS Elements; Nikon Instruments, Kanagawa, Japan) on an individual fruit basis. The number of fruit analyzed per treatment and sampling date ranged from 33 to 66 fruit.

WATER-INDUCED MICROCRACKING. Formation of microcracks in the CM was studied using epidermal segments (ES) excised from GA$_{4+7}$-treated or non-treated control fruit at the Halle site (Knoche and Peschel, 2006). Generally, fruit were sampled in 2-week intervals between 33 and 138 DAFB. Stainless steel washers (6.4 mm i.d., 17.8 mm o.d.) were mounted in the equatorial region using an ethyl-cyanacrylate adhesive (Loctite 406; Henkel Loctite Deutschland, Munich, Germany). The purpose of the washer was to maintain any strain of the dermal system after excising of the ES by cutting tangentially underneath the washer using a razor blade. These ES consisted of CM plus epidermal, hypodermal, and some parenchyma tissue held in a washer. Frequency of microcracks in the CM before exposure to water was established by pipetting ≈100 µL of a 0.1% acridine orange solution on the outer surface of the ES exposed through the washer hole. After 10 min, the dye solution was removed by carefully blotting with tissue paper. The ES were then transferred to the stage of a fluorescence microscope (Model BX-60; Olympus, Hamburg, Germany) and viewed at ×100 [filter module U-MWU, Olympus (330 to 385 nm excitation wavelength, 420 nm or greater emission wavelength)]. The number of fluorescing microcracks in the cuticle within the hole of the washer was counted. The ES with washers attached were subsequently incubated at 22 ± 0.5 °C in deionized water containing 30 mM NaN$_3$. ES were either completely submerged, thereby exposing both inner and outer sides of the ES to water, or incubated such that only the inner surface was in contact with water while the outer side in the washer hole remained dry. Unless specified otherwise, ES with the washers attached were removed from the solution after 48 h and blotted, and the dye solution was reapplied to the surface exposed through the washer hole. The ES were then reinspected for microcracks as described previously. Using this procedure, the increase in frequency of microcracks on exposure to water was calculated on an individual ES basis. The minimum number of replications was 10.

Initially, a time course experiment was conducted to establish a suitable sampling window for determining effects. ES from GA$_{4+7}$-treated and non-treated control fruit were inspected for microcracks after 0, 4, 8, 24, 48, and 144 h of exposure of the outer side of the ES to water or to the ambient atmosphere.

To identify interactions between the development stage and GA$_{4+7}$ treatment on water-induced microcracking, fruit were sampled from GA$_{4+7}$-treated or non-treated control trees between 33 and 138 DAFB. Water-induced microcracking was studied as described using 10 replicates.

The effect of GA$_{4+7}$ on water vapor-induced formation of microcracks was established by exposing the outer surface of ES excised from GA$_{4+7}$-treated or non-treated control fruit to defined humidities. Here, fruit was sampled at 159 DAFB. The inner surface of the segments remained in contact with deionized water. Relative humidity (RH) was adjusted by incubating the outer surface of the ES above dry silica (0% RH), saturated KCl solutions [85% RH (Wexler, 1995)], or deionized water (100% RH). A fourth treatment comprised ES with outer surfaces in direct contact with deionized water. The number of replications per treatment was 10.

FRUIT MASS AND SURFACE AREA. Mass, equatorial diameters, and length of developing fruit were determined in GA$_{4+7}$-treated and non-treated control fruit. Two equatorial diameters perpendicular to each other and fruit height were quantified using electronic calipers and subsequently averaged on an individual fruit basis. Fruit surface area was calculated from average diameter assuming a spherical fruit as a first approximation.

