Increase in Epidermal Planar Cell Density Accompanies Decreased Russetting of ‘Golden Delicious’ Apples Treated with Gibberellin A₄₊₇

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Abstract. A 2-year study was conducted in a ‘Golden Delicious’ (*Malus ×domestica* Borkh.) orchard with a high incidence of physiological fruit russetting to examine the effect of gibberellin A₄₊₇ (GA₄₊₇) on apple epidermal cell size. Beginning at petal fall, four sequential applications of GA₄₊₇ (0, 15, or 30 mg·L⁻¹) were applied to whole trees every 7–10 days with an orchard air-blast sprayer at a volume of ≈1000 L·ha⁻¹. Fruit epidermal tissue samples were taken approximately monthly beginning 1 week after the fourth application. Tissue was treated in the laboratory with an enzyme mixture to remove cellular debris in preparation for examination using either light or scanning electron microscopy (SEM). In 2007, because russetting was insignificant, treatment differences could not be established. Moreover, transillumination microscopy did not permit accurate measurement of ‘Golden Delicious’ fruit epidermal planar cell area beyond midseason because the isolated cuticle became thick and multilayered. In 2008, however, respective treatments of 15 and 30 mg·L⁻¹ reduced calyx end russetting by 40% and 83% and increased epidermal planar cell density by 14% and 27% as measured using SEM.

The presence of russet on ‘Golden Delicious’ (*Malus ×domestica* Borkh.) apples has long been a concern to producers and marketers of fresh fruit throughout the world because it detracts from the smooth, uniform finish of the fruit and results in economic loss resulting from grade reduction (Faust and Shear, 1972a). Physical russetting is the result of irreversible injury to the peel by impact, abrasion, or repeated agitation by another material such as might occur with hail or limb and leaf rubbing. Physiological russetting, on the other hand, although foundationally genetic (Eccher et al., 2008), may be induced or aggravated by various factors including: 1) xenobiotics (Creasy and Swartz, 1981; Hatch, 1975; Lallu et al., 2010; McCormick and Streif, 2008; Sanchez et al., 1992); 2) changes in ambient temperature, humidity, and aridity (Creasy, 1980; Faust and Shear, 1972b; Fogelman et al., 2009; Meyer, 1944; Tukey, 1959); and 3) insects and pathogens such as rust mite, ring virus, and various bacteria, yeasts, and fungi (Daines et al., 1984; Duso et al., 2010; Easterbrook and Fuller, 1986; Heidenreich et al., 1997; Lindow et al., 1998; Wood, 2010). In the orchard,

these factors often are related and/or co-dependent.

Many methods have been evaluated to alleviate physiological russetting in apple. Like other environmentally based peel disorders such as superficial scald, sunburn, scarfskin, or flecking, a consistent response to treatment is often difficult to achieve or predict. One of the more successful methods of reducing physiological russetting is use of multiple topical applications of gibberellins during early fruit development. Since the first published reports over 35 years ago (Eccher, 1975; Eccher and Boffelli, 1978; Edgerton and Veinbrants, 1979; Taylor, 1975), researchers have conducted numerous studies to better understand the mechanisms underlying the beneficial effects of gibberellins on apple peel finish.

Eccher (1975) first observed less irregularity among epidermal cells and reduced cuticle cracking in fruit treated with GA₄₊₇ than in untreated fruit. Skene and Greene (1982) recognized the relationship between microcracking in the cuticle and regions of russetting in ‘Cox’s Orange Pippin’. [Also, see the review regarding fruit skin splitting and cracking (Opara et al., 1997).] Microcracking in ‘Golden Delicious’ peel tissue was reported to increase as a result of surface wetting (Knoche and Grimm, 2008). More recently, Knoche et al. (2011) found water-induced russetting and microcracking of ‘Golden Delicious’ apples decreased on fruit pretreated with multiple applications of GA₄₊₇. They also reasoned the effect of GA₄₊₇ on microcracking and, therefore, russetting must reside with the epidermal and hypodermal cell layers.

