APPLE (*MALUS DOMESTICA* BORKH.) FRUIT SKIN DISORDERS AND CHANGES IN PIGMENT CONCENTRATIONS ASSOCIATED WITH THE DISORDERS

By

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A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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May 2007
To the Faculty of Washington State University:

The members of the committee appointed to examine the dissertation of David Andrew Felicetti find it satisfactory and recommend that it be accepted.

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...
ACKNOWLEDGMENTS

First and foremost I would like to extend great thanks to Dr. Larry E. Schrader for his guidance and support and for allowing me to develop and explore ideas freely while not letting me stray too far. Many thanks are extended to Dr. Gerry E. Edwards, Dr. N. Richard Knowles, and Dr. John K. Fellman for their time spent as members of my Graduate Advisory Committee and for their invaluable insight and suggestions over the course of this research. The financial support of the Washington Tree Fruit Research Commission, the teaching assistantships provided by the Department of Horticulture and Landscape Architecture, and the graduate research assistantship provided by the Agricultural Research Center, without which this research could not have been completed, is gratefully acknowledged. I would also like to thank Dr. James P. Mattheis at the USDA/ARS Tree Fruit Research Laboratory in Wenatchee, Washington for not only providing advice on HPLC analyses but for providing the HPLC itself. Additionally, I would like to thank Dr. David R. Rudell and David Buchanan from the USDA/ARS Tree Fruit Research Laboratory for the many helpful conversations about analyses of various compounds in apple fruit and for helping with the occasional HPLC emergency.

I would also like to thank my family. The financial and emotional support provided by my parents, Linda and Carmen, throughout the years has been tremendous and I cannot thank them enough. I could not have made it this far without them. My life partner, Erin Beneski, has been invaluable with her professional criticism (and at times not so professional), emotional support, and overall tolerance of my mood swings. Finally, I would like to thank my sister, Laura, for encouraging me to apply to WSU and for the long walks through the stubble fields with muddy dogs.
VITA

I was born July 12, 1973 in DuBois, Pennsylvania just 20 miles from Brookville, Pennsylvania where I spent the first 18 years of my life. After graduating from Brookville Area High School in June of 1991 I deferred my matriculation into Hobart College (a small liberal arts college in central New York State) for one year and spent six month traveling in Australia. After my travels I began my undergraduate education at Hobart College, Geneva, NY in September of 1992, and in June of 1996 I graduated with my B.S. in Biology. I spent the next five years working at the Plant Genetics Research Unit, USDA, ARS located on the campus of Cornell University’s New York State Agricultural Experiment Station, in Geneva, New York where I acquired my appreciation for tree fruit horticulture. In the spring of 2001 I applied to Washington State University, Pullman, Washington and in August of 2001 I enrolled in the Department of Horticulture and Landscape Architecture in the College of Agriculture and Home Economics (CAHE) which is now the College of Agricultural, Human, and Natural Resource Sciences. In May of 2003, I obtained my M.S. in Horticulture under the supervision of Dr. Larry E. Schrader. Since the completion of my Masters Degree program I have continued my education and research under the supervision of Dr. Larry E. Schrader at Washington State University.
APPLE (*MALUS DOMESTICA* BORKH.) FRUIT SKIN DISORDERS AND CHANGES IN PIGMENT CONCENTRATIONS ASSOCIATED WITH THE DISORDERS

Abstract

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Apple fruit sunburn and 'Fuji' stain cause great economic losses, and are a direct result of pigment changes that cause discoloration. 'Fuji' stain appears during cold storage on the sun-exposed side of apples that have been sunburned. These disorders were characterized with in-depth analyses of compounds involved in apple color development and how changes in the concentrations of these compounds are associated with sunburn and 'Fuji' stain.

As severity of sunburn increased in 'Fuji' apples, concentrations of chlorophylls a and b, and idaein decreased. A sudden increase in total quercetin glycosides and β-carotene upon initial sunburn was observed. The hue angle (h°) was highly correlated to the concentrations of total chlorophylls, idaein, and total quercetin glycosides.

In a second study, comparison of the sun-exposed side of non-sunburned apples to three different areas of the sun-exposed side of sunburned 'Fuji', 'Gala', 'Delicious', 'Golden Delicious', and 'Granny Smith' apples revealed differential responses of carotenoids to sunburn. β-carotene concentrations in the peel of 'Fuji' and 'Delicious' apples decreased as the distance from the sunburned area increased while in 'Granny Smith' it increased. The chlorophyll concentrations of all five cultivars and the idaein concentration of the red
cultivars increased as the distance away from the sunburned area increased, while the quercetin glycoside concentrations decreased. The $h^\circ$ was highly correlated to the chlorophyll, quercetin glycoside, and idaein concentrations (red cultivars only). The $h^\circ$ was also highly correlated to the carotenoid concentration for 'Fuji', 'Delicious' and 'Golden Delicious'.

In a third study, discolored peel from apples with 'Fuji' stain was compared to various peel types from apples not exhibiting stain [i.e. sunburned peel, area around sunburned peel (halo), and area around halo (OH); the sun-exposed side of non-stained non-sunburned apples (NSNB) and non-discolored peel outside stained area (OS)]. Concomitant low concentrations of idaein, quercetin glycosides, and epicatechin in peels with stain are in contrast to my earlier studies regarding sunburn and appear to be a unique characteristic of the stained peel of 'Fuji'.

Results of these studies demonstrate the important role of pigments in the development of two important apple skin disorders.
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Dedication

This dissertation is dedicated to my son Ethan
GENERAL INTRODUCTION

Like any product, apples are subject to the laws of supply and demand and over the years as the supply of apples has increased, the prices have decreased. In an attempt to overcome decreasing prices, quality standards have increased and various grading systems have been established. This is to help ensure that the highest quality fruit is sold at the highest price and overall encourages the production of high quality fruit. Because consumers choose their apples based on appearance, appearance has become increasingly important in determining the overall quality of apples. One important aspect of appearance is color. The result is the downgrading of apples based only on color. This is not to say that apples are not downgraded for other reasons, but if apples do not meet the color standard set for that cultivar they are downgraded.

The importance placed on color has encouraged research looking into disorders that affect apple color. Two such color disorders are sunburn and 'Fuji' stain. Sunburn is a disorder that develops in the orchard before harvest and can affect all apple cultivars grown in high light and high temperature conditions. Sunburn is not unique to apples and has been reported and researched in many crops. The term “'Fuji' stain” as used here refers specifically to the post-harvest color disorder affecting the sun-exposed side of 'Fuji’ apples. 'Fuji’ stain is neither well researched nor well understood. It typically develops after one or two months of cold storage and although little is known about it, reports have associated high temperatures and ultraviolet-B radiation with its formation (Gong and Mattheis, 2003; Schrader et al., 2003a; Schrader et al., 2004).

There are no official reports on the incidence of sunburn or 'Fuji’ stain but it has been estimated that at least 8% of the apples grown in the Pacific Northwest (PNW) are affected
by sunburn annually. This is believed to be a conservative estimate with the real incidence of
sunburn being much higher. According to a report by Brunner et al. (2003), warehouses
reported sunburn to be the leading cause of cullage and accounted for over 25% of the culls.
Analyses of the culls conducted by Brunner et al. revealed that sunburn was indeed found on
over 25% of the culls. 'Fuji' stain is not as prevalent as sunburn on an industry-wide basis,
but for some growers the incidence of 'Fuji' stain has been in excess of 50%. The result of
these two disorders is severe economic losses to both individual growers and the apple
industry of the PNW. These economic losses provided the impetus for this research.

The studies in chapters 2, 3, and 4 are designed to gain a better understanding of the
discolorations associated with sunburn and 'Fuji' stain. These studies were not designed to
answer the overall question of “why are these changes occurring?” They were designed to
determine if there were differences in concentrations of peel pigments and other associated
compounds among different peel types. The pigments of interest were chlorophylls a and b,
various carotenoids including β-carotene and the xanthophylls, idaein (anthocyanin),
quercetin glycosides (flavonol), chlorogenic acid, and epicatechin.

The study in chapter 2 was specifically designed to determine if the concentrations of
the aforementioned compounds changed with severity of sunburn browning in 'Fuji' apples.
The classes or degrees of sunburn severity were based on the classes of sunburn as reported
by Schrader et al. (2003a). Schrader et al. categorized six different classes of sunburn (0-5).
They defined 0 as no sunburn, 1 to 4 as increasing severities of sunburn browning, and 5 as
sunburn necrosis. Class 5 was omitted from this study as it is a different type of sunburn.

The study in chapter 3 took a different approach at investigating changes in pigment
concentrations. When looking at the sun-exposed side of a sunburned apple there is a color
gradient across the front of the apple that blends the color of the sunburned area into the peel surrounding the sunburned area. Pigment concentrations associated with this color gradient were specifically examined in Chapter 3. In doing so only the sunburn browning class 2 (SB-2) was used, and the gradient was divided into three areas. The three areas consisted of the SB-2 area, the area immediately outside the SB-2 area (halo), and the area immediately surrounding the halo (OH). In order to determine if changes in concentration were consistent among cultivars, five cultivars with obviously different pigmentation patterns (i.e. 'Fuji', 'Gala', 'Red Delicious', 'Golden Delicious', and 'Granny Smith') were studied.

