Antioxidant Metabolism and Gene Expression during “Stain” Development on ‘Fuji’ Apples during Cold Storage

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Abstract. A distinct type of postharvest skin browning on apple (*Malus domestica* Borkh.) fruit called “stain” is a frequent disorder in ‘Fuji’ grown under high light and elevated temperatures. Symptoms typically develop only on sun-exposed sections of the fruit regardless of the presence of sunburn symptoms, and sometimes only in the margins of this area. The role of different antioxidant systems in tissue exposed to different levels of sunlight and having different degrees of sun injury were investigated during cold storage [1 °C, >90% (relative humidity) RH]. Ascorbic acid (AsA) and glutathione (GSH) concentrations, AsA–GSH recycling enzyme activities and gene expression, and flavonoids and carotenoid concentrations were determined every 30 days. “Stain” incidence increased with sun exposure and sunburn level. Both shaded and exposed fruit peel without sunburn symptoms had the highest AsA content. The AsA–GSH recycling enzyme activities and gene expression levels had no clear relationship with sun exposure during cold storage. Chlorophyll a (chl a) and chlorophyll b (chl b) levels diminished over time and were higher in tissue without any type of sun injury. In contrast, carotenoid levels increased as sun injury incidence increased and remained relatively stable during storage. Total phenolics and quercetin glycoside levels changed coincidentally during storage. Results indicate that the AsA–GSH cycle does not have a clear role in “stain” development. Nevertheless, reduced ascorbate levels may reduce the capacity to prevent oxidative stress–provoked damage which may, in turn, result in oxidation of quercetin glycosides, which would then lead to skin browning.

Sun-related physiological disorders have a substantial annual economic impact on the worldwide apple (*M. domestica*) industry, particularly when grown in semiarid climates where losses can be from 10% to 50% of total production (Racsko and Schrader, 2012; Yuri et al., 2000b). Climatic conditions in these regions can elevate photooxidative and heat stress throughout the growing season affecting the tree and

fruit physiology. Skin browning or “stain” can develop on ‘Fuji’ apples during cold storage (Mattheis, 1996; Schrader et al., 2008). “Stain” symptoms are typically browning or discoloration that begin to appear 1–2 months after harvest (Schrader et al., 2008) on the periphery of sunburned or sun-exposed areas of the peel (Felicetti and Schrader, 2010; Kupferman, 1994). Symptoms are very superficial and only occur in the first epidermal cell layer of the peel. To date, there are no known postharvest crop protectant or storage regimes that reduce this problem. “Stain” is not prevented by postharvest diphenylamine or AsA (0.2%) treatments at harvest (Kupferman, 1994).

As with sunscald of ‘Granny Smith’ apples (Contreras et al., 2008; Hernandez et al., 2014; Lurie et al., 1991), “stain” development is associated with sun exposure in the orchard as well as sunburn incidence and severity (Fan and Mattheis, 1998; Felicetti and Schrader, 2010; Schrader et al., 2003, 2008). Fuji apples are highly susceptible to “stain,” although it can occur on other cultivars including Royal Gala and Honeycrisp apples. Limiting sunlight exposure on ‘Fuji’ apples using bags prevents “stain” development (Fan and Mattheis, 1998). Raynox® is an orchard-applied carnauba-based coating that blocks ultraviolet-B and, consequently, reduces sunburn and “stain” incidence (Schrader et al., 2008).

Sun injury or sunburn of fleshy fruit is caused by absorption of excess solar energy by exposed tissue, leading to photoinhibition and oxidative stress (Ma and Cheng, 2003; 2004; Torres et al., 2006). Defense mechanisms activated in tissue exposed to direct sunlight or during early stages of sunburn include antioxidants (i.e., AsA, GSH) and enzymes functioning as antioxidants or regeneration of antioxidants such as, ascorbate peroxidase (APX; EC 1.11.1.11), dehydroascorbate reductase (DHAR; EC 1.8.5.1), monodehydroascorbate reductase (MDHAR; EC 1.6.5.4), glutathione reductase (GR; EC 1.6.4.2), catalase (CAT; EC 1.11.1.6), and superoxide dismutase (EC 1.15.1.1) (Chen et al., 2008; Ma and Cheng, 2003, 2004). However, activity of these recycling enzymes is not always higher in sun-damaged apple peel (Zhang et al., 2014).