Bukovac and Nakagawa (1968) first reported increases in size and number of cortex cells in apples treated with high rates of GA₄₊₇ in lanolin paste applied 2 weeks after anthesis. To my knowledge, the effect on apple epidermal cells, of multiple applications of GA₄₊₇, has not been quantitatively examined. Thus, the objective of this work was to determine whether the decrease in russetting of ‘Golden Delicious’ apples from multiple applications of GA₄₊₇ was accompanied by an increase in epidermal planar cell density (number of epidermal cells per unit peel surface area).

Materials and Methods

Experiments were conducted in 2007 and 2008 in a uniform, mature, commercial orchard of ‘Golden Delicious’/‘M.M.106’ (‘MM.106’) trees growing in loamy sand near Mattawa, WA (lat. 46°39’16.58” N, long. 119°52’32.12” W; elevation 192 m). Trees were irrigated by undertree impact sprinklers and fruit from trees in most areas within this 13.2-ha block had a 70% to 80% historical incidence of russetting (≈7 of 10 years 50% or greater of the harvested fruit had calyx and/or shoulder russetting sufficient to cause reduction in grade).

Treatment dosages of 0, 15, or 30 mg·L⁻¹ GA₄₊₇ (ProVide[®]; Valent Biosciences Corp., Libertyville, IL) were applied topically to whole trees at first petal fall (FPF) and every 7–10 d thereafter for four sequential applications. Each treatment was applied to three rows of ≈20 trees per row with an orchard air-blast sprayer at a volume of ≈1000 L·ha⁻¹. Treatment at the rate of 0 mg·L⁻¹ was water only. Two untreated buffer rows separated each treatment row in a randomized block design.

Fruit tissue sampling. Fruitlet collection for peel tissue sampling began 1 week after the fourth (final) GA₄₊₇ application. In 2007, the sampling dates were 8 June, 18 June, 17 July, and 17 Aug. In 2008, the sampling dates were 12 June, 16 July, 13 Aug., and 10 Sept. Four fruit of similar size on the east side of each of four trees within each treatment row were removed and handled only by the stem and calyx end so as not to disturb the epidermal tissue. During the first two sampling dates, whole fruit were too small to be held securely in foam-pocketed trays; thus, fiber trays were modified as follows. To hold the apple securely on the tray and keep it from rolling around and disturbing the surface, a small hole ≈5 mm in diameter was punched through the bottom of each cup on the fiber tray. The stem of the apple was pushed through the hole and clamped on the opposite side with a small binder clip to secure it snugly against the fiber tray. In July and August, fruit were large enough to fit snugly in foam cells with the interior side of the fruit (side facing the vertical axis of the tree trunk) facing up. Fruit fitted in foam cells were placed in fiber cartons with lids and transported to the laboratory.

Fruit sampling for russet evaluations. At harvest, ≈3–5 d after the last tissue sample

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date, 10 apples of approximately the same size were removed from 10 trees from each treated row. Fruit were transported to the laboratory and kept at 1.1 °C until further examination. Each apple was rated for russet severity (percent of fruit surface) by location (stem end or calyx end) within 10 d of harvest.

2007: sample preparation for light microscopy. In the laboratory, the diameter of each apple was measured across the widest part of the shoulder. Two fruit per tree closest to the mean fruit diameter within each treatment replication were selected for epidermal tissue excision and processing ($n = 24$). From each apple, a longitudinal slice 5–8 mm wide was cut from the middle of each whole fruit roughly perpendicular to the axis of greatest sun exposure. A single epidermal section 8 mm long and 2 mm thick was excised from opposite sides of each slice at the widest diameter. Both sections were placed into a single plastic vial containing 5 mL of an enzyme solution containing pectinases [Enzyme Commission (EC) 3.2.1.15], cellulases (EC 3.2.1.4), and pectin lyases (EC 4.2.2.10) (Sigma-Aldrich, St. Louis, MO) formulated according to the method of Ju and Bramlage (1999) to remove cellular material. This enzyme solution was kept at 23 °C and changed weekly for 3 weeks. After enzymatic removal of extracellular debris, isolated cuticles were kept at 23 °C in distilled water containing 0.01% sodium azide (NaN_3) as an aid in controlling microbial growth (Lichstein and Soule, 1943).

