Peel Injury on 'Gala' and 'Golden Delicious' Apples from Aqueous Hypochlorite Solutions

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ADDITIONAL INDEX WORDS. *Malus × domestica*, chemical, storage, cuticle, wax, lipids

SUMMARY. Experiments were conducted over several years to distinguish symptoms of sodium hypochlorite- or calcium hypochlorite-induced peel injury from other superficial maladies on 'Gala' and 'Golden Delicious' apples (*Malus domestica*), and to evaluate factors implicated in injury incidence and severity. 'Royal Gala' apples treated by dipping in freshly prepared aqueous sodium hypochlorite for 10 min showed moderate peel injury at the lowest treatment concentration of 150 mg·L⁻¹ when treated immediately after harvest, whereas no injury was observed on fruit treated at even the highest concentration of 2400 mg·L⁻¹ when fruit were kept at -1 °C for 3 months before treatment. At lower rates, rinsing fruit after treatment reduced injury on fruit dipped for 2 min, whereas after a 10-minute treatment, rinsing had no effect. Warm fruit (field heat) generally had a higher incidence of peel injury compared with fruit placed at -1 °C for 24 h before treatment. When 'Golden Delicious' apples were treated by dipping for 2 min in freshly prepared aqueous solutions of sodium hypochlorite or calcium hypochlorite at an equimolar hypochlorite ion concentration of 0.008 M (600 mg·L⁻¹ sodium hypochlorite), treatment temperature had a greater influence on incidence of peel injury with sodium hypochlorite than with calcium hypochlorite. Analysis of nonpolar solvent-extractable epicuticular waxes indicated differences due to treatment among several extracted compounds. Microscopic examination of injured peel tissue indicated altered appearance of wax platelets.

Sodium hypochlorite (NaOCl) is a common chemical used in raw and processed fruit production for disinfection (Dunstone et al., 2003), sanitation (Kreske et al., 2006; Podolak et al., 2009; Salomão et al., 2008; Stopforth et al., 2004), and reduction of pesticides (Hwang et al., 2001; Ong et al., 1996), mycotoxins (Hasan, 2000), and enzymatic processes (Fu et al., 2007; Wang et al., 2007). In fresh apple processing, it is most often used at harvest as a bin drench before the apples are placed in cold storage, during initial processing as bins are unloaded into dump tanks, and in presizing flume water during fruit segregation. Although there are several chlorinated compounds designated for such uses (U.S. Code of Federal Regulations, 2009; U.S. Food and Drug Administration, 2001), sodium hypochlorite is one of the least expensive. Rates of NaOCl

in dump tanks and flume water range from 20 to 200 mg·L⁻¹ and, although 10 min may be typical of the length of time an apple may be subjected to the treatment, times may vary from 2 to 40 min depending on logistics (E.A. Curry, unpublished data). Nevertheless, because the liquid form is quite easy to use, it may not be monitored as carefully as it should be. Thus, there may be potential for accidental overdosing.

During the course of examining numerous fruit maladies and symptom scenarios, the question often arises whether there is peel injury as a result of sodium hypochlorite treatment, especially on cultivars with little or no red pigmentation such as Royal Gala or Golden Delicious (E.A. Curry, unpublished data). In large part, these experiments were conducted to clarify the nature and appearance of peel injury induced by normal or excessive concentrations of aqueous hypochlorite salt solutions. A wide range of concentrations was used to explore the full array of symptoms and to avoid equivocation.

Materials and methods

Fruit for experiments conducted in 2005 and 2006 were from commercial orchards of mature 'Royal

Gala'/'Malling 106' ('M.106') trees grown near East Wenatchee, WA, and Orondo, WA, respectively. No related experiments were conducted in 2007. In 2008, fruit were harvested from mature 'Golden Delicious'/'M.106' trees grown commercially in Malaga, WA. All plantings were trained to a modified central leader, were uniform in vegetative vigor and cropping, similar in management strategy, and were irrigated with undertree impact sprinklers. In 2009, 'Golden Delicious'/'M.106' apples from trees growing near Orondo, WA, were treated in situ approximately 3 weeks before harvest. All orchards were located such that fruit could be transported to the laboratory within 20 min. For laboratory experiments, fruit selected were free from sunburn or other surface blemishes and were harvested when internal ethylene concentration (IEC) of 20 randomly harvested apples was 0.1 to 1.0 µL·L⁻¹. IEC was measured using standard methods (Curry, 2008).