Levels of certain pigments, including chlorophylls and carotenoids, decrease as sun-injury symptoms increase, whereas others, including xanthophylls, increase with light stress, possibly as a photoprotective mechanism (Chen et al., 2008; Ma and Cheng, 2003; Tartachnyk et al., 2012; Torres et al., 2006; Wünsche et al., 2001). Phenolic compounds rapidly accumulate on sun-exposed peel in response to direct sunlight (Felicetti and Schrader, 2008; Yuri et al., 2010). Phenolic compounds also appear to be directly involved in sunscald development in ‘Granny Smith’ apples during cold storage (Hernandez et al., 2014). The antioxidative capacity and regeneration of ascorbate by the AsA–GSH pathway ostensibly has an indirect role in sunscald development by improving defense against postharvest oxidative stresses (Hernandez et al., 2014). During this process, quercetin oxidation and accumulation of its brown products may be responsible for the symptoms (Jimenez and Garcia-Carmona, 1999). Flavonols, including quercetin can be substrates for polyphenol oxidase from a variety of plant species (Gasic et al., 2004; Jimenez and Garcia-Carmona, 1999) indicating that this defense-related process may, likewise, have a similar function in apple in this instance. We hypothesize that, while mechanisms resulting in sunscald may be similar, they can culminate in different symptoms on ‘Fuji’ peel. Accordingly, our objective was to evaluate

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antioxidant metabolism to compare “stain” and sunscald development.

Materials and Methods

Plant material and fruit sampling. ‘Fuji’ apples (*M. domestica*) were harvested in 2011 from a commercial orchard grafted on a seedling rootstock, planted in 1990 (San Clemente, Maule, Chile).

At harvest, fruit were divided into four different categories according to the severity of sun damage. These were 1) Shaded—fruit nonexposed to direct sunlight, usually located in the internal part of the tree; 2) Exposed—fruit exposed to direct sunlight without sun-injury symptoms, usually located in the periphery of the tree; 3) Mild—fruit with slight discoloration on the sun-exposed skin; 4) Moderate-Severe (Mod-Sev)—presence of yellowing and browning on the fruit surface, with or without dark brown patches (Fig. 1).

Fruit were stored in air (0 °C, >90% RH) for up to 4 months. Every 30 d, peel (up to 4 mm thick) from the damaged area in each fruit included in replicates from each sun-injury category (five replicates of five fruit each) was sampled using a scalpel, and immediately frozen in liquid nitrogen. Frozen peel was ground using a mortar and pestle and stored at −80 °C until analysis. “Stain” incidence from all of the sun-injury categories was evaluated every 15 d during the

storage period (120 d). This was carried out on three replicates of 100 fruit per category.

Antioxidant metabolites. Ascorbic acid (AsA) and total glutathione (GSH) determinations were carried out by extracting peel tissue (five replicates of five fruit each) with 1 M HClO₄ as described by Hernandez et al. (2014). In brief, supernatants were divided into two aliquots of 400 µL for AsA and GSH determinations, 200 µL of 0.1 M HEPES/KOH (pH 7.0) added, and pH adjusted with 6 M K₂CO₃ to pH 4.0–5.0 for AsA and 6.0–7.0 for GSH. Both metabolites were determined spectrophotometrically with a kinetic reaction at 265 nm for AsA and 412 nm for GSH according to Torres et al. (2006).

Antioxidant enzyme activities. For all enzyme activity assays, frozen peel tissue (five replicates of five fruit each) was extracted using MES/KOH buffer (pH 6.0) containing KCl, CaCl₂, and AsA as described by Hernandez et al. (2014). Catalytic activity for each enzyme was determined by monitoring the change in optical density (OD) using a spectrophotometer (Agilent Technologies, model 8453, Santa Clara, CA). APX activity was monitored at 290 nm, MDHAR at 340 nm, DHAR at 265 nm, CAT at 240 nm, and GR activity at 340 nm as described by Torres et al. (2006).

Expression analysis of antioxidant enzymes by real-time polymerase chain reaction. Total RNA was extracted from powdered frozen peel (4 g) and first-strand complementary DNA synthesis carried out as described by

Hernandez et al. (2014). Real-time polymerase chain reaction analyses were performed using sets of specific primers designed by Zubini et al. (2007) for antioxidant enzymes (APX, DHAR, MDHAR, and GR) and employed by Hernandez et al. (2014). Relative expression levels of target genes were calculated according to Pfaffl (2001), and were normalized against the expression of eukaryotic initiation factor-4A and elongation factor-1 (Zubini et al., 2007).