ISOLATION OF CUTICULAR MEMBRANES. The CMs were isolated enzymatically from GA$_{4+7}$-treated and control fruit at the Halle and the Ljubljana sites according to standard protocols (Orgell, 1955). Epidermal discs and strips were excised from the equatorial region using a cork borer (i.d. 8.9 mm) and parallel razor blades (6 mm width), respectively. Specimens were incubated in 50 mM citric acid buffer (pH 4.0) containing pectinase [90 mL·L^{-1} (Panzym Super E flüssig; Novozymes, Bagdsvaerd, Denmark)] and cellulase [5 mL·L^{-1} (Celluclast; Novozymes)]. NaN$_3$ was added at 30 mM to prevent microbial activity. Enzyme solutions were refreshed repeatedly until CM separated from adhering dermal tissue. Isolated CMs were thoroughly rinsed in deionized water and air dried. Dewaxed CMs (DCMs) were prepared by treating CMs with 10 consecutive 0.5-h washes in CHCl$_3$/methanol (1:1, v/v) at 80 °C. Masses of CM or DCM discs were determined gravimetrically on a minimum of 10 discs per replicate for five replications.

STRAIN OF THE CUTICLE. For quantifying strain, isolated CM discs were spread on a glass slide and photographed under a microscope at 40× magnification (MZ6 microscope; Leica Mikrosysteme, Bensheim, Germany). The CM area was quantified by image analysis (analySIS 3.0; Soft Imaging System, Münster, Germany) and strain [ε (%)] calculated according to the equation:

$$\varepsilon = \frac{A - A_O}{A_O} \cdot 100 \qquad [1]$$

where A represents the area of the CM on the fruit before excision (equivalent to the area of the cork borer) and A_0 is equal to the area of the relaxed CM after isolation. The number of replicates was 10.

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TENSILE TESTS. Uniaxial tensile tests were performed using CM strips (20 × 6 mm²) excised and isolated at 75 and 138 DAFB. CM strips were mounted in a frame made from paper and masking tape (Tesa Krepp[R]; tesa Werk Hamburg, Hamburg, Germany). The frames were used to prevent unintentional stress of the CM during handling and mounting. Frames with CM strips were equilibrated either at 22 °C and 50% RH or hydrated by incubating in deionized water at 22 °C for 16 h. After equilibration, frames were mounted in a universal material testing machine (Z 0.5; Zwick Roell, Ulm, Germany). Immediately before testing, frames were cut open. Specimens were subjected to uniaxial tensile tests at a test velocity of 200 mm·min^{-1} and grip distance of 12 mm until failure occurred. The strain until failure (%), force at failure (Newtons), and the modulus of elasticity (Newtons) were determined for dry and hydrated CM strips. The modulus was obtained from the maximum slope of a linear regression line fitted through a plot of force (Newtons) versus strain (percent/100). Coefficients of determination averaged 0.99. Occasionally, cracks in the CM occurred in or adjacent to clamps. Data on these specimens were excluded from analysis, because these specimens may have been damaged during clamping. The minimum number of replicates used in the final analysis was 18.

DATA ANALYSIS AND TERMINOLOGY. Experiments on the effects of GA$_{4+7}$ on water vapor-induced formation of microcracks and on the mechanical properties of isolated CMs were analyzed by analysis of variance (ANOVA). ANOVA, multiple comparisons of means, and regression analysis were carried out using SAS (Version 9.1.3; SAS Institute, Cary, NC). Data expressed on a percentage basis were arcsin-transformed before ANOVA. Data in figures are presented as means ± SE of means. Where not shown, error bars were smaller than the data symbols used on the figures.

Throughout our study we refer to minute fractures in the cuticle as microcracks. These cracks usually do not traverse the epidermal and hypodermal cell layers. Microcracks may be infiltrated using the fluorescence tracer acridine orange. After infiltration, microcracks larger than ≈50 μm length and longer are detectable by fluorescence microscopy.

Results

RUSSETING. Immersing fruit for 48 h in water induced russeting in non-treated control fruit but not in GA$_{4+7}$-treated fruit (Fig. 1). Russeting was more severe when fruit were immersed in water during early stages of development as compared with later stages. Visual russeting estimation showed that the percentage of fruit having less than 5%, 10%, or 15% of the fruit surface russeted was always lower in the GA$_{4+7}$ treatment than in the non-treated control (M. Stopar, data not shown).