No attempt was made to measure or monitor any of the aqueous molecular species generated from the chlorinated salts. By using distilled water to prepare solutions and by carefully adjusting initial pH, it was assumed the initial concentrations of hypochlorite ion (OCl⁻) and hypochlorous acid (HOCl) (both of which will hereinafter be referred to simply as "hypochlorite") were governed according to the acid ionization constant (Morris, 1966).

2005: 'ROYAL GALA' AND SODIUM HYPOCHLORITE: RATES, RINSING, AND PRESTORAGE VERSUS POSTSTORAGE TREATMENT. About 900 'Royal Gala' apples were harvested on 24 Aug. from a commercial orchard near East Wenatchee, WA, around 1500 HR, at which time the mean ambient temperature was 29 °C. Harvested fruit were gently placed in open plastic containers and transported to the laboratory where two-thirds were placed immediately in storage at -1 °C for later treatment (cold fruit). Remaining fruit were kept outside in the shade until further processing (warm fruit), during which time fruit cortex temperature at a depth of 5 mm did not fall below 26 °C. These fruit were dipped for 10 min in fresh aqueous solutions of sodium hypochlorite kept at

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4.5 ± 2.0 °C. All fruit were treated within 2 h of harvest. Hypochlorite solutions were made using liquid commercial bleach to final concentrations of 0, 300, 600, 1200, or 2400 mg·L⁻¹ and pH adjusted to 6.5 using 0.5 N HCl hydrochloric acid (HCl) (initial pH = 10.8). There were three replications of 20 fruit per treatment. Within each group of 20 fruit, 10 were rinsed in cool tap water for about 5 s after treatment, whereas the remaining 10 were not rinsed. All fruit were then allowed to dry on fiber trays, after which they were transferred to fresh trays and placed in closed cardboard cartons at -1 °C for 5 months. About 24 h after being placed in storage at -1 °C the previous day, a second set of fruit (cold fruit) was removed and treated as were warm fruit on the day of harvest. A third set of previously untreated cold fruit was removed from storage after 3 months at -1 °C, and treated similarly to those processed the day after harvest. All treated fruit were examined for peel injury 1 month after treatment and 5 months after harvest. Fruit were visually inspected and separate ratings were recorded for peel injury in the calyx bowl, stem bowl, and shoulder (the rest of the fruit surface). Injury was rated as 1 = none, 2 = slight but marketable, or 3 = brown and unmarketable. The number of fruit receiving a rating of 3 within any site was converted to percentage of injury per treatment replication.

2006: 'ROYAL GALA': FRUIT TEMPERATURE VERSUS DRENCH TEMPERATURE. About 800 'Royal Gala' apples were harvested on 4 Sept. around 1500 HR, at which time the mean ambient temperature was 35 °C. Harvested fruit were gently placed in open plastic bins and were transported to the laboratory. Half of the fruit was placed immediately in storage at -1 °C for later treatment (cold fruit). The rest were kept outside under shade such that fruit temperature at a cortex depth of 5 mm did not fall below 29 °C until further processing (warm fruit). This fruit was dipped, in groups of 20 apples, for 10 min in fresh aqueous solutions of sodium hypochlorite kept at 2 °C or 22 ± 2.0 °C within 2 h of harvest. There were three replications per treatment. Fresh sodium hypochlorite solutions were made using commercial bleach to concentrations of 0,

150, 300, or 600 mg·L⁻¹ and pH adjusted to 6.5 using 0.5 N HCl. Fruit were placed to dry on fiber trays, after which they were transferred to fresh trays and placed in closed cardboard cartons at -1 °C for 5 months. About 24 h after being placed in storage at -1 °C, a second set of apples (cold fruit) was removed and treated similarly to those treated immediately after harvest. All fruit were examined for peel injury 1 month after treatment and 5 months after harvest as described above.