Pigment analysis. Total chlorophylls and carotenoids were extracted using 100% acetone in a 2:0.1 tissue ratio. Concentrations were estimated spectrophotometrically by measuring the OD at 470 nm, 645 nm, and 662 nm and calculating chl a, chl b, xanthophyll, and carotenoid concentrations using equations presented in Lichtenthaler and Wellburn (1983).

Using the same extract (40 µL), individual carotenoid and chlorophyll concentrations were determined using a high-performance liquid chromatography photodiode array (HPLC-PDA) system (model Smartline; Knauer, Berlin, Germany) as described by Hernandez et al. (2014). Quantification was carried out comparing peak areas with standard curves using authentic standards purchased from Merck (Darmstadt, Germany) and Extrasynthese (Lyon, France).

Phenolic compounds. Total phenolic content was estimated using the Folin–Ciocalteu reagent and ethanolic tissue extractions as described by Hernandez et al. (2014). Total phenolic concentration was expressed as equivalents of chlorogenic acid (CAE) per kg fresh weight (FW). Individual flavonoids were determined using an HPLC-PDA system (Knauer) as described by Hernandez et al. (2014). Quantification was carried out comparing peak areas from samples with standard curves using authentic standards (quercetin glycosides) purchased from Merck.

Statistical analysis. Analysis of variance and mean separation (Tukey’s honestly significant difference, $P < 0.05$) was carried out using Statistica v.7.0 (Tulsa, OK). Nonparametric analysis was performed using the

Fig. 1. Fruit nonexposed to direct sunlight [Shaded (1)], fruit exposed to direct sunlight without sun-injury symptoms [Exposed (2)], fruit with slight discoloration on the sun-exposed skin [Mild (3)], and presence of yellowing and browning on the fruit surface, with or without dark brown patches [Mod-Sev (4)]. Mod-Sev = Moderate-Severe.

Fig. 2. (A) “Stain” incidence (%) and (B) its probability during regular air storage (0 °C, >90% relative humidity) of ‘Fuji’.

Scheirer-Ray-Hare test. SAS logistic procedure (version 8.02; SAS Institute, Cary, NC) was used for logistic regressions.

Results

“Stain” incidence. Total “stain” incidence increased alongside initial sunburn severity (Fig. 2). It reached 10% after 120 d in cold storage on the Sev sunburn category, linearly increasing ($y = 1.0136x - 7.8425$, $R^2 = 0.966$) from <1% starting at 60 d. There was no “stain” on Exposed or Shaded fruit. By contrast, symptoms started to appear beginning at 45 d in Mod and Sev sunburned fruit, and 75 d in the Mild category (Fig. 2). Logistic regressions using sunburn categories at harvest to predict “stain” appearance postharvest are shown in Table 1.

Ascorbate–glutathione cycle. Total AsA was significantly higher in Exposed and Shaded peel compared with that of sun-injured (Mild, Mod-Sev) for most of the storage period (Fig. 3).

In general, AsA content decreased as storage duration increased in nonsunburned peel and remained low and relatively stable in sunburned tissue (Fig. 3). At harvest, healthy fruit had in average 950% more

ascorbate than the sun-injured ones. This difference almost disappeared by 120 d of storage (Fig. 3). Total glutathione (reduced + oxidized) was higher in healthy peel ($0.045 \text{ mmol}\cdot\text{kg}^{-1} \text{ FW}$, in average) compared with sun-injured tissue ($0.020 \text{ mmol}\cdot\text{kg}^{-1} \text{ FW}$, in average), and remained stable during most part of the storage period. There were no clear differences in any of the AsA–GSH recycling enzyme activities or transcription levels between sun exposure or sun-injury levels (Fig. 4A–J). Nevertheless, Shaded peel showed higher activity of DHAR, GR, and MDHAR between 0 and 30 d than sun-damaged tissue (Fig. 4B–D).

Chlorophyll and carotenoids. Chl a and Chl b levels decreased in peel from all exposure categories with storage duration (Fig. 5). Sunburned peel generally had lower chlorophyll contents than healthy fruit (Exposed and Shaded) (Fig. 5) with higher degradation rates (10 and $4 \text{ mg}\cdot\text{kg}^{-1} \text{ FW}$ per month for Chl a and Chl b, respectively) than sunburned fruit (5 and $1.3 \text{ mg}\cdot\text{kg}^{-1} \text{ FW}$ per month in average for Chl a and Chl b, respectively).