MICROCRACKS IN THE CUTICLE. The frequency of microcracks in the cuticle increased rapidly with time when the outer side of the ES was in contact with deionized water but not when exposed to the ambient atmosphere (Fig. 2). The frequency of water-induced microcracks was lower in GA$_{4+7}$-treated than in non-treated fruit.

Water-induced microcracking of the CM increased in the course of development and was consistently higher in control as compared with GA$_{4+7}$-treated fruit (Fig. 3A). The relative decrease in microcracking by GA$_{4+7}$ was larger during early as compared with later developmental stages. The frequency of
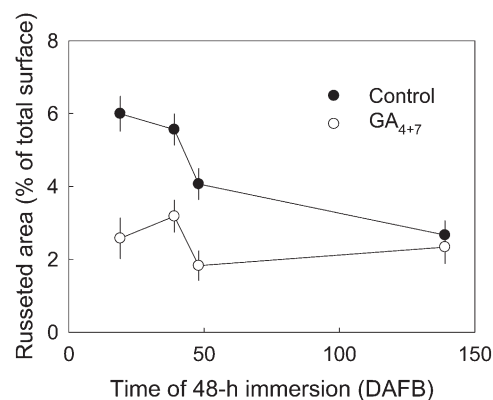

Fig. 1. Effect of a 48-h immersion of developing 'Golden Delicious' apple into deionized water on russeting. Trees were sprayed with gibberellin A$_{4+7}$ [GA$_{4+7}$ (4 × 10 mg·L^{-1})]; non-treated trees served as controls. Russeting was expressed as percent of the total fruit surface area with russet. Vertical bars represent ± SE. DAFB = days after full bloom.
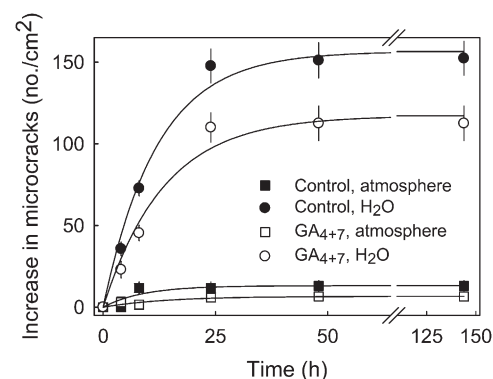

Fig. 2. Time course of change in frequency of microcracks in the cuticle of epidermal segments of mature 'Golden Delicious' apple during exposure of the outer surface to the ambient atmosphere (air) or water (H$_2$O). Fruit were sampled from trees treated with gibberellin A$_{4+7}$ (GA$_{4+7}$) or from non-treated control trees. Vertical bars represent ± SE.

microcracks before exposure to water in the in vitro assay increased continuously throughout development and was consistently lower for GA$_{4+7}$ than for control fruit (Fig. 3B).

Increasing RH above the outer surface of ES increased formation of microcracks in non-treated control fruit but to a lesser degree in GA$_{4+7}$-treated fruit (Table 1).

FRUIT GROWTH, CUTICLE DEPOSITION, AND STRAIN. Fruit mass and surface area of developing 'Golden Delicious' fruit increased in a sigmoidal pattern with time (Fig. 4A–B). There was no effect of GA$_{4+7}$ on fruit mass, surface area, or on maximum rates of change in mass or surface area at the Halle or Ljubljana site (data not shown).

Mass of CM and DCM calculated on a unit fruit surface area (Fig. 5A) and a whole fruit basis increased throughout development (Fig. 5B) indicating that CM and DCM thickness increased. There was no effect of GA$_{4+7}$ (Fig. 5). Qualitatively and quantitatively similar data were obtained at Ljubljana (data not shown).