To prepare for imaging, isolated cuticles were rinsed for several minutes in clean distilled water. Each cuticle was then mounted in water on a glass slide with a glass coverslip and examined using a stereomicroscope (Model SZX12; Olympus Corp., Tokyo, Japan) fitted with a CCD digital camera (Model CoolSNAP *cf*; Photometrics, Tuscon, AZ). The ocular reticle (100 divisions) was calibrated with a 10-mm stage micrometer (pitch 0.1 mm) at the magnification used for image capture. After selecting a representative area, a brightfield image was recorded for further image processing. All cuticle sections were imaged using the same magnification.

Images were processed using Image-Pro Plus (Version 4.5; Media Cybernetics, Inc., Bethesda, MD). The 24-bit TIFF color images were separated into individual eight-bit RGB (red, green, blue) channels and the green channel selected for measurements. Gray-scale images were printed and quadrants drawn on each print. To minimize observer bias, the top right quadrant of each printed image was always used for counting individual cells. Two observers counted the number of individual cells within the chosen quadrant by placing a red dot on each counted whole cell and a green dot on each partial cell using a felt tip marker.

2008: sample preparation for scanning electron microscopy. Additional tissue samples were taken every 7–10 d, beginning at petal fall, using fruit from untreated trees. After the last treatment application, samples were taken every 3–4 weeks on the dates

indicated previously. Samples were processed similarly to those of the previous year with several modifications. For fruit 15 mm or less in diameter, the fruit was cut equatorially into three sections of equal (visually) thickness. The middle third of the fruitlet was quartered longitudinally. Individual quarter sections were then processed as described subsequently. From fruit greater than 15 mm in diameter, a longitudinal slice 5–8 mm wide was cut from the center of each whole fruit, roughly perpendicular to the axis of greatest sun exposure. A single epidermal section 8 mm long and 2 mm thick was excised from each (opposite) side of each slice at the widest diameter. One section was set aside momentarily for further processing in preparation for examination using SEM, and the other section was placed into a single plastic vial containing 5 mL of the enzyme mixture as described previously. Vials containing apple tissue sections in enzyme solution were shaken gently using an environmental shaker kept at 38 °C. After each week in enzyme solution, samples in vials were sonicated for 10 min in a water bath kept at 38 °C before changing the solution. This was repeated until the cuticle sections were clear or there was no discernible particulate matter in the solution after sonication. Isolated cuticles were rinsed for several minutes in clean distilled water before air drying on a clean glass slide with a glass coverslip to prevent excessive curling.

Evaluation of fruit cuticles using scanning electron microscopy. Surface wax morphology was examined from the remaining section of peel tissue excised previously according to the method of Curry (2005) with the following modifications. From the center of each piece of peel tissue, a section of cuticle ≈ 4 –5 mm in diameter and 0.2 mm thick was shaved by hand using a 0.012-mm thick double-edge stainless steel razor previously rinsed with acetone and air-dried to remove any residual oil. The shaved cuticle sections were fixed to a 24-mm aluminum stub using double-sided carbon tape by pressing the edges of the section onto the tape using a pair of fine-tipped tweezers under a stereomicroscope. The stub was placed in a small glass vacuum desiccator containing packaged silica gel and kept at 20 °C and 1.3×10^4 Pa for 48 h or until further treatment. The time between first fruit incision and placing tissue under low vacuum in the glass desiccator was less than 2 min.

Before SEM evaluation, mounted tissue was coated with a gold/palladium alloy using a Desk II cold sputter coater (Denton Vacuum Inc., Morristown, NJ) fitted with a tilting omni-rotating head. With the sample 47 mm from the gold/palladium target, a coating thickness of ≈ 20 nm was achieved after 70 s at 40 mA and 2.6 Pa. Coated samples were kept in a vacuum desiccator and held under low vacuum at 1.3×10^4 Pa and 20 °C until microscopically examined using a Tescan Vega-II Model 5136LM Scanning Electron Microscope (Tescan, s.r.o., Brno, Czech Republic) equipped with both secondary and back-scattered electron detectors. Unless otherwise noted, images were obtained at 10 kV and 7.4×10^{-3} Pa.