α - and β -carotene were significantly higher in Mod-Sev fruit tissue during most of the storage period (Fig. 5C and D), and reached higher Car:Chl ratios during the storage period. In general, the more the sunburn, the higher the overall carotenoid concentration. α -Carotene was 91% higher in sunburn peel, whereas β -carotene was 178% higher in the same tissue. Levels of both carotenoids remained relatively stable throughout storage for all sun exposure categories, with the exception of a sharp increase of β -carotene from 0 d to 30 d (Fig. 5C

and D). By contrast, lutein + zeaxanthin levels were similar for all sun exposure categories while it decreased over time (Fig. 5F).

Phenolics and quercetin glycosides. Total phenolics were significantly higher (115% in average) in Mod-Sev fruit tissue during most of the storage period while decreasing over time (Fig. 6). Levels decreased at a rate of 1.2 and $0.3 \text{ g CAE kg}^{-1} \text{ FW}$ per month in both sunburned and healthy tissue, respectively.

Consequently, all quercetin glycoside levels were elevated in peel with any sunburn compared with sun-exposed and shaded peel during storage (Fig. 7A–F). Amounts at harvest increased with sun exposure and sunburn severity (Fig. 7A–F). Quercetin 3-O-gal was the most abundant quercetin glycoside. It represented around 20% and 30% in healthy and sunburned tissue, respectively. Quercetin 3-O-rut and quercetin 3-O-glu represented 4% and 8% in healthy and 17% and 21% in sunburned tissue, respectively. The at-harvest levels of these three quercetin glycosides have the highest correlation with “stain.”

By contrast, healthy tissue had a higher proportion out of total quercetin glycosides of quercetin 3-O-ram (30%), quercetin 3-O-xil (19%), and quercetin 3-O-ara (16%) than sunburned tissue (13%, 10%, and 8%, respectively). A reduction of all quercetin glycosides was observed until 90 d of storage, especially in Mod-Sev fruit (Fig. 7). The highest reduction was observed in quercetin 3-O-gal for Mod-Sev peel ($574 \text{ mg}\cdot\text{kg}^{-1} \text{ FW}$ per month) compared with $188 \text{ mg}\cdot\text{kg}^{-1} \text{ FW}$ per month for Mild, $256 \text{ mg}\cdot\text{kg}^{-1} \text{ FW}$ per month for Exposed, and $67 \text{ mg}\cdot\text{kg}^{-1} \text{ FW}$ per month for Shaded peel.

Table 1. Logistic regression equations for “stain” incidence versus sun exposure category of ‘Fuji’ stored in regular air.

Condition	Regression equation
Mild	$f(x) = 0.2721 (\text{days}) - 9.3820$
Moderate	$f(x) = 0.2350 (\text{days}) - 7.1738$
Severe	$f(x) = 0.2385 (\text{days}) - 7.4172$

Fig. 3. Ascorbic acid (A) concentrations in ‘Fuji’ apple peel from different sun exposure categories (Shaded, Exposed, Mild, and Mod-Sev) during regular air storage. Different letters within the same storage time indicate values are different according to Tukey’s honestly significant difference ($P < 0.05$) test.

Discussion

Our results indicate that “stain” on ‘Fuji’ apples, as well as sunscald on ‘Granny Smith’ (Hernandez et al., 2014), develops only on sun-exposed and damaged sections of the fruit. Therefore, preharvest factors, such as tree vigor, nutrition, and seasonal climatic conditions that affect sunburn development while on the tree, directly influence “stain” development after harvest (Felicetti and Schrader, 2010; Schrader et al., 2003, 2004). In our study, “stain” incidence increased linearly as sunburn severity increased, supporting previous findings (Schrader et al., 2003). However, Schrader et al. (2003) reported 38% incidence after 4 months of air storage compared with 10% in our study for the same sunburn category (Sev). This may have resulted from genotype and environmental/seasonal differences between studies. Our results show that “stain” symptoms can appear as early as 6 weeks after harvest in Sev sunburned fruit (Fig. 2A). Unlike sunscald on ‘Granny Smith’ apples, “stain” symptoms did not appear on exposed fruit during storage, perhaps due to biochemical difference involving genotype differences as well as antioxidant levels (Ma and Cheng, 2003, 2004; Merzlyak et al., 2002b).