The study in chapter 4 focused on 'Fuji' stain and investigated the possibility that changes in pigment concentrations were responsible for the discoloration. Over the course of the study two experiments with distinctly different approaches were conducted. The first experiment compared stained peel to SB-2, halo, OH, and non-stained non-sunburned (NSNB) peel types. This was done because 'Fuji' stain has been associated with sunburn and reported to occur in the halo of the sunburned area (Schrader et al., 2003a; 2004). It was thought that direct comparisons between the stained peel and the different peel types (e.g. SB-2, halo, OH, and NB) would allow changes in pigment concentration associated with stain to be differentiated from changes associated with sunburn. The second experiment took a much simpler approach and compared the pigment concentrations in stained peel to peel outside the stained area (OS) and to NSNB peel.

The remainder of this chapter provides a brief review of sunburn in apples. Because little is known about 'Fuji' stain a complete review of it has been included in the chapter 4 manuscript and will not be included here.
Review of Sunburn

The terms sunburn and sunscald have been used interchangeably over the years to describe disorders of various fruits caused by solar radiation. According to the Compendium of Apple and Pear Diseases published by the American Phytopathological Society (Jones and Aldwinckle, 1990), the “damage to fruit by exposure to solar radiation is usually described as sunburn, where as sunscald is injury to the bark and underlying tissues caused by freezing”. The lack of consistency has caused some confusion as to what is actually being studied. This is mentioned only to clarify why the term sunburn is used and that it will be used consistently in this review.

Sunburn affects many fruits and was reported as early as 1918 on beans (*Phaseolus vulgaris* L.) (Macmillan, 1918). In a series of experiments, Macmillan (1918) determined it was the result of exposure to the sun and concluded that it was caused by light of short wavelength (MacMillan, 1923). Since then, sunburn has been noted and studied in many fruits that are grown in high heat and high sunlight areas (Felicetti, 2003; Rabinowitch et al., 1986; Rabinowitch et al., 1974; Schrader et al., 2001).

Over the years different types of sunburn have been reported. Barber and Sharpe (1971) distinguished three types of sunburn in peppers and squash: 1) heat injury sunscald (i.e. sunburn) (HIS), 2) ultraviolet sunscald (UVS), and 3) photodynamic sunscald of heated tissue (PSHT). Studies on apple sunburn at Washington State University have led to the classification of three types of sunburn: 1) sunburn browning, 2) sunburn necrosis, 3) photo-oxidative sunburn. It is important to note that the three types of sunburn described by Barber and Sharpe (1971) do not directly coincide with the three types described by Schrader et al. (2001) for apples.
The three types of sunburn that have been reported in apples are sunburn necrosis, sunburn browning (Schrader et al., 2001), and photo-oxidative sunburn (Felicetti, 2003). Sunburn necrosis occurs when fruit skin temperatures (FST) reach $52 \pm 1 \, ^\circ C$ and is the result of heat-induced cell death (Schrader et al., 2001). Schrader et al. determined that sunburn necrosis could be induced in the dark, and thus concluded that light was not required for its formation. Sunburn necrosis appears as a dark brown or black spot on the surface of the apple and can be several millimeters thick.

The second type of sunburn, sunburn browning, does not result in cell death and is characterized by a yellowing or browning of the skin. The discoloration does not penetrate into the flesh of the apple. Sunburn browning occurs at lower temperatures (46 to 49 °C) than those required for sunburn necrosis and requires sunlight (Schrader et al., 2001). The minimum temperature needed for sunburn browning to occur is referred to as the threshold temperature. The threshold temperature is specifically defined as the minimum temperature needed to induce sunburn browning when fruit are exposed to sunlight for one hour (Schrader et al., 2001; 2003b). Ultraviolet-B (UV-B) radiation is suspected to play a role in the formation of sunburn browning because applications of UV-B filtering compounds, RAYNOX® and p-aminobenzoic acid (PABA), reduce the incidence of sunburn browning (Schrader et al., 2001). Schrader et al. (2003a) later classified different degrees of sunburn on a scale from 0 to 5. They defined 0 as no sunburn, 1 to 4 as increasing severities of sunburn browning, and 5 as sunburn necrosis.

The third type of sunburn is photo-oxidative sunburn (Felicetti, 2003). It is light-induced cell death of shaded apples that suddenly become exposed to sunlight. The initial damage is photo-bleaching of the peel, which turns brown upon continued exposure to
sunlight. There does not appear to be a minimum temperature requirement for the formation of photo-oxidative sunburn as is the case with sunburn necrosis and sunburn browning. However, the influence of temperature has not been fully explored (i.e. higher temperatures may cause more damage faster). Photo-oxidative sunburn has been observed to occur as a result of hand thinning in mid to late June when the top fruit of a cluster is removed, exposing shaded apples to full sunlight. Summer pruning and shifting of a tree limb with a heavy crop load can also suddenly expose shaded fruit to full sunlight and cause this type of sunburn. Photo-oxidative sunburn has also been observed in late October and early November as a result of turning apples to expose the shaded side to sunlight to induce red color development.

In summary, three distinctly different types of sunburn have been characterized in apples and the factors that induce each type have been distinguished. However, little is known about the role of pigments in determining the appearances of the different types of sunburn as well as the appearances of the different severities of sunburn browning.
Literature Cited


Changes in Pigment Concentrations Associated with the Degree of
Sunburn Browning of 'Fuji' Apple (Malus domestica Borkh cv. 'Fuji')

ADDITIONAL INDEX WORDS. Chlorophyll, carotenoid, anthocyanin, quercetin, HPLC

ABSTRACT. Significant changes in pigments have been identified, quantified and correlated to the changes in color associated with sunburn browning of 'Fuji' apples in the 2005 and 2006 growing seasons. Apples were sorted into five classes (NB = no sunburn and SB-1 to SB-4 indicates increasing severity of sunburn browning). Both years indicate a decline in chlorophylls a and b and reduced anthocyanin accumulation with increased sunburn severity. Both years showed a sudden increase in total quercetin glycosides upon initial sunburn (NB to SB-1) with the majority of the increase coming as a result of increased quercetin 3-galactoside and quercetin 3-glucoside/quercetin 3-rutinoside. Modest and generally statistically insignificant changes in quercetin glycosides occurred from the SB-1 to SB-4 samples. β-carotene showed an initial increase in both years, but changes in the SB-3 and SB-4 treatments were inconsistent between the two years. The xanthophylls, violaxanthin and antheraxanthin, were significantly higher in SB-1 than NB, but no difference was detected from SB-1 to SB-4. Lutein, a xanthophyll, showed no change as a result of sunburn in either year. The hue angle (h°) was highly correlated to the concentrations of total chlorophylls, idaein, and total quercetin glycosides. Despite minor discrepancies between the 2005 and 2006 growing seasons the overall trends of decreased chlorophyll and idaein, and increased quercetin glycosides and carotenoids persist. The lower chlorophyll and anthocyanin concentrations observed in the sunburned apples allow
the yellows from the carotenoids and quercetin glycosides to be more prominent. Additionally, the increases in carotenoid and quercetin glycoside concentrations make the change in color more striking.
Apple sunburn is a serious problem in many parts of the world where apples are grown in the presence of high solar irradiance and high temperature. Three types of apple sunburn have been characterized in this laboratory. Sunburn necrosis and sunburn browning were described by Schrader et al. (2003a; 2001; 2003b), and photo-oxidative sunburn was first described by Felicetti (2003). Sunburn necrosis occurs when fruit surface temperatures (FST) reach 52 ± 1 °C, is the result of heat induced cell death, and does not require direct sunlight (other than as a heat source). Sunburn necrosis appears on the surface of the apple as a dark brown or black spot, which may be several millimeters thick. The second type of sunburn, sunburn browning, does not result in cell death and is characterized by a yellowing or browning of the skin. It occurs at lower FST (46 to 49 °C) than those required for sunburn necrosis and requires sunlight (Schrader et al., 2001; 2003b). The minimum FST needed for sunburn browning is referred to as the threshold temperature (Schrader et al., 2001). The threshold temperature is specifically defined as the minimum FST needed to induce sunburn browning when fruit are exposed to sunlight for one hour (Schrader et al., 2001). The third type of sunburn is photo-oxidative sunburn which appears to be a purely photo-oxidative process that results in photo-bleaching of shaded peel that is suddenly exposed to sunlight (Felicetti, 2003).

The degree of sunburn can be classified into six classes based on appearance (Schrader et al., 2003a). In this system, a rating of 0 is equal to no sunburn, a rating of 5 is equal to sunburn necrosis, and ratings of 1 to 4 refer to different degrees of sunburn browning. In the red cultivars, the degrees of sunburn browning (1 to 4) are based on reduced red color and increasing yellow and brown colors. In all cultivars, an increased sunburned area, increased browning, and increased blackened lenticels in the affected area are observed as the degree of
sunburn browning increases (i.e., 1 has more red color, less browning, fewer blackened lenticels, and a smaller affected area than 2 to 4). Schrader et al. (2003a) implied that degrees 1 to 4 are the result of increased exposure to sunburn browning induction factors (e.g., sunlight and heat).

This study focuses specifically on degrees 0 to 4, with 5 omitted because it is a different type of sunburn. The intent of this study was to characterize the changes in pigment concentrations between sunburn degrees 0 to 4. A better understanding of these pigment changes will facilitate the development of new technologies and methods for sunburn remediation.