Similarly, isolated, air-dried cuticles from the enzymatic treatment were fixed onto the aluminum stub with the cortical side facing up. Tissue was then processed for SEM examination as previously described.

Epidermal cells were counted by printing the gray-scale SEM image and tagging individual cells with colored markers as previously described. Individual cell measurements were made using the SEM software (Vega) by outlining the cell “pocket” just inside the raised portion (cell imprint) of the cuticle with the drawing tool. The program calculated the perimeter and planar area of each epidermal cell imprint. At least 20 cells per image were measured from three areas of each isolated cuticle and the means and errors recorded. Analysis of variance was performed and graphs generated using Systat statistical software (Version 12.0; Systat Software, Inc., Chicago, IL). Error bars within each graph represent $+ \text{ or } \pm \text{ SEM (SE)}$.

Results and Discussion

Fruit russetting. In 2007, fruit russetting in the section of orchard used for this study was insufficient to establish treatment differences (data not shown). Because russetting across the region was less, this effect was likely related to climate. Nevertheless, epidermal cell counts were recorded so that planar cell density could be calculated and compared. In 2008, treatment differences in russet reduction were apparent. Most of the russetting occurred on the distal half of the fruit (calyx end) with 77%, 31%, and 13% of the fruit having more than 20% russeted peel for GA_{4+7} treatments concentrations of 0, 15, and 30 $\text{mg}\cdot\text{L}^{-1}$, respectively. At the highest dosage concentration of applied GA_{4+7} , 74% of apples had no russetting.

Epidermal cell density by transillumination microscopy, 2007. A gray-scale image of the green channel (RGB) from a representative brightfield image similar to those from which planar epidermal cell density was determined for the three treatments in 2007 is shown in Figure 1. After examining all three channels, the green channel was chosen because of its slightly improved clarity (data not shown). The white dotted lines indicate the quadrant from which cells were counted. Obviously, the lines transected many cells. Therefore, transected cells were counted as half and the number of half cells was divided by two and added to the number of whole cells in the quadrant. Subsequent analysis indicated the planar cell density (number of cells per mm^2) determined by this “half-cell” method was within 14% of the planar cell density determined by counting only the number of whole cells within the same approximate quadrant and then determining the exact area encompassing the cells to use as the divisor for density calculations (data not shown).

Planar cell densities for ‘Golden Delicious’ epidermal tissue 1 week after the fourth GA_{4+7} treatment are shown in Figure 2. Analysis of variance indicated no difference between observers. Only results from Observer B indicated

Fig. 1. Gray-scale image of the green channel (RGB) from a representative brightfield TIFF image similar to those from which planar epidermal cell density was determined for gibberellin A_{4+7} treatments on 'Golden Delicious' apple in 2007. Dotted lines indicate quadrant from which number of cells was counted. Bar is 10 μ m.

Fig. 2. Graph of planar cell density vs. treatment application rate (two independent observers) from 'Golden Delicious' apple epidermal tissue sampled on 8 June 2007 and enzymatically treated to remove cell debris. Bars indicate + SE.

a difference among treatments; namely, the highest treatment rate resulted in the greatest epidermal planar cell density. It is likely there was insufficient definition in the images from which to gather accurate data verifying treatment differences, although the pattern of increasing cell density with increasing treatment rate was suggested by data from both observers. Indeed, this was the only sample date in 2007 from which data were recorded. Attempts to count cell number using cuticles isolated on subsequent sample dates failed because the individual cell definition using this method was poor (data not shown). This may have occurred as a result of 1) thickening cuticle + external amorphous wax layers attenuating and scattering the transmitted light; 2) ineffective or insufficient removal of cellular material; and/or 3) inability to identify newly forming cell imprints because of the thinness of the cutin outlining the new cells. That is, when epidermal cells divide, presumably each cell generates its own cutin pocket, and with this method of

Fig. 3. Scanning electron micrograph of the interior surface (flesh side) of a representative section of untreated 'Golden Delicious' apple fruit cuticle sampled on 12 June 2008 and enzymatically treated to remove cell debris. Example of individual cell area measurement outlines are in black. Arrow indicates developing cutin from recent cell division. Bar is 10 μ m.