Fig. 4. (A–E) Antioxidant enzyme [ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), and catalase (CAT)] activities and (F–J) gene expression in ‘Fuji’ apple peel from different sun exposure categories (Shaded, Exposed, Mild, and Mod-Sev) during regular air storage. Different letters within the same storage time indicate values are different according to Tukey’s honestly significant difference ($P < 0.05$) test.

Logistic regression prediction models developed for “stain” incidence probability (Fig. 2) could be potentially used by fruit producers for making storage and marketing decisions. The same models have been successfully developed

for sunscald prediction on ‘Granny Smith’ apples (Hernandez et al., 2014), another high-light and heat stress-related postharvest disorder.

AsA content, a major polar antioxidant, was lower in sun-injured tissue and remained

relatively stable throughout storage (Fig. 3). The same dynamic was observed during sunscald development in ‘Granny Smith’ apples (Hernandez et al., 2014). This indicates that high solar environments lead to

Fig. 5. (A) Chlorophyll a, (B) chlorophyll b, (C) carotenoids:chlorophyll (Car:Chl) ratio, (D) total chlorophyll, (E) α-carotene, (F) β-carotene, (G) lutein + zeaxanthin, and (H) total carotenoids concentration of 'Fuji' apple peel from different sun exposure categories (Shaded, Exposed, Mild, and Mod-Sev) during regular air storage. Different letters within the same storage time indicate values are different according to Tukey's honestly significant difference ($P < 0.05$) test.

low levels of ascorbate in fruit tissues, possibly decreasing their capability to overcome oxidative stress during cold storage. Chen et al. (2008) found that sunburned peel had higher total AsA and GSH than non-sunburned peel. However, Zhang et al. (2014) recently reported that total ascorbate concentrations decrease in 'Fuji' apples in response to photooxidative stress in the field. Furthermore, the ascorbate de novo synthesis pathway appears to be fully functional during the first stages of sunburn development, but later, in severely damaged peel, L-galactose dehydrogenase and L-galactono-1,4-lactone dehydrogenase activities decrease (Zhang et al., 2014).

Unlike results published by Hernandez et al. (2014) in 'Granny Smith' apples, Exposed peel from 'Fuji' apples, except at harvest, had similar L-AsA levels than Shaded peel (Fig. 3). These authors, as well as

Davey et al. (2007), Ma and Cheng (2003), and Li et al. (2008) have found that apple peel exposed to high light have higher L-AsA content than shaded peel. In agreement with previous work by Felicetti and Mattheis (2010) and Hernandez et al. (2014), L-AsA decreased during storage in Exposed and Shaded peel most likely due to a lower capacity of apple tissue to synthesize AsA when detached from the tree (Felicetti and Mattheis, 2010).

Our results support those of Davey et al. (2007) who reported that GSH levels were lower in apple tissue acclimated to high light compared with shaded peel. By contrast, Ma and Cheng (2004) found higher GSH levels on sun-exposed sections, along with higher APX, MDHAR, DHAR, and GR activities, AsA-GSH recycling enzymes. In our study, these antioxidant enzymes (activities and transcription levels) did not vary between

sun exposure and sunburn levels (Fig. 4). Similar results have been found during sunscald development in 'Granny Smith' apples (Hernandez et al., 2014), indicating sun-injured tissue has limited capacity to overcome oxidative stress during low temperature storage.

In our study, Chl a was significantly higher in sunburned compared with healthy peel, although it remained relatively constant throughout storage, as it did during sunscald development on 'Granny Smith' apples (Hernandez et al., 2014), although both chl a and b concentrations in this cultivar were double those found in 'Fuji' apples (Fig. 5). This difference was previously reported (Felicetti and Schrader, 2009a).

In agreement with Merzlyak and Solovchenko (2002a) and Torres et al. (2006), α- and β carotene levels were higher in sunburned compared with healthy peel

Fig. 6. Total phenolic content of 'Fuji' apple peel from different sun exposure categories (Shaded, Exposed, Mild, and Mod-Sev) during regular air storage. Different letters within the same storage time indicate values are different according to Tukey's honestly significant difference ($P < 0.05$) test.