Materials and Methods

This study was conducted in the 2005 and 2006 growing seasons on 'Fuji' apples. In 2005, an orchard located in Wenatchee, Wash. at the Washington State University – Tree Fruit Research and Extension Center (WSU-TFREC) was used. In 2006, a commercial orchard west of Yakima, Wash. was used. Both orchards received standard horticultural practices and disease and pest control. Apples were collected on 14 Oct. 2005 and 18 Oct. 2006 from their respective orchards, placed in cold storage at 0.5 °C, and sampled the following day. The classification used to separate sunburn browning into classes was modified from Schrader et al. (2003a). The highest classification (5) was eliminated, the non-sunburned apple was labeled “NB”, and increasing sunburn severity was labeled SB-1, SB-2, SB-3 and SB-4, respectively (Plate 1).

Sampling. In 2005, 10 apples of sunburn degrees 0 to 4 were selected and four peel disks (16 mm diameter, 1mm thick) were taken from each apple. Two disks were used for chlorophyll and carotenoid analyses and two for phenolic analysis. Thus, each degree (class)
of sunburn had 10 repetitions with each repetition composed of two peel disks from the same apple. In 2006, 20 apples of sunburn degrees 0 to 4 were selected and two peel disks (12 mm diameter, 1 mm thick) were taken from the sun-exposed side of each apple. One of the disks was for chlorophyll and carotenoid analyses and the other for phenolic analysis. Four disks from four different apples were pooled to make five repetitions per degree of sunburn, with each repetition composed of four disks from four apples. All samples were immediately frozen in liquid nitrogen and stored at -80 °C until analyses were performed.

**PEEL COLOR ANALYSES.** Before peel disk samples were taken, the color of the area to be sampled on the sun-exposed side of each apple was determined using a colorimeter (CR-300 Chroma Meter, Minolta Corp., Osaka, Japan). The CIE L*a*b* (L*, lightness factor) color space was used and the hue angle (h°, tan⁻¹ (b*/a*)) and chroma (C*, \sqrt{(a^*)^2 + (b^*)^2} ) were calculated (McGuire, 1992).

**EXTRACTIONS, GENERAL.** Frozen samples were crushed and ground into a fine powder using a mortar, pestle, and liquid nitrogen immediately prior to the extractions. All extracts were filtered (0.45 μm PTFE membrane) into amber vials. Light exposure was minimized throughout the extraction process by performing extractions in near dark conditions and keeping samples covered when not handled.

**CHLOROPHYLL AND CAROTENOID EXTRACTIONS.** Buffer (0.5 ml 0.1 M HEPES, adjusted to pH 7 using 0.5 M KOH), was added to mortar followed by 1 ml 100% acetone and the tissue was ground for an additional 1 min. The resultant homogenate was centrifuged at 12,000 g for 2 min. The mortar and pestle were rinsed thrice with 1 ml 100% acetone and the rinsate was used to resuspend the pellet. The resultant suspension was centrifuged and the supernatants were combined and partitioned into hexanes (3 x 1 ml). The hexanes phase
was dried using a roto-evaporator (vacuum of 760 mm Hg; water bath at 20 °C) and reconstituted in 100 μl of 100% acetone.

**Phenolic Extractions.** The phenolic extraction was modified from Rudell et al. (2002). 1 ml of acidified methanol (1% HCl v/v) was added to the mortar and the tissue was ground for an additional 1 min. The homogenate was centrifuged at 12,000 g for 2 min. The mortar and pestle were washed with acidified methanol (3 x 1 ml) and washes were used to re-suspend the pellet. This suspension was centrifuged for 2 min and the supernatant combined with the first. The combined supernatants were washed with 1 x 3 ml hexanes and the hexanes layers were discarded. The aqueous phase was roto-evaporated under a vacuum of 760 mm Hg to concentrate phenolics and remove any residual hexanes.

**Pigment Analyses.** 25 μl samples were analyzed using a reverse-phase high performance liquid chromatography (HPLC) system equipped with a Series 1100 Hewlett Packard injector, a Hypersil ODS guard column (5 μm, 4.0 x 4 mm; Agilent Technologies, Palo Alto, CA), a Hypersil ODS column (5 μm, 4.0 x 125 mm; Agilent Technologies, Palo Alto, CA) and a photodiode array (PDA) detector (model 996; Waters, Milford, Mass.). Pigments were eluted at a flow rate of 1 ml min⁻¹ and a column temperature of 25 °C using a binary gradient.

The chlorophyll and carotenoid gradient used was modified from Rudell et al. (2002) and consisted of 100% solvent A (80:20 Methanol-0.1 M HEPES buffer (pH 7.0, titrated with 0.5 M KOH) (v/v)) for the first 2 min, then solvent B (ethyl acetate) increased linearly and reached 50% at 21 min. This mixture was maintained until 40 min at which time the column was re-equilibrated with 100% solvent A for 12 min. The 0.1 M HEPES buffer was adjusted to pH 7.0 before mixing with methanol.
The phenolic gradient used was modified from Rudell et al. (2002) and consisted of 100% solvent A (1:10:89 H₃P0₄-methanol-deionized water v/v) for the first 2 min and then decreased linearly to 20% solvent A and 80% solvent B (1:70:29 H₃P0₄-methanol-deionized water v/v) at 36 min. From 36 to 40 min solvent B increased linearly to 100%. The run ended at 40 min at which time the column was re-equilibrated with 100% solvent A for 13 min.

**Peak Identification and Quantification.** Peaks were identified by retention time and spectral comparison to authentic standards. Peaks were quantified using molar absorption coefficients derived from authentic standards. The following exceptions apply. Violaxanthin and antheraxanthin were identified based on the elution order and relative peak heights of similar HPLC methods (Thayer and Bjorkman, 1990; Yamauchi and Watada, 1991). The lutein molar extinction coefficient was used to quantify violaxanthin and antheraxanthin. Quercetin 3-xyloside (reinutrin or reynoutrin), quercetin 3-arabinopentoside (guajaverin), and quercetin 3-arabinofuranoside (avicolarin) have been tentatively identified based on their retention times, uv/vis spectra, elution order, and their presence in apple compared to other reports (Rudell et al., 2002; Schieber et al., 2002). The xanthophylls and β-carotene were quantified at 446 nm, chlorophyll b (chl b) at 466 nm, and chlorophyll a (chl a) at 660 nm. Epicatechin was quantified at 280 nm, chlorogenic acid at 328 nm, the quercetin glycosides were quantified at 357 nm, and idaein was quantified at 519 nm. Lutein, β-carotene, chl a, and chl b, quercetin 3-β-D-glucoside (isoquercitrin), quercetin rutinoside (rutin), quercetin 3-D-galactoside (hyperin or hyperoside), quercetin 3-L rhamnoside (quercitrin), epicatechin, and chlorogenic acid were purchased from Sigma-Aldrich Co.
STATISTICAL ANALYSES. One-way analysis of variance (ANOVA) was performed to determine if significant differences existed among the pigment concentrations of the peel types. Fisher’s least significant differences (LSD) were calculated when appropriate to determine which means were statistically different (P < 0.05; Proc GLM; Means/LSD; SAS Institute Inc.). The mean h° was regressed against the mean concentrations of total chlorophylls, total carotenoids, total quercetin glycosides, and idaein to determine linear R² (Proc REG; SAS Institute Inc.).

Results

Both years showed trends of decreasing chl a and chl b concentrations with increased severity of sunburn (Fig. 1). However, the decrease in chl b was not proportional to the decrease in chl a as is demonstrated by a marked increase in chl a/b ratios with increased sunburn severity (Table 1). In 2005, the chl a concentration in NB apples was significantly higher than in SB-3 and SB-4, while chl b concentrations in SB-1 to SB-4 were significantly lower than in NB. In 2006, the concentration of chl a and chl b in SB-1 to SB-4 were significantly lower than in NB, with SB-3 and SB-4 being significantly lower than SB-1 and SB-2. In 2006, there was no detectable chl b in SB-3 and SB-4.

Both years had consistent xanthophyll results (Fig. 2A and B). Violaxanthin (V), antheraxanthin (A), and total xanthophylls in burned apples were higher than in NB apples, but little difference was observed among burned apples (SB-1 to SB-4). Zeaxanthin was not detected in either year, likely because the apples were cold stored in the dark over night before peel disks were collected. In 2005, there were no significant differences in lutein concentrations. In 2006, SB-3 and SB-4 were significantly lower than SB-1 and SB-2. β-carotene showed inconsistent results between the two years. In 2005, SB-3 was no different
than NB but was lower than SB-2 and SB-4. In 2006, this was not seen but rather a substantial increase in β-carotene was seen in the SB-4.

Idaein is the only anthocyanin reported here as it is the main anthocyanin in apple. Other anthocyanins that have been previously reported in apple are not reported in this study because their peaks could not be quantified consistently. Idaein concentrations decreased with increased sunburn severity (Fig. 3). In 2005, idaein concentration steadily decreased with sunburn severity. In 2006, the decrease was very large between NB and SB-1 after which modest decreases were detected. In both years the idaein concentrations of SB-4 were lower than SB-3 but not significantly lower.

The quercetin glycosides showed consistent increases between the 2 years (Fig. 4A and B). The main glycosides, quercetin 3-galactoside and quercetin 3-glucoside, were dramatically higher in the SB-1 when compared to the NB samples and showed increases of 69% and 183% in 2006, and 116% and 307% in 2005, respectively. More modest increases in these two quercetin glycosides were detected from SB-1 to SB-4. The other quercetin glycosides increased more modestly over all treatments. The concentrations of quercetin, the aglycone of the quercetin glycosides, are reported here even though it is believed that no free quercetin is stored in apple peel. Its presence is believed to be an artifact of the method of analysis and mainly a direct result of the acid hydrolysis of quercetin 3-arabinofuranoside and to a lesser extent quercetin 3-arabinopyranoside (Table 2).