2008: 'GOLDEN DELICIOUS': SODIUM HYPOCHLORITE VERSUS CALCIUM HYPOCHLORITE. About 960 'Golden Delicious' apples were harvested on the morning of 18 Sept. Ambient temperature was about 23 °C. Selected fruit were placed in open plastic containers and transported to the laboratory. One-fourth of the fruit was moved inside the building (23 °C) and the remainder was placed in storage at -1 °C for later treatment. In groups of 20, apples were dipped for 2 min in fresh aqueous solutions of sodium hypochlorite or calcium hypochlorite. There were three replications per treatment. Fresh sodium hypochlorite solution was made using commercial bleach to final concentrations of 600 mg·L⁻¹ (0.008 M); pH was adjusted to 6.5 using 0.1 N HCl. To keep the concentration of hypochlorite ion the same, calcium hypochlorite solution was made using 572 mg of calcium hypochlorite [Ca(OCl)₂ (Sigma-Aldrich, St. Louis)] per liter of water to a final hypochlorite ion concentration of 0.008 M; pH was adjusted to 6.5 using 0.1 N HCl. Similar to previous experiments, temperature of the treatment solution was 2 °C or 22 °C ± 2 °C. Unrinsed fruit were placed to dry on fiber trays, after which they were transferred to fresh trays and placed in closed cardboard cartons at -1 °C for 5 months. About 24 h after being placed in storage at -1 °C the previous day, another set equal to the first set was treated the same as those the previous day. After 10 d in storage, the entire experiment was repeated with the remaining fruit. Cold fruit were treated as described above and "warm" fruit were removed from -1 °C and kept at 22 °C for approximately 24 h before being treated as cold fruit the previous day. All fruit were examined for peel injury as described above after 2,

4, and 20 weeks at -1 °C. The amount of injured peel tissue was estimated and recorded independently by two people and the results were combined for analysis.

NONPOLAR ORGANIC SOLVENT-EXTRACTABLE LIPID EXTRACTION AND ANALYSIS. Fruit treated at harvest were rated for injury 1 month after treatment, after which two fruit from each treatment replication were selected for epicuticular wax analysis. From an area of the peel visually unaffected, a disk was excised using a 20-mm-diameter cork borer and the flesh was removed to a final thickness of about 1 mm. Each tissue disk was sliced in half with a clean razor blade and both halves were placed in a 16 × 125-mL glass test tube. Samples were extracted three times for 1 h, each with 10 mL of hexane (Mallinckrodt Baker, Phillipsburg, NJ). Extracts were transferred sequentially to a 16-mL collection vial, evaporated to dryness at 40 °C under a stream of nitrogen (N₂) using a Reacti-ThermTM (Thermo Fisher Scientific, Rockford, IL) manifold, and sealed with a polytetrafluoroethylene (PTFE)-lined screw cap. Evaporated samples were placed in a -80 °C freezer until analysis. Compounds extracted in this manner are hereinafter referred to as nonpolar organic solvent-extractable (NPOSE) lipids. Wax samples were analyzed underivatized by adding 1 mL of hexane that included an internal standard of

Fig. 1. Percentage of 'Royal Gala' apples showing peel injury in the calyx bowl, stem bowl, or shoulder (rest of the fruit surface) from a 10-min dip in sodium hypochlorite (NaOCl) at increasing concentrations immediately after harvest. Injury was evaluated after 1 month of storage at -1 °C (30.2 °F). Bars represent ±SE; 1 mg·L⁻¹ = 1 ppm.

heptadecane such that final concentration after addition of all reactants was 100 ng·μL⁻¹. Samples were capped and heated to 55 °C for 1 h to

resolubilize the wax. Once cooled to 23 °C, a 1-μL sample was analyzed using a gas chromatograph mass spectrometer (HP 5890; Agilent, Santa

Clara, CA) fitted with a 30-m capillary column (0.25 mm × 0.25 μm, ZB-5; Phenomenex, Torrance, CA) by means of split injection (1:50) at a temperature of 150 °C. The temperature program was 150 to 300 °C at a rate of 5 °C·min⁻¹ with a 12-min hold at 300 °C. Helium was used as carrier gas at a flow rate of 1 mL·min⁻¹. Several major peaks were identified by comparison of mass spectra with known standards and each identified peak was quantified by interpolation using a calibration curve corrected according to the internal standard. The amount of unknown peaks was estimated relative to nonacosane. Samples were analyzed twice and all measurements were used in deriving standard errors. Relative concentration of components was calculated by dividing the area of the integrated peak area by the total area of all integrated peaks.