Strain of the CM remained constant between 33 and 97 DAFB averaging 12.2% and 12.4% for control and GA$_{4+7}$-treated fruit, respectively, and thereafter decreased toward maturity (Fig. 6). There was no difference between control and GA$_{4+7}$-treated fruit.
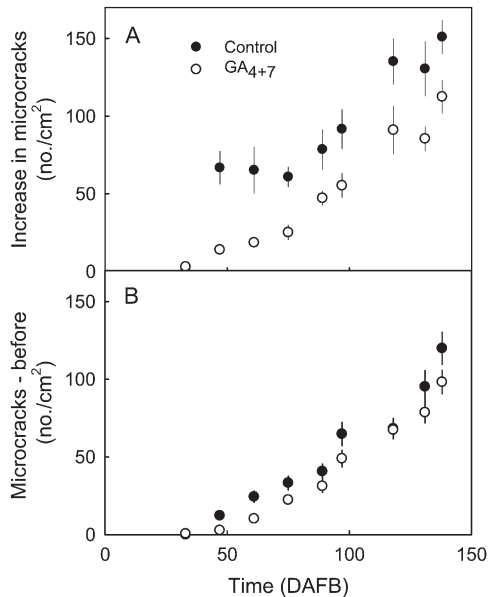
Fig. 3. (**A**) Increase in microcracks in the cuticular membrane (CM) of epidermal segments (ES) of developing 'Golden Delicious' apple during a 48-h exposure of the outer surface of the ES to water. (**B**) Frequency of microcracks in the CM before exposure to water. Fruit were sampled from trees treated with gibberellin A_{4+7} (GA_{4+7}) or from non-treated control trees. Vertical bars represent ± SE. DAFB = days after full bloom.

Table 1. Effect of gibberellin A_{4+7} (GA_{4+7}), water vapor, and liquid water on the formation of microcracks in the cuticle of epidermal segments (ES) excised from GA_{4+7}-treated and non-treated 'Golden Delicious' apple.[z]

Relative humidity (%)	Increase in microcracks (no./cm²)[y]		
	Control	GA_{4+7}	Mean
0	4.7	2.5	3.6 d[x]
84.2	65.0	48.8	56.9 c
100	109.7	96.1	102.9 b
Liquid water	167.2	124.0	145.6 a
Mean	86.6 a	67.8 b	

[z]Trees were sprayed with gibberellin A_{4+7} [GA_{4+7} (4×10 mg·L⁻¹ at 6, 16, 26, and 38 d after full bloom)]; non-treated trees served as controls.
[y]The increase in microcracks was calculated as the difference in microcracks after minus that before a 48-h exposure to defined humidities or liquid water.
[x]Interaction humidity × GA_{4+7} not significant at $P = 0.1079$. Mean separation within main effect humidity by Tukey's Studentized range test at $P < 0.05$.

TENSILE TESTS. Fracture force, strain, and the modulus of elasticity all increased from 75 to 138 DAFB (Table 2). Hydrating the CMs generally decreased fracture forces and moduli; there was no effect on fracture strain. With few exceptions, fracture force, fracture strain, and the modulus of elasticity of isolated CM strips were unaffected by GA_{4+7} (Table 2). The only exceptions were a small but significant increase (+5%) in the modulus at 138 DAFB and a decrease of 15% in fracture strain at 75 DAFB.

Discussion

Our experiments established that the effects of GA_{4+7} and water on the formation of microcracks in in vitro assays largely
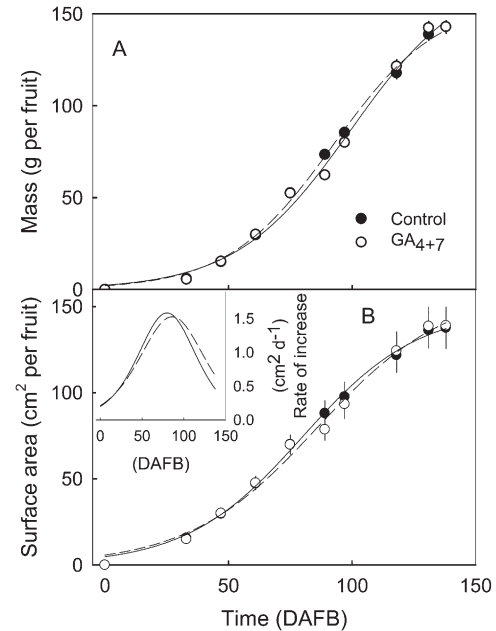