Chlorogenic acid and epicatechin increased with increased sunburn severity (Fig. 5). Chlorogenic acid in SB-1 was 50% and 70% higher than in NB in 2005 and 2006, respectively. Chlorogenic acid in SB-4 was 99% and 138% higher than in SB-3 in 2005 and 2006, respectively. In both years, the most severe sunburn (SB-4) had a higher chlorogenic
acid concentration than all other degrees of sunburn. In 2005, SB-1 and SB-2 were higher than NB, lower than SB-4 but no different from each other. In 2006, only SB-1 was higher than NB, but both SB-1 and SB-2 were lower than SB-3 and SB-4.

In 2005, the epicatechin concentration of the NB peel was significantly lower than that of SB-3 and SB-4, but in 2006 NB was significantly lower than all the burned peel types (Fig. 5). In both years SB-2 and SB-3 epicatechin concentrations were no different from each other, but in 2005 they were significantly lower than SB-3 and SB-4 and in 2006 they were significantly higher than NB.

The colorimetric data had general increases in the L* and the h° and a general decrease in C* as sunburn severity increases (Table 3). The exception to this was the 2006 SB-4 peel which had a lower L* value than SB-1, and h° and C* values that were between the SB-2 and SB-3 values. The change in hue angle is linearly correlated to the changes in total quercetin glycosides, idaein, and total chlorophyll concentrations (Table 4), but not to changes in total carotenoid concentration (Table 4). Quadratic relationships were not significant (P > 0.25).

Discussion

Using the extraction and analysis methods described by Rudell et al. (2002) we experienced the same chl a degradation that prevented them from quantifying chl a using HPLC. We determined that the degradation was the result of acid hydrolysis in extracts that were awaiting HPLC analysis. As a result, a modified procedure using a buffered extraction solvent and a buffered eluting solvent was developed to minimize this hydrolysis.

The data clearly show significant concentration changes in pigments that are associated with changes in the degree of sunburn. The results of the chlorophyll and
carotenoid analyses are consistent with previous reports on the responses of leaves and apple fruit to increasing light levels (Demmig-Adams, 1998; Demmig-Adams and Adams, 1992; Ma and Cheng, 2004, 2003; Thayer and Bjorkman, 1990). These reports compared shaded vs. sun-exposed tissues and reported that shaded tissues contained more chlorophyll, less violaxanthin (V) and antheraxanthin (A), and the same amount of lutein. The increase in chl a/b ratio is particularly indicative of a light acclimation response and indicates a reduction in the amount of light harvesting complex II (LHCII), which contains most of the chl b (Green and Durnford, 1996; Kitajima and Hogan, 2003).

The higher concentrations of V and A seen in SB-1 to SB-4 as compared to NB suggest that an up-regulation of these pigments occurred. This increase indicates a need for increased non-photochemical quenching (NPQ) capacity which suggests that the SB treatments received more light than the NB treatment. However, the relative lack of differences among the SB treatments suggests 1) there is no need for further up-regulation or 2) there is a limited capacity of the xanthophyll pigments to up-regulate. The stepwise decline of the chlorophylls suggests a need for further up-regulation of the xanthophyll cycle, but the data show a limited capacity to up-regulate. This limitation could be due to limited finite resources (i.e. enzymes and precursors) that are needed to synthesize the xanthophylls. Since β-carotene is an immediate precursor to V and A, the fact that it was detected indicates that the limitation is not due to limited precursors. Not only was β-carotene found, it was also found in higher concentration in the burned apples. It is important to note that the percent increase of β-carotene was greater than V and A. This is important because it indicates that more β-carotene has been produced than has been converted to xanthophylls.
The increase in β-carotene under these conditions suggests that it is not being converted to xanthophylls. One possible explanation for this is the deactivation of carotenoid b-ring hydroxylase which is the enzyme responsible for the conversion of β-carotene to zeaxanthin. The deactivation of this enzyme would prohibit the formation of V and A.

Another explanation lies in understanding that β-carotene does not solely exist as a precursor to the xanthophylls. It is an accessory pigment that is found in the P680 reaction centers where it scavenges singlet oxygen and quenches triplet state chlorophyll to protect photosystem II (PSII) (Demmig-Adams et al., 1996). Hence, β-carotene may be up-regulated to help protect PSII under such conditions.

The earlier examination of the decline in chlorophyll concentration suggested that the decline was the result of increased light levels and this is still a good explanation. However, under sunburn conditions the fruit surface reaches temperatures in excess of 44 °C. Reports linking photo-oxidative stress to lower chlorophyll levels (Merzlyak et al., 1998), and reports that high temperatures alone can reduce photosynthetic efficiency by 53% thus implying increased photo-oxidative stress (Torres et al., 2006), raise the question of whether the decline in chlorophyll concentration is due solely to the increased light or to increased photo-oxidative stress resulting from high temperatures and high light. Both are possible explanations and given the conditions under which sunburn occurs it would be easy to assume the differences in chl concentration were the result of photo-oxidative stress. However, this assumption is confounded by the fact that the chl a/b ratio dramatically increases. The increase in chl a/b would require that LHCII be preferentially oxidized over the other chlorophyll containing protein complex. While LHCII is photo-oxidized, reports indicate that the oxidation results in widespread photo-oxidation and photo-bleaching of all
of the chlorophyll containing protein complexes (Olszowka et al., 2003; Zucchelli et al., 1988). The change in chl a/b ratio indicates that the change is more likely the result of light levels. However, it is not possible to conclude from this experiment whether the changes are the result of varying light levels or varying degrees of photo-oxidative stress.

The phenolic results are particularly intriguing. The most dramatic changes observed were the increases in quercetin glycoside concentrations and the decrease in anthocyanin (idaein) concentration with increased severity of sunburn. The increases in chlorogenic acid, epicatechin, and quercetin glycosides are not surprising given the condition under which sunburn occurs, because their induction by light is well documented (Bruns et al., 1986; Dixon and Paiva, 1995; Feinbaum et al., 1991; Schmelzer et al., 1988; Spayd et al., 2002; Tattini et al., 2004; Vanderauwera et al., 2005). Although ultraviolet-B (UV-B) radiation seems to be a common source of induction for these compounds, anthocyanins are synergistically induced by red light (Arakawa et al., 1985). The accumulation of these compounds has been linked to protection against UV radiation and visible light with quercetin glycosides protecting against the former and anthocyanins protecting against the latter (Li et al., 1993; Smillie and Hetherington, 1999).

The light induction of these compounds explains the increased chlorogenic acid, epicatechin, and quercetin glycosides concentrations but not the decreased anthocyanin concentrations. This can be partially explained by examining the effect of temperature on anthocyanin accumulation. Along with light induction, anthocyanins are induced by cold and inhibited by warm temperatures (Creasy, 1968; Curry, 1997; Dela et al., 2003; Faragher, 1983). This temperature relationship seems to indicate that heat stress would suppress anthocyanin development in apples. However, the literature suggests that this suppression is
not permanent (Dela et al., 2003). It is important to keep this in mind when discussing the temporal separation between the dates of sunburn events, dates of normal anthocyanin accumulation and the date of sample harvest.

Air temperature data (WSU-AgWeatherNet) near the orchards in 2005 and 2006 indicate that ambient temperatures in 2005 did not exceed 27.2 °C from September 8 to October 14 (2005 harvest date). In 2006, the temperature did not exceed 31.1 °C from September 9 to October 18 (2006 harvest date). In the Pacific Northwest, 'Fuji' apples start to develop color in early September and continue to develop color until harvest. It is not known when the sunburn occurred but given the ambient temperatures it likely did not occur after September 9th both years. This provided approximately 5 weeks of sunburn-free conditions for the apples to accumulate anthocyanins during their typical peak anthocyanin accumulation period. The fact that anthocyanin accumulation is depressed indicates a long term heat stress effect on anthocyanin accumulation in apples.

The decreased anthocyanin concentration is not surprising given the appearance of sunburned apples, but given the current state of knowledge about the biosynthetic pathway of phenolic compounds in apples it is particularly intriguing that anthocyanin concentration decreased while epicatechin concentrations increased. Both anthocyanins and epicatechin are synthesized from cyanidin. UDP-glycosyl:flavonoid-3-O-glycosyltransferases (UFGT) transfer glycosides to cyanidin to form anthocyanins, while anthocyanidin reductase (ANR) reduces cyanidin to epicatechin. It might be expected that the difference lies in UFGT. However, UFGT is also required and used to glycosylate quercetin to form the quercetin glycosides. Because the quercetin glycosides increased, it appears that UFGTs were present and active. The lack of anthocyanins despite the availability of cyanidin, as demonstrated by
the presence of epicatechin, and UFGT has been reported in green leaves (Pfeiffer et al., 2006). They hypothesized that relative activities of ANR and UFGTs could account for such a dichotomy. Pfeiffer et al. (2006) also suggested that temporal and spatial separations in compound formation as well as the transport or the lack of transport of anthocyanins to the vacuole were considerations. The data presented here show increases in epicatechin that pale in comparison to the increases in quercetin glycosides. This suggests that UFGT activities were at least comparable to that of ANR. It must be conceded that because these data represent only one point in time and ANR and UFGTs were not assayed, it is not known when these compounds were formed or that there were indeed coincidental enzyme activities. Thus, the increases in various compounds may have occurred at different times. Despite this concession, the data do not favor an explanation involving differential enzyme activities, but rather spatial or temporal separation, or issues involving the transport to the vacuole.