Fig. 2. Peel injury on 'Royal Gala' (A and B) and 'Golden Delicious' (C and D) apples from a 10-min dip in 1200 mg·L⁻¹ (ppm) sodium hypochlorite immediately after harvest. Photographs were taken at 2 weeks (A and C) or 5 months (B and D) after storage at -1 °C (30.2 °F).

Fig. 3. Percentage of 'Royal Gala' apples showing peel injury in the calyx bowl, stem bowl, or shoulder (rest of the fruit surface) from a 10-min dip in 1200 mg·L⁻¹ (ppm) sodium hypochlorite immediately after harvest (0 months) or after 3 months at -1 °C (30.2 °F). Injury was evaluated on all fruit 1 month after treatment during which time they were kept at -1 °C. Bars represent ±SE.

Fig. 4. Percentage of 'Royal Gala' apples showing peel injury from a 10-min dip in sodium hypochlorite (NaOCl) at increasing concentrations immediately after harvest with or without (■) a 5-s rinse with distilled water. Injury was evaluated after 1 month of storage at -1 °C (30.2 °F). Bars represent ±SE; 1 mg·L⁻¹ = 1 ppm.

Fig. 5. Percentage of 'Royal Gala' apples showing peel injury after 1 month of storage at -1 °C from a 10-min dip in sodium hypochlorite (NaOCl) at increasing concentrations immediately after harvest. Warm fruit (26 °C) were treated the day of harvest in treatment solutions kept at 4.5 ± 2 °C, whereas cold fruit were placed at -1 °C 24 h before treatment. Bars represent ±SE; (1.8 × °C) + 32 = °F, 1 mg·L⁻¹ = 1 ppm.

2009: 'GOLDEN DELICIOUS' TREATED IN VIVO. At 0900 HR on the morning of 4 Aug., five fruit on each of six 'Golden Delicious'/'MM.106' trees were treated with freshly made aqueous solutions containing 0, 100, 200, 400, or 600 mg·L⁻¹ of sodium hypochlorite by enclosing the entire fruit within small plastic bags filled with treatment solution for 2 min. Air temperature was 24 °C and treatment solution temperature was 4 °C ± 1 °C. After 2 min, bags were removed and fruit remained untouched on the tree.

EFFECT OF HYPOCHLORITE ON EPICUTICULAR WAX MORPHOLOGY BY SCANNING ELECTRON MICROSCOPY (SEM). Fruit treated in vivo on 4 Aug. were harvested 7 d later and transported to the laboratory for examination and further analysis. Fruit were sampled according to the methods of Curry (2008) with the following modifications. Portions of

treated fruit peel with obvious hypochlorite injury ≈4 to 5 mm diameter and 0.2 mm thick were shaved from the fruit by hand using a 0.012-mm-thick double-edge stainless steel razor previously rinsed with acetone and air-dried to remove residual oil. The shaved cuticle section was fixed to a 24-mm aluminum stub using double-sided carbon tape by pressing the edges of the entire 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 10 °C and 13 kPa for 24 h or until further treatment. Before SEM evaluation, mounted tissue was coated with platinum using a Desk II cold sputter coater (Denton Vacuum, Morristown, NJ) fitted with a tilting omni-rotating head. With the sample 47 mm from the platinum target, a coating thickness of ≈20 nm was achieved after 75 s at 40 mA

and 2.6 Pa. Coated samples were kept in a vacuum desiccator and held under low vacuum at 13 kPa and 10 °C until microscopically examined using a SEM (Vega-II model 5136LM; Tescan, Brno, Czech Republic) equipped with secondary and back-scattered electron detectors. Unless otherwise noted, images were obtained at 10 kV and 0.0074 Pa.

DATA ANALYSIS. Statistics were performed using Systat 12 (Systat Software, San Jose, CA). Where appropriate, percentage of injury was transformed using arcsine transformation (Ahrens et al., 1990). Normally distributed data were subjected to analysis of variance and means were separated using Tukey's Studentized range test (HSD). Data not normally distributed were subjected to the Kruskal-Wallis test. Differences among treatment means were assessed at $P \leq 0.05$. Unless otherwise noted, error bars on graphs represent ±SE.