Fig. 4. Time course of change in (**A**) fruit mass and (**B**) calculated fruit surface area of developing 'Golden Delicious' apple from trees treated with gibberellin A_{4+7} [GA_{4+7} (4×10 mg·L⁻¹)] or from non-treated controls. Inset: Rate of increase in fruit surface area as affected by GA_{4+7} (dash line) or non-treated control (full line). Vertical bars present ± SE. DAFB = days after full bloom.

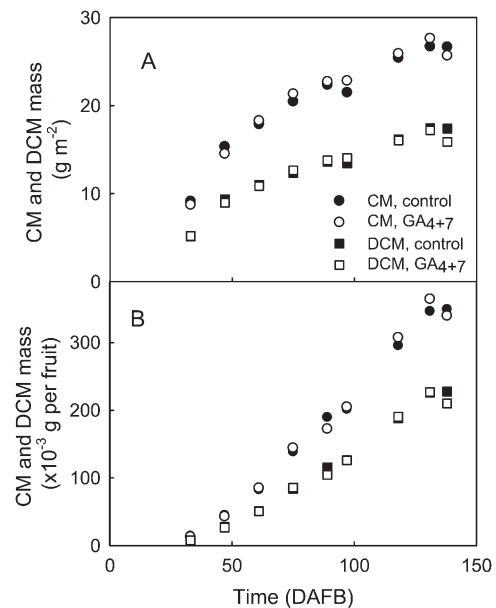

Fig. 5. Time course of change in mass of cuticular membrane (CM) and dewaxed cuticular membrane (DCM) of developing 'Golden Delicious' apple on (**A**) a unit fruit surface area basis and (**B**) a whole fruit basis. Fruit were sampled from trees treated with gibberellin A_{4+7} [GA_{4+7} (4×10 mg·L⁻¹)] or from non-treated control trees. DAFB = days after full bloom.

paralleled their effects on russeting in vivo under orchard conditions. This would be expected if microscopic cracks in the cuticle are an early symptom in the formation of russeting (Faust and Shear, 1972a, 1972b; Verner, 1938).

Theoretically, the effects of GA_{4+7} and water on formation of microscopic cracks in the cuticle may result from effects on

1) tension in the cuticle that represents the driving force for formation of cracks; 2) rheological properties of the cuticle; and/or 3) effects on "repair" mechanisms that affect the initiation and/or the extension of cracks in the cuticle.

DRIVING FORCE FOR MICROCRACKING OF THE CUTICULAR MEMBRANE. Tensional forces in the dermal system represent the driving force for crack formation. These forces result from an increase in fruit volume and, hence, surface area that subjects the dermal tissue and the cuticle to a tangential stress (Curry, 2005; Skene, 1982; Verner, 1938).

The cuticle, as the outermost layer of this composite, is subjected to the largest stress. Cuticles are viscoelastic polymers that respond to stress by reversible elastic and/or irreversible plastic deformation or strain (Petracek and Bukovac, 1995). In such polymers, the ratio of elastic to plastic strain depends on the rate of strain. High rates primarily cause elastic strain and a proportional stress, whereas low rates favor time-dependent plastic deformation that thins the polymer and alleviates stress. These points demonstrate that the rate of strain is an important factor in skin failure. The rate of strain for a growing fruit equals the relative growth rate in surface area that is defined as the increase in surface area per unit of time and per unit of surface area present at that time. The relative growth rate is at maximum during early development when fruit is most sensitive to russeting (Creasy, 1980). Because GA_{4+7} had no effect on fruit growth, the relative growth rates in surface area and the strain of the CM did not differ between GA_{4+7}-treated and non-treated control fruit.