As mentioned earlier, quercetin is an artifact of analysis, and is mainly a direct result of the acid hydrolysis of quercetin 3-arabinofuranoside and to a lesser extent quercetin 3-arabinopyranoside. This degradation process explains the significant decrease in quercetin 3-arabinofuranoside concentration that was seen in 2006 as it was paralleled by a significant increase in quercetin.

Aside from providing an objective measure of the color of the varying degrees of sunburn, the colorimetric data allows probing into the relationship between changes in peel pigments and changes in color. The high correlations between changes in total quercetin glycosides, idaein, and chlorophylls indicate that these pigments are likely contributing to the change in color, while the changes in total carotenoids are not. These relationships should be interpreted cautiously and are not meant to be used to calculate pigment concentrations based
on hue angle as it is possible to achieve the same hue angle with multiple combinations of pigments (Lancaster et al., 1997). Here they are strictly used to explore the nature of the color change in relationship to changes in the individual pigments.

Although the exact conditions that caused the varying degrees of sunburn are not known, sunburn of this type is caused by high fruit surface temperatures (FST) and high sunlight. As such, it is not unreasonable to speculate that the varying degrees discussed here are the result of differences in FST, irradiance, and/or time of exposure to these factors (i.e. SB-1 was exposed to a lower FST and/or less sunlight than SB-2, 3 or 4). Although it was not the intent of this study to determine the specific conditions at which the varying degrees of sunburn occur, it is important to note that the results support the notion that increased sunburn severity is the result of increased exposure to light and/or heat stress.

In conclusion, there are indeed significant changes in pigment concentrations that are associated with changes in sunburn severity and these changes in concentrations help explain the color change. The loss of chlorophylls (green) and the decreased anthocyanin (red) accumulation in the sunburned peel allow the colors of the carotenoids (yellow) and quercetin glycosides (yellow, tan) to be more apparent. In addition, the increases in carotenoids and quercetin glycosides cause the discoloration to be even more dramatic.
Literature Cited


Plate 1. Degrees of sunburn browning as modified from Schrader et al. (2003a). NB, no sunburn; SB-1 to SB-4, sunburn severity 1 to 4 respectively.
Table 1. 2005 and 2006 Chlorophyll a/b ratios for NB, SB-1, SB-2, SB-3, and SB-4 peel types. Chl, chlorophyll; NB, no sunburn; SB-1 to SB-4, sunburn severity 1 to 4 respectively.

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Table 2. Concentrations of individual quercetin glycosides and quercetin in the final extract in mg·g⁻¹ fresh wt. Min., minutes after extraction completion; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside, Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification

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<td>3.08</td>
<td>0.63</td>
<td>0.43</td>
<td>0.19</td>
<td>0.51</td>
<td>0.34</td>
<td>0.47</td>
</tr>
</tbody>
</table>
Table 3. 2005 and 2006 colorimetric data. Means with the same letter in parenthesis are not significantly different ($P \leq 0.05$). $L^*$, lightness factor; $h^\circ$, hue angle; $C^*$, chroma; NB, no sunburn; SB-1 to SB-4, sunburn severity 1 to 4 respectively.

<table>
<thead>
<tr>
<th></th>
<th>$L^*$</th>
<th></th>
<th>$h^\circ$</th>
<th></th>
<th>$C^*$</th>
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<tr>
<td>NB</td>
<td>34.9 (d)</td>
<td>39.4 (d)</td>
<td>24.7 (d)</td>
<td>21.6 (d)</td>
<td>33.1 (a)</td>
<td>34.2 (a)</td>
</tr>
<tr>
<td>SB-1</td>
<td>44.0 (c)</td>
<td>50.3 (c)</td>
<td>39.6 (c)</td>
<td>44.8 (c)</td>
<td>32.9 (a)</td>
<td>31.4 (b)</td>
</tr>
<tr>
<td>SB-2</td>
<td>47.3 (b)</td>
<td>55.3 (b)</td>
<td>45.8 (b)</td>
<td>56.9 (b)</td>
<td>31.5 (a)</td>
<td>26.2 (c)</td>
</tr>
<tr>
<td>SB-3</td>
<td>54.1 (a)</td>
<td>61.0 (a)</td>
<td>57.2 (a)</td>
<td>68.0 (a)</td>
<td>25.8 (b)</td>
<td>20.7 (d)</td>
</tr>
<tr>
<td>SB-4</td>
<td>52.9 (a)</td>
<td>48.0 (c)</td>
<td>59.7 (a)</td>
<td>57.1 (b)</td>
<td>24.4 (b)</td>
<td>24.6 (c)</td>
</tr>
</tbody>
</table>

Table 4. Linear equations for the linear regression of hue angle against total chlorophylls, total carotenoids, idaein, and total quercetin glycosides.

<table>
<thead>
<tr>
<th></th>
<th>2005</th>
<th>a</th>
<th>b</th>
<th>$R^2$</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td>Total Chlorophyll</td>
<td>82.41</td>
<td>-2.19</td>
<td>0.97</td>
<td>0.0019</td>
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<tr>
<td>Total Carotenoids</td>
<td>3.22</td>
<td>1.74</td>
<td>0.29</td>
<td>0.3461</td>
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<tr>
<td>Idaein</td>
<td>66.89</td>
<td>-238.09</td>
<td>0.94</td>
<td>0.0056</td>
<td></td>
</tr>
<tr>
<td>Total Quercetin Glycosides</td>
<td>3.65</td>
<td>6.76</td>
<td>0.85</td>
<td>0.0251</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>2006</th>
<th>a</th>
<th>b</th>
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<th>$P$</th>
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</thead>
<tbody>
<tr>
<td>Total Chlorophyll</td>
<td>72.92</td>
<td>-1.76</td>
<td>0.93</td>
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<tr>
<td>Total Carotenoids</td>
<td>21.9</td>
<td>0.63</td>
<td>0.35</td>
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<tr>
<td>Idaein</td>
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<tr>
<td>Total Quercetin Glycosides</td>
<td>-17.88</td>
<td>6.16</td>
<td>0.95</td>
<td>0.0048</td>
<td></td>
</tr>
</tbody>
</table>

† Hue angle = $a + b$(concentration)
Fig. 1. 2005 and 2006 chlorophyll a (Chl a) and chlorophyll b (Chl b) concentrations in µg·g⁻¹ fresh wt. for 'Fuji' NB, SB-1, SB-2, SB-3, and SB-4 peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds in individual years. NB, non-sunburned; SB-1, sunburn degree of 1; SB-2, sunburn degree of 2; SB-3, sunburn degree of 3; SB-4, sunburn degree of 4.
Fig. 2A. 2005 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in μg·g⁻¹ fresh wt. for 'Fuji' NB, SB-1, SB-2, SB-3, and SB-4 peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. NB, non-sunburned; SB-1, sunburn degree of 1; SB-2, sunburn degree of 2; SB-3, sunburn degree of 3; SB-4, sunburn degree of 4.
Fig. 2B. 2006 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in µg·g⁻¹ fresh wt. for 'Fuji' NB, SB-1, SB-2, SB-3, and SB-4 peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. NB, non-sunburned; SB-1, sunburn degree of 1; SB-2, sunburn degree of 2; SB-3, sunburn degree of 3; SB-4, sunburn degree of 4.
Fig. 3. 2005 and 2006 idaein concentrations for ‘Fuji’ NB, SB-1, SB-2, SB-3, and SB-4 peel types in mg·g⁻¹ fresh wt. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within years. NB, non-sunburned; SB-1, sunburn degree of 1; SB-2, sunburn degree of 2; SB-3, sunburn degree of 3; SB-4, sunburn degree of 4.
Fig. 4A. 2005 concentrations of individual quercetin glycosides for 'Fuji' NB, SB-1, SB-2, SB-3, and SB-4 peel types in mg·g⁻¹ fresh wt. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds. NB, non-sunburned; SB-1, sunburn degree of 1; SB-2, sunburn degree of 2; SB-3, sunburn degree of 3; SB-4, sunburn degree of 4; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 4B. 2006 concentrations of individual quercetin glycosides for 'Fuji' NB, SB-1, SB-2, SB-3, and SB-4 peel types in mg·g⁻¹ fresh wt. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. NB, non-sunburned; SB-1, sunburn degree of 1; SB-2, sunburn degree of 2; SB-3, sunburn degree of 3; SB-4, sunburn degree of 4; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 5. 2005 and 2006 chlorogenic acid and epicatechin concentrations for 'Fuji' NB, SB-1, SB-2, SB-3, and SB-4 peel types in mg·g⁻¹ fresh wt. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds in individual years. NB, non-sunburned; SB-1, sunburn degree of 1; SB-2, sunburn degree of 2; SB-3, sunburn degree of 3; SB-4, sunburn degree of 4; CA, chlorogenic acid.
Changes in Pigment Concentrations Associated with Sunburn Browning of Five Apple Cultivars

ADDITIONAL INDEX WORDS. *Malus domestica*, Fuji, Gala, Granny Smith, Golden Delicious, Red Delicious, chlorophyll, carotenoids, anthocyanins, quercetin