Fig. 6. Percentage of 'Royal Gala' apples showing peel injury after 1 month of storage at -1 °C from a 10-min dip in sodium hypochlorite (NaOCl) immediately after harvest. Warm fruit (29 °C) were treated the day of harvest in treatment solutions kept at 2 °C or 22 ± 2 °C. Cold fruit were placed at -1 °C 24 h before treatment at 2 °C or 22 ± 2 °C. Bars represent ±SE; (1.8 × °C) + 32 = °F, 1 mg·L⁻¹ = 1 ppm.

Fig. 7. Percentage of 'Golden Delicious' apples showing peel injury after 5 months of storage at -1 °C from a 2-min dip in water (H₂O), sodium hypochlorite (NaOCl), or calcium hypochlorite [Ca(OCl)₂] at 0.008 M hypochlorite ion concentration immediately after harvest. Warm fruit (29 °C) were treated the day of harvest in treatment solutions kept at 2 °C or 22 ± 2 °C. Cold fruit were placed at -1 °C 24 h before treatment also at 2 or 22 ± 2 °C. Bars represent ±SE; (1.8 × °C) + 32 = °F.

Results and discussion

‘ROYAL GALA’ AND SODIUM HYPOCHLORITE: RATES, RINSING, AND PRESTORAGE VERSUS POSTSTORAGE TREATMENT. When dipped in fresh aqueous solutions of NaOCl for 10 min, fruit treated immediately after harvest showed signs of injury at or above the lowest treatment dosage of 300 mg·L⁻¹ (Fig. 1). Injury appeared first, and generally was most severe, in and/or around the calyx bowl (Fig. 2). Injury was evident at the time of the first examination, namely, 1 month after treatment, but subsequent experiments indicated that the time of symptom expression was somewhat concentration dependent. With a 10-min exposure and treatment concentrations ≥400 mg·L⁻¹, injury was expressed within 48 h, whereas injury from lower concentrations often took as long as 3 weeks to express fully (data not shown). Fruit that was stored at -1 °C for 3 months before imposing treatment showed almost no injury whatsoever, even at the highest rate of 2400 mg·L⁻¹ (Fig. 3). This suggests that the natural epicuticular wax production by epidermal cells continued to such a degree sufficient to protect underlying cells, as demonstrated previously (Belding et al., 1998; Curry, 2008; Veraverbeke et al., 2001). The time interval between hypochlorite-induced peel injury and absence of injury due to continued epicuticular wax biosynthesis would likely depend on a number of factors such as cultivar, growth rate and growing conditions, fruit maturity at harvest, postharvest treatment, storage conditions, and processing methods. This list is similar in many respects to that enumerated by Michailides and Manganaris (2009) regarding factors during harvest that affect postharvest decay.

Data also suggest that rinsing fruit may reduce peel injury if the concentration of hypochlorite in the treatment is sufficiently low (Fig. 4) or the time of contact with the solution is reduced (data not shown). In these trials, unrinsed fruit placed with the stem/calyx axis horizontal dried within about 25 to 35 min at 23 °C and relative humidity of about 30%; however, often this is not the case. Where there is high humidity, such as the center of the drenched or refilled bin where fruit are sequestered from

voluminous air movement and there are many fruit to fruit contact droplets, drying may indeed take hours and, therefore, injury may result from prolonged exposure to slowly increasing concentration of hypochlorite in the shrinking droplet. Warm fruit (retained field heat) treated within 2 h of harvest in cold water showed the greatest amount of injury, whereas fruit kept at -1 °C for 24 h before treatment showed little or no injury (Fig. 5). Because these results were so dramatic, the experiment was expanded the following year.

In 2006, fruit from a different orchard was harvested at about the same physiological stage according to IEC; however, the maximum daytime temperature in 2006 was about 6 °C higher than during harvest 2005. At the lowest rate used in this trial, 150 mg·L⁻¹, the greatest percentage of fruit with peel injury occurred when warm (22 °C) fruit was treated in cold (2 °C) water (Fig. 6). Moreover, when cold water was used for the treatment, warm fruit consistently showed more injury, regardless of rate. When warm water was used for treatment, however, results were inconsistent at the low rates of 150 and 300 mg·L⁻¹ and indistinguishable at rates higher than 300 mg·L⁻¹. Generally, warm fruit treated in cold water showed about 30% to 40% greater incidence of hypochlorite-induced peel injury than when warm water was used.