The apple fruit CM is less strained as compared with that of soft berries (*Ribes uva-crispa* L., *Ribes nidrigrolaria* B.), sweet cherry (*Prunus avium* L.), or plum (*Prunus ×domestica* L.) (Khanal et al., 2011; Knoche and Peschel, 2006). Apple differed from these crops in that cuticle thickness increased

Fig. 6. Strain of the cuticular membrane (CM) of developing 'Golden Delicious' apple. Trees were treated with gibberellin A_{4+7} [GA_{4+7} (4×10 mg·L^{-1})]. Non-treated trees served as controls. Vertical bars represent ± SE. DAFB = days after full bloom.

throughout development (Fig. 5), but that of the berries and stone fruit decreased as a result of a cessation of CM deposition already during early development. Thus, in apple, the deposition of newly formed CM on older, more strained layers of the CM fixed the strain of the older layers and prevented strain relaxation on isolation. From these considerations, the following conclusions may be drawn. First, the driving force for formation of microcracks in the cuticle depends on the balance between the relative growth rate in the surface area of the fruit on the one hand and the rate of CM deposition on the other hand. Second, the decrease in microcracking resulting from GA_{4+7} cannot be accounted for by a decrease in the driving force for cracking because neither the relative growth rate in surface area nor the rate nor amount of CM deposition was affected by GA_{4+7}. Thus, the driving forces for microcracking of the CM must have been similar for GA_{4+7}-treated and non-treated control fruit.

RHEOLOGICAL PROPERTIES. According to Vincent (1990), the best resistance to failure is obtained in a "tough" skin where resistance to crack initiation and propagation is high. Vincent (1990) considered crack initiation as the critical factor in fruit skins and crack initiation is governed by the presence and orientation of stress-concentrating defects.

In our experiments, there was no consistent difference in force or strain at fracture or the modulus of elasticity of CM isolated from GA_{4+7}-treated versus non-treated control fruit at 75 and 138 DAFB. Thus, the decrease in formation of microcracks caused by GA_{4+7} cannot be explained by effects on CM rheology.

Hydration decreased fracture force and moduli of the hydrated cuticle, but fracture strains remained constant. These observations are consistent with those obtained for CM from other fruit crops [tomato (Petracek and Bukovac, 1995), sweet cherry (Knoche and Peschel, 2006)] and would account for water-induced microcracking provided that water increased hydration of the non-isolated CM of the ES.

REPAIR MECHANISMS ON FAILURE. In our study, the presence of microcracks in the cuticle was deduced from the infiltration zones of the fluorescent tracer acridine orange. This technique only detects microcracks that traverse the cuticle. Cracks that do not traverse the cuticle (Maguire et al., 1999) or those that "healed" by filling with wax (Curry, 2008) are not infiltrated and would pass undetected. The latter repair mechanism will depend on the rate of wax deposition at the site of cracking and/or compositional changes within the wax fraction, particularly those that favor constituents of high diffusivity within the cuticle. Effects of GA_{4+7} on these latter processes are not necessarily detected when quantifying rates and amounts of deposition of total wax. However, we would expect these processes to be too slow to account for a decrease in microcracking by GA_{4+7} in the 48-h incubation period of our washer
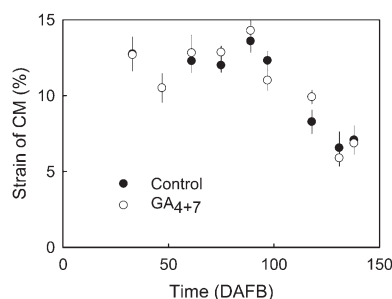
Table 2. Effect of gibberellin A_{4+7} (GA_{4+7}) on selected rheological properties of dry and hydrated, enzymatically isolated 'Golden Delicious' apple cuticles sampled at 75 and 138 d after full bloom (DAFB).