ABSTRACT. Peel disks were taken from three different areas of the sun-exposed side of sunburned 'Fuji', 'Gala', 'Delicious', 'Golden Delicious', and 'Granny Smith' apples (*Malus domestica* Borkh): the sunburned area (SB-2), the area around the sunburned area (halo), and the area around the halo (OH). Additionally, samples were taken from the center of the sun-exposed side of non-sunburned apples (NB). The SB-2 chlorophyll a and b concentrations of 'Fuji', 'Delicious', 'Golden Delicious', and 'Granny Smith' were generally significantly (P < 0.05) lower than those in the halo, OH and NB peels. Differences observed in 'Gala' chlorophyll concentrations in 2005 were mostly insignificant; in 2005 chlorophyll a and b concentrations of SB-2 were significantly lower than in OH and NB. Concentrations of total quercetin glycosides increased significantly from OH to halo to SB-2 in 'Fuji', 'Gala', 'Golden Delicious', and 'Granny Smith'; the increases were not always significant in 'Delicious' apples. Idaein concentrations in the red cultivars (i.e. 'Fuji', 'Gala', and 'Delicious') generally decreased significantly from NB to OH to halo to SB-2. Significantly higher levels of β-carotene, violaxanthin, and antheraxanthin were observed in 'Fuji' and 'Delicious' SB-2 peel as compared to OH and NB peels. In 2005, neither 'Gala' nor 'Golden Delicious' had significant differences in β-carotene concentrations among their own peel types. Concentrations of violaxanthin and antheraxanthin in 'Gala' SB-2 were higher than in halo,
OH and NB in 2005 but not in 2006. 'Golden Delicious' SB-2 concentrations of violaxanthin were higher than in halo, OH, and NB in 2005, but in 2006 lower than those in OH, and no different from halo and NB. Differences in 'Granny Smith' carotenoid concentrations were consistent from year to year but different from 'Fuji' and 'Delicious' with no differences in violaxanthin and antheraxanthin and lower concentrations of β-carotene and lutein in the SB-2 and halo peels as compared to the OH and NB peels. These results indicate changes in pigment concentrations associated with sunburn. Changes in chlorophylls, idaein, and quercetin glycosides generally responded similarly among the cultivars while changes in carotenoids did not.
Previous work on apple sunburn has revealed three distinct types of sunburn: sunburn necrosis, sunburn browning and photo-oxidative sunburn (Felicetti, 2003; Schrader et al., 2001). Schrader et al. (2003b) discussed in detail the differences between them and the causes and factors that influence their incidence. The most prevalent and costly is sunburn browning and as such it has been the focus of most research on sunburn. Although much is known about the factors that cause sunburn browning, very little is known about how it affects the apple. The most obvious effect that sunburn browning has on the apple is its discoloration which results in reduced marketability.

Despite being the most obvious characteristic of sunburn browning, changes in color associated with this disorder are poorly understood. To better understand the discoloration of sunburn browning, changes in pigment concentrations with respect to changes in 'Fuji' sunburn browning severity were described earlier (chapter 2). In that study 'Fuji' sunburn was graded on a five-point scale that was based on the classes of sunburn browning described by Schrader et al. (2003a). In general, the results showed that as 'Fuji' sunburn severity increased, chlorophyll and idaein concentrations decreased while carotenoids and quercetin glycosides concentrations increased. The hue angle (h°) was well correlated to the chlorophyll, quercetin glycoside, and idaein concentrations. Although the study provided insight into how the pigment concentrations changed with respect to sunburn and how those changes were related to the discoloration, the study was limited to only 'Fuji' apples.

The objective of the current study was to compare changes in pigment concentrations of several apple cultivars with respect to sunburn. In this study a different approach was used. Instead of looking at changes in concentrations with respect to the degree of sunburn we looked at changes with respect to distance away from the sunburned area. When looking
at a sunburned apple, the front can be divided into three sections or areas. The first is the area that is sunburned and shows the most severe discoloration. The second is the area that immediately surrounds the sunburned area and shows moderate discoloration. The third section is the area immediately surrounding the halo and shows no signs of sunburn. For purposes of this study these three sections were respectively identified as the burned area (SB-2), the halo, and the area outside the halo (OH). This study also compared SB-2, halo, and OH peel types of sunburned apples with the sun-exposed non-sunburned (NB) peel type from non-sunburned apples for 'Fuji', 'Gala', 'Delicious', 'Golden Delicious', and 'Granny Smith' apples.

**Materials and Methods**

This study was conducted in the 2005 and 2006 growing seasons. 'Gala', 'Granny Smith', 'Golden Delicious', and 'Delicious' apples were harvested from various orchards within or near Wenatchee, Wash. All cultivars except 'Fuji' were harvested from only one orchard and the same orchard was used in both years. 'Fuji' apples were harvested from different orchards each year and samples were taken from only one orchard each year. In 2005 the 'Fuji' orchard used was located in Wenatchee, Wash. and in 2006 the orchard used was 10 miles west of Yakima, Wash. All orchards received standard horticultural practices and disease and pest control. All samples were taken from apples harvested near commercial harvest maturity as determined by the managers of the individual orchards. 'Gala' apples were harvested on 24 Aug. 2005 and 31 Aug. 2006. 'Granny Smith' apples were harvested on 20 Sept. 2005 and 27 Sept. 2006. 'Delicious' apples were harvested on 16 Sept. 2005 and 26 Sept. 2006; 'Golden Delicious' apples were harvested on 10 Sept. 2005 and 16 Sept. 2006. 'Fuji' apples were harvested on 14 Oct. 2005 and 18 Oct. 2006.
**Sampling.** In 2005, 10 apples with no sunburn and 10 apples with SB-2 sunburn were harvested. The non-sunburned 'Granny Smith' and 'Golden Delicious' had two categories: those that had a ‘red blush’ on the sun-exposed side and those that did not. Thus 'Granny Smith' and 'Golden Delicious' had an additional 10 apples and one more treatment than the red cultivars. The sun-exposed side of each sunburned apple was divided into three areas: the SB-2, the halo, and the OH (Plate 1). The SB-2 zone was defined as the center of the sunburned area and generally was a circle 3 to 4 cm in diameter. The halo was defined as the area immediately surrounding the SB-2 zone and was approximately 1.5 cm wide. The area outside the halo was the area immediately surrounding the halo and was approximately 1.5 cm wide. Four peel disks (16 mm diameter, 1mm thick) were taken from each zone in each apple. For the halo and OH zones, a total of four disks were taken from each of the halo and OH zones. Two disks from each zone were taken from the left side of the SB-2 zone and two disks were taken from the right side of the SB-2 zone. The two disks were taken in a column orientation rather than a row orientation. Two disks from each zone were used for chlorophyll and carotenoid analysis and two were used for phenolic analysis. Overall each SB-2 apple had 12 peel disks removed. Additionally, the sun-exposed side of 10 non-sunburned apples were sampled (NB), with only four peel disks taken from the center of the sun-exposed side of each apple. Each peel type had 10 repetitions with each repetition composed of two peel disks.

In 2006, the repetitions were reduced from 10 to five, but each repetition contained one disk (12 mm diameter, 1mm thick) from multiple apples. For each of the analyses (i.e the chlorophyll and carotenoid analysis and the phenolic analysis), each pooled repetition of 'Gala', 'Granny Smith', and 'Delicious' contained one disk from three different apples. Pooled
repetitions of 'Golden Delicious' contained one disk from five different apples and repetitions of ‘Fuji’ apples contained one disk from four different apples. The same treatments were sampled as in 2005; however, half the number of disks were taken from each apple. Two disks were taken from each zone with one disk used for chlorophyll and carotenoid analysis and the other for phenolic analysis. Both years, all samples were immediately frozen in liquid nitrogen and stored at -80 °C until analysis.

**Peel Color Analyses.** In 2006, before peel disk samples were taken, the color of the SB-2, halo, OH, NB, and blushed ('Granny Smith' and 'Golden Delicious') zones of each apple to be sampled was determined using a colorimeter (CR-300 Chroma Meter, Minolta Corp., Osaka, Japan). The CIE L* a* b* (L*, lightness factor) color space was used and the hue angle (h°, tan⁻¹ (b*/a*)) and chroma (C*, \(\sqrt{(a^*)^2 + (b^*)^2}\)) were calculated (McGuire, 1992).

**Extractions and Pigment Analyses.** Methods for extraction and pigment analyses were the same as in chapter 2.

**Statistical Analyses.** One-way analysis of variance (ANOVA) was performed to determine if significant differences existed among concentrations of pigments in the peel types. Fisher’s least significant differences (LSD) were calculated when appropriate to determine which means were statistically different (P < 0.05; Proc GLM; Means/LSD; SAS Institute Inc.). The mean h° was regressed against the mean concentrations of total chlorophylls, total carotenoids, total quercetin glycosides, and idaein to determine if a linear correlation existed (Proc REG; SAS Institute Inc.).
Results

Chlorophyll Analyses. The lowest concentrations of chlorophyll a (chl a) and chlorophyll b (chl b) were observed in the SB-2 peel in both years for all five cultivars (Fig. 1A–E) but they were not always significantly lower than the concentrations observed in the halo, OH, and NB peels. In 2005 and 2006, 'Fuji' SB-2 chl a concentrations were significantly lower than in halo and OH (Fig. 1A). In 2006, the chl a concentrations in SB-2 peel was significantly lower than in NB peel. In 2005 and 2006, 'Fuji' chl b concentrations in SB-2 were significantly lower than in OH and NB. In 2005, the SB-2 chl b concentration was significantly lower than in halo as well. In both years the chl a/b ratios in SB-2 and halo were significantly greater than in OH and NB (Table 1).