Fig. 4. Graph of planar cell area and planar cell density vs. treatment application rate from 'Golden Delicious' apple epidermal tissue sampled on 12 June 2008 and enzymatically treated to remove cell debris. Scanning electron micrographs were used for measurements. Bars indicate + SE.

imaging, thickness of this pocket was insufficient to reduce transmitted light and generate the contrast necessary to identify newly forming cells. Nevertheless, the pattern of increasing number of smaller cells with increasing dosage of GA₄₊₇ was sufficient to warrant a second attempt with improved cuticle isolation methodology and enhanced imaging, thereby affording greater magnification and detail resolution. (The transillumination method together with improved enzymatic removal of cellular material would be a useful alternative in this type of work where a SEM is not available.)

Examination of cutin imprints by scanning electron microscopy, 2008. Cuticles isolated and cleared of cellular material using the improved enzymatic treatment protocol proved to be superior with regard to identifying individual epidermal cell imprints using SEM (data not shown). Figure 3 shows such a

Fig. 5. Graph of planar cell surface area vs. fruitlet diameter of untreated 'Golden Delicious' apple cuticles isolated during the first ~5 weeks of fruitlet development. Inset scanning electron micrographs indicate representative cutin imprints of epidermal cells from which measurements were made. Bars indicate $\pm$ SE.

representative cuticle section from untreated fruit together with the overlay of individual cell area measurement outlines. Using this technique, a graph of planar cell area and cell density was constructed for cuticles sampled on 18 June 2008, 1 week after the fourth application of GA₄₊₇ (Fig. 4). Differences among treatments are clear. Epidermal planar cell density increased by 14% and 27%, whereas epidermal planar cell area decreased by 18% and 31% for application rates of 15 mg·L⁻¹ and 30 mg·L⁻¹, respectively.

Not unexpectedly, there was greater variability in both planar cell area and planar cell density for the GA₄₊₇ application rate of 15 mg·L⁻¹ than for both higher and lower rates. Possibly, this is related to activation threshold. As the apple enlarges, 1) cuticle thickens and may reduce penetration of aqueous a.i.; and 2) number of epidermal cells beneath each microdroplet decreases, thereby reducing number of affected cells. This is made clearer by the data in Figure 5, which shows cutin imprints of epidermal cells during the first 30 d of fruitlet development. The area within the 100- μ m diameter circle (e.g., microdroplet) encompasses fewer, albeit larger, epidermal cells as fruitlet diameter increases. Thus, less a.i. may contact fewer cells. Whether a threshold exists, however, or whether a threshold changes with development is not known. Other factors that may influence efficacy during the first few weeks of fruit development are ambient conditions that alter drying time, the presence of other topically applied compounds, and increased leaf area (reduced spray penetration). In this particular orchard, canopy development at the FPF stage was quite different from 3 weeks later (E. Curry, personal observation).

Inconsistency of response to plant growth regulators, and particularly to GA₄₊₇, can be frustrating for growers and field staff. Previous work has shown the first or first two applications of GA₄₊₇ are often quite effective for reducing russet (Elfving and Allen, 1987), whereas applying more than four applications has no benefit (Meador and Taylor,

1987; Steenkamp and Westraad, 1988). Consider, for example, if instructions are to apply material beginning at PPF and every 7–10 d thereafter, the time to apply four treatments ranges from PPF + 21 d to PPF + 30 d. Indeed, given this flexibility, by PPF + 14 d, one might have applied one or two additional applications and by PPF + 21 d, two or three additional applications. Nine days' difference for all four applications to have been applied, at this stage of fruit development, may be quite significant.