(Fig. 5). Possibly due to cultivar differences, this difference between both peel conditions was not observed in 'Granny Smith' (Hernandez et al., 2014). Moreover, sunburned 'Fuji' peel had 10 times more β -carotene than that reported for 'Granny Smith' (Hernandez et al., 2014). Differences between cultivars leading to different Car:Chl ratios undoubtedly play a role in tissue stability (photoprotection vs. photooxidation) during sunburn development and perhaps also during sunscald and "stain" development postharvest. In fact, the amount of fruit affected by sunscald in 'Granny Smith' apples (Hernandez et al., 2014) is significantly higher than that of fruit affected by "stain" in 'Fuji' apples (Fig. 2) grown under Chilean conditions. This may suggest that higher carotenoids on 'Fuji' peel provide protection against oxidative stress occurring during cold storage, which leads to less oxidative stress-related disorders such as "stain."

In agreement with Felicetti and Schrader (2008, 2009a) there were no differences in lutein + zeaxanthin concentration among sun exposure categories (Fig. 5). Levels of these xanthophylls were $\approx 50\%$ of those found in

Fig. 7. Quercetin glycoside [quercetin galactoside (Q-Gal), quercetin glucoside (Q-Glu), quercetin rutinoside (Q-Rut), quercetin ramnoside (Q-Ram), quercetin xylose (Q-Xyl), and quercetin arabinose (Q-Ara)] content of 'Fuji' apple peel from different sun exposure categories (Shaded, Exposed, Mild, and Mod-Sev) during regular air storage. Different letters within the same storage time indicate values are different according to Tukey's honestly significant difference ($P < 0.05$) test.

Table 2. Correlation coefficients of linear regressions of quercetin glycosides at harvest versus “stain” development probability during regular air storage.

Quercetin glycosides	Storage duration (days)			
	30	60	90	120
Q-Rut	0.89	0.93	0.85	0.76
Q-Gal	0.84	0.77	0.64	0.54
Q-Glu	0.87	0.92	0.85	0.77
Q-Xyl	0.70	0.58	0.45	0.36
Q-Ara	0.73	0.62	0.48	0.39
Q-Ram	0.75	0.69	0.57	0.48

‘Granny Smith’ apples (Hernandez et al., 2014), and they sharply decreased at the same rate in all sun exposure categories during storage.

As with ‘Granny Smith’ sunscald (Hernandez et al., 2014), quercetin glycoside concentrations were highly correlated with “stain” appearance probability during cold storage (Table 2). Although in our study quercetin 3-O-rut, quercetin 3-O-glu, and quercetin 3-O-gal were the most highly correlated, as well as in the highest proportion compared with other quercetin glycosides in sun-injured peel.

The latest evidence indicates that sunscald of ‘Granny Smith’ and well as “stain” in ‘Fuji’ apples, both peel browning disorders, may be derived from similar biochemical processes involving flavonoids, and in particular quercetin glycosides, which are major phenolics in this species. One of these processes could be quercetin oxidation by polyphenol oxidase, previously reported on in vitro studies (Jimenez and Garcia-Carmona, 1999). Differences of incidence and severity of both disorders may derive from differences of pigments (chlorophylls and carotenoids) and quercetins levels among cultivars (Felicetti and Schrader, 2009b; Van der Sluis et al., 2001). In ‘Fuji’ peel, total phenolics decreased 30% during the first 60 d in storage (Fig. 6), while only decreasing 12% in ‘Granny Smith’ peel (Hernandez et al., 2014). Moreover, the Mod-Sev category had seven times more total quercetin glycoside content than Shaded peel ‘Granny Smith’ (Hernandez et al., 2014) compared with only two times more in ‘Fuji’.

In general, “stain” appears less often than sunscald, later in storage (after 45 d in Sev sunburned fruit), in fewer fruit, and with less severity than sunscald (Fig. 2). The latter appears most every season, relatively early during storage, on sun-exposed and sunburned portions of the peel, early in storage, and at rates as high as in 100% by 3 months in storage (Hernandez et al., 2014).

Our results indicate that the AsA-GSH may play an indirect role in “stain” development on ‘Fuji’, as it does with sunscald of ‘Granny Smith’ (Hernandez et al., 2014). Low ascorbate levels at harvest on sunburned tissue, as well as low antioxidant enzymes activities, may hinder the protective role of this key antioxidant system during cold storage. This may, in turn, result in oxidation of quercetin glycosides, then, skin browning. Therefore, ‘Fuji’ “stain” and sunscald in ‘Granny Smith’ from prior reports, appear

to be related disorders metabolically although symptoms developed differently in these different apple genotypes.

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