In 2005, the concentrations of chl a and chl b in 'Gala' peel types were not significantly different from each other (Fig. 1B). In 2006, SB-2 chl a and chl b concentrations were significantly lower than in OH and NB. Additionally, NB chl a concentration was significantly greater than in SB-2, halo, and OH. In 2005, the SB-2 and halo peels had significantly greater chl a/b ratios than OH (Table 1). In 2006, the SB-2 chl a/b ratio was significantly lower than those in the halo, OH, and NB peel types.

In both years, concentrations of chl a and chl b in the SB-2 of ‘Delicious’ were significantly lower than the concentrations in the halo, OH, and NB peel types (Fig. 1C). In 2006, the concentrations of chl a and chl b had significant stepwise increases from SB-2 to halo to OH. Additionally, the increase in chl b concentration from OH to NB in 2006 was also significant. In 2005, the chl a/b ratios in the SB-2 and halo peel types were significantly greater than those in the OH and NB peel types (Table 1). In 2006, the chl a/b ratio in the SB-2 peel type was significantly lower than that in the halo and OH peel types.
In both years 'Golden Delicious' had significant stepwise increases in both chl a and chl b concentrations from SB-2 to halo to OH (Fig. 1D). In both years, the chl a and chl b concentrations of the halo and the blushed peel were not different. The chl a and chl b concentrations in the blushed peel were significantly lower than concentrations in non-blushed peel (i.e. NB). The chl a and chl b concentrations in the blushed peel were less than those in the OH peel, but this decrease was not significant in 2005 for the chl a concentrations. In 2005, the chl a/b ratio of SB-2 peel was no different than that of the other peel types (Table 1). In 2006, the SB-2 chl a/b ratio was significantly lower than that in the halo peel, but significantly greater than that in the NB and OH peel types.

In both years 'Granny Smith' had significant stepwise increases in both chl a and chl b concentrations from SB-2 to halo to OH (Fig. 1E). The only significant difference in chl a and chl b concentrations observed among the OH, blush, and NB peel types was found in the 2006 chl b concentrations where the concentration of chl b in the blush peel was significantly lower than in the NB peel. In 2005, the chl a/b ratios in SB-2, halo, and OH were no different from each other, but all three were significantly greater than that in NB and blush (Table 1). In 2006, the chl a/b ratio in SB-2 was significantly greater than that of the halo, OH, NB, and blush peel types which were not significantly different from each other.

**CAROTENOID ANALYSES.** The decrease in β-carotene concentration from OH to NB in 2005 was not significant (Fig. 2A). In 2005, β-carotene was significantly higher in SB-2 than in all other peel types (Fig 2A). In 2006, 'Fuji' had significant decreases in β-carotene concentration from SB-2 to halo to OH peels, and OH and NB were not significantly different (Fig. 2B). Both years showed no significant differences in lutein concentrations among the peel types (Fig. 2A and B). The violaxanthin (V) and antheraxanthin (A)
concentrations showed similar trends in both years. For both of these xanthophylls, the concentrations in the SB-2 and halo peels were significantly greater than in the OH and the NB peels.

The 2 years of 'Gala' data are inconsistent. In 2005, there were no significant differences in \( \beta \)-carotene concentrations among the peel types, with the OH peel having the lowest concentration (Fig. 2C). Additionally, the concentrations of \( \beta \)-carotene were similar to the concentrations of the individual xanthophylls. In 2006, the \( \beta \)-carotene concentrations are three to six times greater than the concentrations of the individual xanthophylls as well as the 2005 \( \beta \)-carotene concentrations (Fig. 2C and D) and the \( \beta \)-carotene concentrations in the OH peel was significantly greater than in the NB peel. In 2006, no changes in lutein, V, and A were detected in 'Gala' (Fig. 2D). In 2005, lutein, V, and A were found in greater concentrations in the SB-2 peel than in the OH and NB peel (Fig. 2C). The SB-2 peel had greater concentrations of V and A than did the halo.

The \( \beta \)-carotene concentrations in 'Delicious' showed similar trends in both years with a steady decline in concentration from SB-2 to halo to OH to NB peel, although each incremental decrease was not necessarily significant (Fig. 2E and F). In 2005, the V and A concentrations in the SB-2 and the halo peel were greater than in the OH and the NB peels (Fig. 2E). In 2006, there were no significant differences in V concentrations among the peel types, and trends in concentration of A were similar to those seen in 2005 (Fig. 2F).

The 2 years of 'Golden Delicious' data returned variable results. In 2005, no differences were detected in \( \beta \)-carotene concentrations, but in 2006 the \( \beta \)-carotene concentration in SB-2 was significantly lower than in the other peel types (Fig. 2G and H). The halo peel had less than the OH peel, more than the blush and the same as the NB.
xanthophylls were no more consistent than β-carotene from year to year. In 2005, no differences were seen in lutein concentrations. In 2006, SB-2 had significantly lower concentrations of lutein than the other peel types. Additionally, the concentration of lutein in the NB peel was significantly higher than that in the OH and blush peel types (Fig. 2 G and H). In 2005, V concentrations decreased incrementally from SB-2 to halo to OH peel, but in 2006 concentrations in SB-2 and the halo peels were significantly lower than in the OH peel.

The 2 years of 'Granny Smith' data yielded similar trends. In both years, there was a general increase in β-carotene and lutein concentrations from the SB-2 peel, to the halo peel, to the OH peel (Fig. 2I and J). Both years, lutein was higher in the blush and NB peels than in the other peel types. In 2005, the sum of the V and A concentrations in the blushed peel was higher than in all other peel types (Fig. 2I). In 2006, the concentration of V + A in the halo peel was lower than in NB peel (Fig. 2J).

Zeaxanthin was not detected in any of the cultivars in both years. This is likely because the apples were cold stored in the dark over night before peel disks were collected.

**Idaein Analyses.** For 'Fuji', 'Gala', and 'Delicious' there was a consistent trend of increasing idaein concentration from the SB-2 to halo to OH to NB peel (Fig. 3A-C). In most cases these increases were significant.

Not surprisingly the blushed peel of 'Golden Delicious' and 'Granny Smith' apples showed significantly higher concentrations of idaein than SB-2, halo, OH, and NB peels (Fig. 3D and E). There was no idaein detected in the NB and OH peels of 'Golden Delicious' both years, as well as in the halo peel in 2006 (Fig. 3D). In 2005, the idaein concentrations in the halo peels of 'Golden Delicious' and 'Granny Smith' were significantly higher than the concentration in the SB-2 peel. In 2005, the SB-2, OH, and NB peels of 'Granny Smith' were
not significantly different and there was no detectable idaein in the OH and NB peels (Fig. 3E). In 2006, the NB peel of 'Granny Smith' had a higher idaein concentration than the SB-2, halo, and OH peels, which had no detectable idaein.

**Quercetin Glycoside Analyses.** In both years, concentrations of total quercetin glycosides in 'Granny Smith', 'Golden Delicious', 'Gala', and 'Fuji' apples were significantly lower in the halo peel than in the SB-2 peel (Fig. 4A and B). In 2005 and 2006 the concentration of total quercetin glycosides in the halo of 'Delicious' apples was lower than in the SB-2 peel, but not significantly so. All five cultivars had significantly lower concentrations of total quercetin glycosides in the OH peel than in the halo peel, in 2005 and 2006. In both years, 'Granny Smith', 'Fuji', and 'Delicious' had no difference in total quercetin glycoside concentrations between OH and NB peels. The total quercetin glycosides in the 'Granny Smith' blush peel were significantly higher than in the OH and the NB peels, but no different from the halo peel, in both 2005 and 2006. In both years, the blushed peel of 'Golden Delicious' apples contained intermediate concentrations of total quercetin glycosides to the Halo and the OH peel, but the concentration in the blush peel was not significantly higher than in the OH peel in 2006. Over the course of both years and all five cultivars there was only one instance where the concentrations of total quercetin glycosides in the SB-2 peel and the Halo peel were not significantly higher than in the OH and the NB peels. In that one instance (2005 'Gala'), the concentration in the NB peel was lower than in the halo peel, but not significantly lower than in the halo. The greatest differences in total quercetin glycoside concentrations were generally between the SB-2 peel and the NB peel, exceptions being 'Gala' and 2005 ‘Delicious’ which had a lower concentration in the OH peel than the NB peel.
The previously mentioned trends of the total quercetin glycosides were generally mimicked by the individual quercetin glycosides for their respective cultivars (Fig. 5A-J). Despite the similar trends, the individual quercetin glycosides did not change proportionally and the largest increases were not observed in the same quercetin glycosides in all cultivars. 'Granny Smith' SB-2 peel (both years) and halo peel (2005 only) were the only instances where quercetin 3-glucoside/rutinoside (Glu/Rut) was higher than the other quercetin glycosides (Fig. 5I and J). In all five cultivars, the quercetin glycosides with the greatest differences in concentrations between the different peel types were quercetin 3-galactoside (Gal) and quercetin 3-glucoside/rutinoside (Fig. 5A-J).

**CHLOROGENIC ACID ANALYSES.** The 'Fuji', 'Gala', 'Delicious', and 'Golden Delicious', had general trends of decreasing chlorogenic acid concentration from SB-2 to halo to OH to NB peel (Fig. 6A-D). No chlorogenic acid was detected in 'Granny Smith' apples (Fig. 6E). The four cultivars in which chlorogenic acid was detected had significantly higher concentrations of chlorogenic acid in the SB-2 peel than in the OH and NB peel, and in general had higher concentrations in the SB-2 peel than in the OH peel.