Again, attempts to count the number of epidermal cells on cuticles from successive sampling dates by examining the cutin imprints became more difficult (data not shown). With each successive sample (monthly intervals), the cutin surrounding the cells became increasingly thicker and multilayered (Fig. 6). By 10 Sept., the enzymatically isolated cutin was at least three cell layers deep in places (Fig. 6D), which precluded accurate area measurements of the epidermal cell layer. Possibly, this multilayering is a response to arid conditions typical of those found in central Washington State and other hot, dry regions. Conditions of high humidity during the last month of fruit development tend to reduce cuticle thickness in such cultivars as Gala and Golden Delicious (E. Curry, personal observation). Thus, it is not surprising that fruit exposed to conditions of high

desiccation stress during the final month of development, which, in this location, is often the hottest month of the year, would tend to develop a epicuticular layer optimized to reduce water loss both pre- and postharvest.

This also may raise concern regarding effect of overhead cooling on cuticle thickness of certain cultivars and the resultant water loss during storage. It stands to reason that fruit grown in an arid environment should be the most well protected against water loss during fruit development and, therefore, during storage where subfreezing fan coil temperatures continually extract ambient water vapor from the room. In contrast, fruit developing under conditions of high humidity, precipitation, or prolonged overhead cooling could develop a thinner cuticle less likely to protect adequately against fruit water loss, especially if the fruit were harvested during or shortly after such moisture events. Preliminary observations in which portions of individual fruit peel were subjected to high humidity (greater than 95%) during the last 4 weeks of development suggest humid microenvironments of individual fruits (e.g., leaf-to-fruit or fruit-to-fruit contact areas) may modify thickness of the newly expanded cuticle matrix, thereby weakening its resistance to desiccation stress (E. Curry, personal observations). Fruit growth rate, ambient humidity, and temperature during this

period would likely contribute to the magnitude of the effects.

Gibberellins have been shown to increase cell division in different species depending on cell location and stage of development (Bangerth and Schröder, 1994; Bradley and Crane, 1957; Sachs and Lang, 1957; Wareing, 1958). Histological study on Japanese pear revealed that parthenocarpic fruits induced by GA₄ and GA₇ had increased cell numbers, but smaller cell size, relative to pollinated fruits (Tsujikawa et al., 1990). As Knoche et al. (2011) suggested, an increase in the planar cell density implies a structurally stronger cuticle. This is especially true if the cuticle surrounding smaller cells is similar to that of their larger counterparts. That is, if treatment with GA₄₊₇ induces cells with smaller planar surface area while maintaining the cuticle membrane at or above thickness levels surrounding untreated cells, it stands to reason that planar surface desiccation stress per cell would also be less. Gibberellins have been reported to increase thickness of cuticle membranes isolated from rice (*Oryza sativa* L.) internodes (Hoffmann-Benning and Kende 1994) as well as overall weight of cuticles isolated from pea (*Pisum sativum*) stems (Bowen and Walton 1988) and developing tomato (*Lycopersicon esculentum* L.) fruit (Knoche and Peschel, 2007). More recently, Knoche et al. (2011) reported no effect of GA₄₊₇ on weight of isolated cuticles in 'Golden Delicious'; however, cutin is not confined to the epidermal cell surface alone; rather, it may develop around hypodermal cell layers as fruit enlarge (Fig. 6). Thus, weight of isolated cuticles may not be the best variable with which to measure the effect of GA₄₊₇ on epidermal cuticle thickness in apples.