With regards to water movement across the peel barrier, differences between fruit temperature and treatment

temperature have been investigated previously using water inoculated with pathogens such as *Mucor piriformis* fungal spores (Combrink and Grobbelaar, 1984) or the bacterium *Escherichia coli* O157:H7 (Buchanan et al., 1999). When warm apples were submerged in *E. coli*-contaminated cold water, such as might occur in apple processing lines where dump tank or flume water is not hygienically maintained, the bacterium was occasionally internalized—most likely

Fig. 8. Nonpolar organic solvent-extractable (NPOSE) lipids from ‘Golden Delicious’ apples 1 month after treatment from a 2-min dip in water (H₂O), sodium hypochlorite (NaOCl), or calcium hypochlorite [Ca(OCl)₂] at 0.008 M hypochlorite ion concentration. Fruit was kept in storage at -1 °C (30.2 °F) until tissue samples were analyzed. Main peaks identified are nonacosane (A) and nonacosanol (B). Bars represent +SE.

Fig. 9. Fruit injury near the calyx end of a ‘Golden Delicious’ apple, sampled 3 d after treating with 100 mg·L⁻¹ (ppm) sodium hypochlorite for 10 min 3 weeks before harvest. A portion of injured peel was excised and examined using the backscattered ion detector of a scanning electron microscope to show residue. The center point and area within the white dotted line and the inset are about the same. The black dotted rectangle on the inset refers to Fig. 10.

through open channels leading from the calyx end into the core region. In another study, Zhuang et al. (1995) found whole fresh tomatoes (*Solanum lycopersicum*) took up greater numbers of cells of *Salmonella* species from an aqueous environment when placed in water that was 15 °C cooler than the fruit. In earlier reports, Showalter (1979) and Bartz and Showalter (1981) found that when tomatoes were dipped in water that was colder than the fruit, creating a negative temperature differential, tomatoes gained up to 4% of the fruit weight in water from the surrounding liquid, most of which was taken up in or around the vascular area beneath the stem scar. In addition to the temperature differential, Bartz (1982) also showed that the amount of water uptake by tomato from an aqueous environment is, in part, dependent on the depth of submersion of the fruit. In apple processing lines, hydrostatic pressure might play a significant role, such as at the bottom of a dump tank 2 m deep, for example, or in vacuum-mediated bin filling systems. In this study, only superficial injury was investigated. Others have shown that treating with hypochlorite may also result in changes in poststorage fruit quality (Heun Hong and Gross, 1998).

‘GOLDEN DELICIOUS’: NaOCl VERSUS $\text{Ca}(\text{OCl})_2$: TREATMENT EFFECTS ON EPICUTICULAR WAX. Although the pattern of peel injury with fruit versus treatment temperature does not appear as simple as that for ‘Royal Gala’ in the previous experiments, the data suggest that ‘Golden Delicious’ apples treated within 24 h of harvest with 0.008 M hypochlorite ion solution generally showed a greater incidence of peel injury than those kept at -1 °C for 10 d before treatment; cold fruit generally showed more injury than warm fruit; peel injury from 0.008 M hypochlorite ion from $\text{Ca}(\text{OCl})_2$ was consistently between 20% and 30%, regardless of fruit of treatment temperature, whereas when the same hypochlorite ion concentration was formulated with NaOCl, temperature had a profound effect, resulting in peel injury from 2% to 40%; and cold treatment solution resulted in more injury with $\text{Ca}(\text{OCl})_2$ than with NaOCl, whereas in warm treatment solution, the reverse was the case

(with one exception of warm fruit treated after 10 d with warm solution) (Fig. 7). These effects may be the result of one of the many factors listed previously, or may be particular to the nature of the ‘Golden Delicious’ fruit epidermal tissue.