Treatment	DAFB	Fracture force [mean ± SE (N)]			Fracture strain [mean ± SE (%)]			Modulus of elasticity [mean ± SE (N)]		
		Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean
Control	75	1.5 ± 0.1	0.9 ± 0.1	1.2 ± 0.1 a	4.8 ± 0.3	4.4 ± 0.4	4.6 ± 0.2 a	45.0 ± 0.9	31.4 ± 0.8	38.6 ± 1.3 a
GA_{4+7}	75	1.4 ± 0.1	0.8 ± 0.1	1.1 ± 0.1 a	4.3 ± 0.2	3.6 ± 0.3	4.0 ± 0.2 b	45.6 ± 0.6	30.1 ± 0.8	38.4 ± 1.3 a
Mean 75 DAFB		1.5 ± 0.0 a^z	0.8 ± 0.0 b		4.6 ± 0.2 a	4.0 ± 0.2 a		45.3 ± 0.5 a	30.7 ± 0.6 b	
Control	138	1.7 ± 0.1	1.1 ± 0.0	1.4 ± 0.1 a	5.4 ± 0.4	4.9 ± 0.3	5.2 ± 0.2 a	54.2 ± 1.0	40.3 ± 1.1	47.4 ± 1.3 b
GA_{4+7}	138	1.9 ± 0.1	1.2 ± 0.1	1.6 ± 0.1 a	5.1 ± 0.3	4.7 ± 0.4	4.9 ± 0.2 a	58.2 ± 1.2	41.8 ± 1.0	50.0 ± 1.5 a
Mean 138 DAFB		1.8 ± 0.1 a	1.2 ± 0.0 b		5.3 ± 0.2 a	4.8 ± 0.2 a		56.2 ± 0.8 a	41.1 ± 0.7 b	

^{z}Interaction hydration × GA_{4+7} not significant. Mean separation within main effects and within DAFB at $P < 0.05$.

assays. Clearly, these arguments are speculative and experimental evidence is currently lacking.

Conclusions

GA_{4+7} consistently decreased microcracking of the CM but had no effect on relative growth rates in surface area or on deposition, strain, or rheological properties of the CM. Therefore, the effect of GA_{4+7} must reside with the epi- and hypodermis underlying the cuticle. Epidermal and hypodermal cells often have thick cell walls and lack gas-filled intercellular spaces, thereby providing strong cell-to-cell adhesion. The increase in aspect ratios of epi- and hypodermal cells is indicative of strain (Meyer, 1944; Verner, 1938). Consistent with a role of epi- and hypodermal tissue in crack formation is the development of cracks in the cuticle primarily along anticlinal cell walls of clusters of epidermal cells (B.P. Khanal, unpublished data). Developing 'Golden Delicious' apple has an ''irregular epidermis'' caused by periclinal cell divisions and cracks in the cuticle (Meyer, 1944; Wertheim, 1982) and this may cause an uneven distribution of stress and localized stress concentration in the CM. Eccher (1975) postulated effects of GA_{4+7} on cell division or cell enlargement in the dermal tissue of 'Golden Delicious' apple. To our knowledge, these effects were often cited but never quantified. For 'Wealthy' apple, Bukovac and Nakagawa (1968) reported increased cortex thickness, increased cell number, and increased sizes after application of GA_4. If dermal cell layers contributed to the fruit skin mechanics, effects of GA_{4+7} on cell division and cell enlargement could alter the susceptibility to microcracking and ultimately to russeting. For example, increased cell division implies more cell walls per unit surface area and, hence, a stronger structural support of the CM. Increased cell enlargement could maintain cell-to-cell bonding under tensional stress thereby preventing failure of apple fruit skin. These subjects merit further investigation, because skin blemishes in many fruit crops, including the russeting of apple, involve failure of the dermal system as the first visible symptom.

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