In pomological science texts, it is often assumed that the duration of cell division in apple fruitlets is 4 to 5 weeks after anthesis, after which fruit growth is primarily a function of cell enlargement (Westwood, 1995). My contention is the period at which "cell division ceases" identifies the developmental stage at which the majority of cortex cells simply enlarge and the epidermal planar cell area is maintained (within certain limits). That is, the planar cell area and, therefore, the epicuticular thickness are optimized according to ambient conditions. Obviously, epidermal cells do not simply enlarge after 5 weeks of fruitlet development. Were this true, epidermal cell planar area when the fruit is 80 mm in diameter would be 16 times that when the fruit was 20 mm in diameter, which, according to the calculations in Figure 5, would be $\approx 3200 \mu\text{m}^2$, or roughly equal to a circle with a diameter of 64 μm . Rather, it appears the cutin polymer, so protective of the epidermal cell, begins to develop around the second, third, and fourth layers of cells (hypodermis), as desiccation stress dictates. Although it is difficult to measure epidermal cell planar area through this tissue at the end of the growing season because of the obstructing cutin multi-layers, the plates in Figure 6 suggest this is the case. Whether the smaller cell size induced by the GA₄₊₇ treatments is maintained through fruit enlargement is not known.

Fig. 6. Scanning electron micrographs of the interior surface (flesh side) of representative sections of untreated 'Golden Delicious' apple fruit cuticle sampled on 12 June (A), 16 July (B), 13 Aug. (C), and 10 Sept. (D) and enzymatically treated to remove cell debris. Note thickening and multilayered cutin on successive sample dates. Bar is 20 μm .

Fig. 7. Effect of four treatment applications of gibberellin A₄₊₇ at 30 mg·L⁻¹ (B, D, F) vs. water only (A, C, E) on 'Golden Delicious' apple calyx-end russet (A–B), cuticular microcracks (C–D), and epidermal cell size (E–F) in 2008. Images of whole fruit calyx end russeting (A–B) were from fruit sampled on 10 Sept. Scanning electron micrographs of epicuticular microcracking (C–D) and epidermal cell cutin imprints (E–F) were from fruit sampled on 12 June. Dashed lines in E and F are yellow enhancements of grayscale overlays representative of individual measured epidermal cell planar areas.

With smaller epidermal cells, one would also expect microcracks to be thinner, shorter in length, and perhaps less invasive as suggested by the images in Figure 7C–D assuming that microcracking is mainly a function of epidermal cell division and/or sub-epidermal cell enlargement.

Generally, environmentally based russet development is either a direct response of the fruit epidermal cell to desiccation or an indirect response to excessive moisture. Response to desiccation may occur by: 1) physical damage resulting in exposure of cells to air with less than 100% humidity; 2) injury of epidermal cells resulting in arrested cuticle development; or 3) disruption of the protective wax layer resulting in a less than optimized water vapor barrier for given conditions. Excessive moisture, in contrast, may elicit russeting by one of two mechanisms. Standing water may increase imbibition and induce enlargement of subcuticular cells, thereby increasing cuticle microcracking against which the cell must protect.

Alternatively, excessive moisture may, temporarily, deacclimate epidermal tissue. For example, when a fruitlet develops in a cool, moist environment, the fruit surface may sense little desiccation stress and, therefore, develop a cuticle optimized for such conditions. If the environment quickly becomes hot and/or arid, the cuticle suddenly is inadequate to manage the loss of water vapor, thereby resulting in "emergency management" of water loss and development of additional suberin. Russeting within the stem bowl, as another example, may be a response of deacclimated peel tissue to a cycle of evaporating moisture and increasing aridity, a response to changing conditions rather than the conditions themselves. Although there are data suggesting apples enclosed in plastic bags (100% humidity) for the entirety of the growing season (Tukey, 1959) develop russet, similar preliminary experiments in arid environments using water-impermeable but breathable resin bags have not been so conclusive (E. Curry, personal observation).

Conclusions

Data from this 2-year study using fruit from an historically high-russetting 'Golden Delicious' orchard suggest GA₄₊₇ reduces environmental peel russetting by reducing epidermal cell size. Transillumination microscopy did not permit accurate measurement of 'Golden Delicious' fruit epidermal cell planar area beyond 6–8 weeks after anthesis because isolated cuticles became thicker and multilayered causing chromatic aberrations. On the other hand, evaluating epidermal cell planar measurements using SEM was clear and more accurate. Further work to examine the effect of localized, high-humidity microenvironments on cuticle thickness and composition as well as sub-epidermal cell organization may provide insight into postharvest disorder development and quality loss in storage.

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