Analysis of epicuticular wax from ‘Golden Delicious’ apples treated within 24 h of harvest with 0.008 M hypochlorite ion solution and kept for 1 month at -1 °C is shown in Fig. 8. Although there are many peaks in the complete chromatogram, only those peaks that comprise >1% of the

total NPOSE wax are shown. The predominant compounds at retention times of 29.5 min (A) and 34.0 min (B) are nonacosane and nonacosanol, respectively. Data suggest treatment has an effect on the extractable amount of nonacosane due, possibly, to a reduction in post-treatment biosynthesis. In contrast, standard errors among means at $P \leq 0.05$ indicate no difference due to treatment in the amount of extracted nonacosanol. However, at $P \leq 0.1$, the trend for amount of extracted nonacosanol is the reverse of that for nonacosane.

Fig. 10. Scanning electron micrograph (A) from the area marked by the black dotted line within the inset in Fig. 9. The white-dotted rectangular boxes in A refer to images B and C. Apple wax platelets from area outside (B) and inside (C) the residue line of demarcation; 1 µm = 1 micron.

Whether the amounts or biosyntheses of these two compounds are inversely related, and whether these trends will persist as time in storage increases or is maximal immediately after treatment is not known.

EFFECT OF HYPOCHLORITE ON EPICUTICULAR WAX MORPHOLOGY BY SEM. Figure 9 shows peel injury on a 'Golden Delicious' apple from a 10-min dip in 100 mg·L⁻¹ NaOCl while still on the tree about 3 weeks before harvest. In earlier work, aqueous sodium chloride (NaCl) at 2400 mg·L⁻¹ induced no cellular or wax platelet injury when examined with SEM compared with considerable tissue necrosis and aberration in platelet morphology with NaOCl at the same sodium ion concentration (data not shown). This experiment was conducted to demonstrate that even low concentrations of hypochlorite ion may affect living tissues. Whereas 100% of fruit treated in this manner were injured similarly with rates at or above 200 mg·L⁻¹, only 30% were injured at 100 mg·L⁻¹ (data not shown). The inset shows an SEM image of that portion of injured tissue within the white dashed circle. This image was generated using the back-scattered ion detector that, under certain conditions, often shows greater contrast for inorganic residue on organic surfaces. It is not difficult to imagine this injury occurring from a solution droplet drying on the fruit surface near the calyx after treatment (warm, dry, and sunny morning). The portion of the SEM image within the black dashed line is magnified in Fig. 10A. At a magnification of ×400, the line of demarcation of the drying residue can be ascertained, moving from the upper right hand corner toward the midsection near the bottom of the image (Fig. 10A). Area B, delineated by the white dashed line, is composed of wax platelets that appear normal and uncontaminated (Fig. 10B), as assessed by images from untreated fruit (data not shown). On the other hand, magnification of area C, just inside the residue line, shows wax platelets with a slightly different or somewhat "melted" appearance together with foreign crystalline structures (Fig. 10C). As shown previously, this foreign matter may be covered by wax platelets, should they continue to grow in spite of the apparent tissue damage beneath. Whether this leads to

dysfunctional "pockets" of epicuticular wax is not known.

Conclusions

Results from this series of experiments indicate the general nature and pattern of peel injury induced by fresh aqueous solutions of NaOCl or Ca(OCl)₂ on 'Royal Gala' and 'Golden Delicious' apples. Data suggest that hypochlorite ion concentration is directly related to incidence and severity. Time of treatment exposure also affects the amount of induced injury, as do temperature of the fruit, temperature of the treatment solution, interval between harvest and treatment, and, obviously, cultivar. Even NaOCl at a rate as low as 100 mg·L⁻¹ may induce injury on fruit at or slightly before harvest. Typically, injury occurs within the calyx bowl or on the shoulder close to the calyx, both of which areas (on these cultivars) have a thinner cuticle/wax layer. Where other areas of the fruit are damaged, the degree of cuticle microcracking may play a role in injury incidence.

Data from these experiments as well as others described in this article strongly suggest that temperature differential may be a key factor in peel barrier response and moisture flux capacitance. Also apparent is the importance of continued epidermal wax biosynthesis after harvest in protecting fruit from external stressors. It is possible that treatments that alter or delay fruit epidermal lipid biosynthesis directly or indirectly (Curry, 2008) may lead to increased injury, depending on the length of treatment effects and length of storage for recovery. Indeed, hypochlorite-induced injury may be a convenient method to assess epidermal wax recovery during regular cold or CA storage.

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