**EPICATECHIN ANALYSES.** Epicatechin showed no uniform trends among the cultivars and very few significant differences. 'Fuji' apples had higher epicatechin concentrations in the SB-2 peel than in the NB peel in both years and greater epicatechin concentrations in the SB-2 peel as compared to the halo peel in 2005 (Fig. 6A). In contrast, the concentration of epicatechin in SB-2 peel of 'Granny Smith' apples was significantly lower than in the other four peel types (halo, OH, blush, and NB) in both years (Fig. 6E). The results for 'Gala' depended on the year. In 2005, the only significant difference was between the SB-2 peel and the OH peel, with the SB-2 peel having a higher concentration...
(Fig. 6B). In 2006, both the SB-2 and NB peels were significantly lower than the halo and OH peels. The only significant difference in 'Delicious' apples was observed in 2005 where the NB peel had a higher concentration than the OH peel (Fig. 6C). The only significant differences in 'Golden Delicious' apples were observed in 2006 when the SB-2 peel had a lower concentration of epicatechin than the other four peel types (halo, OH, blush, and NB) (Fig. 6D).

**COLORIMETRIC DATA.** The colorimetric data show increasing L* from OH to halo to SB-2, and similar L* for the NB and OH peels for all five cultivars (Table 2). The red cultivars ('Fuji', 'Gala', and ‘Delicious’) had increasing h° from NB to OH to halo to SB-2, while the green cultivars ('Golden Delicious', and 'Granny Smith') had decreasing h° from OH to Halo to SB-2. There was a general trend of increasing C* from SB-2 to halo to NB for 'Fuji', 'Gala', 'Golden Delicious', and 'Granny Smith'.

The h° is linearly correlated to the concentrations of total chlorophylls, total carotenoids, idaein, and total quercetin glycosides for 'Fuji' and ‘Delicious’ apples (Table 3). The h° of 'Gala' apples is linearly correlated to the concentrations of total chlorophylls, idaein, and total quercetin glycosides, but not to the concentration of total carotenoids. The h° of 'Golden Delicious' and 'Granny Smith' is linearly correlated to the concentrations of total chlorophylls, total carotenoids, and total quercetin glycosides; however, total carotenoids in ‘Granny Smith' are not as highly correlated to h° as in the other cultivars. Quadratic relationships were not significant (P > 0.15).

**Discussion**

The trends in pigments and chl a/b of the SB-2, halo, and OH peel types of 'Fuji' were similar to those reported in chapter 2. Additionally, comparisons between the OH and NB
peel types of 'Fuji' typically show insignificant differences. It could be argued that the 'Fuji' halo and OH peel types in this experiment could be classified as less severe degrees of sunburn browning than SB-2 based solely on the relative similar trends of changing pigment concentrations. If this were done, the general lack of difference between the OH and NB peel types indicates that the OH peel type could be classified as NB, and that the halo could be classified between NB and SB-2 (e.g. SB-1).

The classification of the halo and OH peel types in this manner is supported by independent studies which showed that the halo was 2 °C cooler than the center of a sunburned area (personal communication, L.E. Schrader). This indicates that the maximum fruit surface temperatures (FST) of the outer peel types (e.g. halo, and OH) did not get as high as the maximum FST of the SB-2 peel. There are many possible reasons why the halo and OH peel types might have lower temperatures and an exhaustive discussion on the factors that are related to apple FST are discussed by Schrader et al. (2003b) and do not need to be reviewed here. Suffice it to say that sunlight is a major factor in determining FST and considering the curvature of the apple surface and the canopy structure of the tree it is fair to suggest that the halo and OH peel types received less direct sunlight than the SB-2 peel.

Assigning specific degrees of sunburn to the 'Fuji' halo and OH peel types was not the intent of this study, but it is relevant to this discussion. It helps explain the results by demonstrating that the different 'Fuji' peel types in this experiment are possibly responding to heat and light stress in the same manner that different degrees of 'Fuji' sunburn browning responded to heat and light stress, and that observed differences in concentrations among the SB-2, halo, and OH peel types can be explained using the same rationale as discussed in
chapter 2. Furthermore, the results of the 'Delicious' pigment analyses of the SB-2, halo, OH, and NB peel types parallel the results of 'Fuji'.

Before moving on in the discussion there is another aspect that should be explored. Although the halo peel appears to be responding to heat and light stress in much the same way that less severe degrees of sunburn would be expected to respond, there are some seemingly minor but potentially important inconsistencies. The results in the previous chapter showed that the SB-1 concentrations of β-carotene, quercetin glycosides, and chlorogenic acid were higher than in the NB but no different from the SB-2 concentrations. This suggested that the slightest burning caused a large initial change in these compounds followed by smaller changes. The results of this study do not fully support this as the trend was not observed in β-carotene (Fig. 2A and B) and quercetin glycosides (Fig. 4A and B), and was observed only in one of the 2 years for the chlorogenic acid (Fig. 6A). If the halo peel were merely a less severely sunburned area than the SB-2 peel (i.e. if it were SB-1), then the β-carotene, chlorogenic acid and quercetin glycoside concentrations might be expected to be non-significantly lower than the concentrations in the SB-2 peel. The results of this experiment hint that changes in the halo and OH peel types may be related to heat and light stress on the SB-2 peel that is transferred to the surrounding peel, and not the result of direct heat and light stress on the halo and OH peels. The physical connection that the SB-2, halo, and OH peel types have in this experiment does not exist between the peels of different degrees of sunburn.

The possibility of indirect effects of heat and light stress could possibly help explain inconsistencies among the OH and NB peel types. The results of this experiment only hint at the possibility that some kind of signaling may play a role. For instance, the relatively low
chl a concentration observed in the 2005 'Fuji' NB peel could indicate that the NB peel was more stressed than both the halo and OH peel types. Additionally, the β-carotene concentrations could be indicating that the OH peel was the least stressed followed by the NB and halo respectively. If some signal from the SB-2 peel promoting the up-regulation of β-carotene synthesis were sent to the surrounding tissue, it could explain why the halo β-carotene concentration is slightly higher than both the OH and NB peels. Furthermore, the 2006 'Fuji' chl a data may imply that the NB and OH peels were stressed equally while the halo received more direct stress. While speculative, a signal from the SB-2 peel could explain higher concentrations of β-carotene in the OH peel than in the NB peel.

The carotenoid concentrations of 'Granny Smith' peel types were consistent between the 2 years; however, they differ from 'Fuji' and 'Delicious'. In fact, the carotenoids trend in the opposite direction. The decrease in both β-carotene and lutein indicate that carotenoid biosynthesis and/or degradation is affected in the SB-2 and OH peels. However, the V + A concentrations in the SB-2, halo, OH, and NB peel types were equal. This indicates that the biosynthetic pathway was not affected, as β-carotene is a precursor to V and A. If biosynthesis is not being affected then the reduction is a result of degradation.

The relative similarity between the decrease in lutein and the reduction in β-carotene indicates a connection between the two. A connection can be found in photosystem II (PSII). Photosystem II is a supramolecular complex of proteins. Some of these proteins contain pigments. Those proteins that contain pigments contain either chl a or both chl a and chl b. The proteins that contain chlorophyll are referred to as chlorophyll-protein complexes (CP). Bassi et al. (1993) determined the carotenoid compositions of the CP complexes and determined that CP47 and CP43 contained β-carotene and lutein and no other carotenoids.
These two CP complexes are closely associated with the reaction center D1 and D2 proteins and upon over-reduction of the reaction center are likely to be damaged. This implies that CP47 and CP43 are the sites of degradation. This is in contrast to 'Fuji' apples where dramatic changes in chl a/b ratios indicated a disproportionate reduction in the amount of light harvesting complex II (LHCII) (Chapter 2). The dramatic increase in chl a/b of 'Fuji' observed again in this study is in stark contrast to the generally insignificant changes in chl a/b of 'Granny Smith' as well as in 'Gala', 'Delicious', and 'Golden Delicious' observed in this study. This provides additional support that a reduction in the amount of LHCII is not the site of chlorophyll degradation in 'Granny Smith'. It also suggests that the disproportionate reduction in the amount of LHCII is not likely the cause of chlorophyll changes in 'Gala', 'Delicious', and 'Golden Delicious'.

The analyses of the chlorophyll and carotenoid concentrations in the various peel types of 'Gala' and 'Golden Delicious' revealed inconsistent results. The results were inconsistent from year to year as well as among cultivars. However, they are somewhat similar to each other. In 2005, neither cultivar had a trend in β-carotene concentrations among the different peel types while in 2006 they had decreasing β-carotene concentrations from OH to SB-2. Additionally, in 2005 both cultivars had higher V + A concentrations in the SB-2 and halo peels than in the OH and NB peels, while in 2006, the concentrations of V + A in the SB-2 and halo peels were either no different, or less than those in the OH and NB peels.

One possible explanation for the inconsistent results of 'Gala' and 'Golden Delicious' from year to year is the prolonged heat wave from 19 July 2006 to 23 July 2006, during which ambient temperatures exceeded 42 °C. This heat wave may have caused carotenoid
degradation similar to that seen in 'Granny Smith' as a result of increased oxidative stress. Although no literature supports this hypothesis; the only two cultivars (i.e. 'Gala' and 'Golden Delicious') that showed inconsistencies between the 2 years were the ones that were harvested the earliest and thus closest to the heat wave. This explanation is incomplete as it does not explain why the 2006 β-carotene concentrations of non-sunburned peel types (NB and blush) of 'Gala' and 'Golden Delicious' are so low compared to the other peel types. At this point there is no simple explanation for these results other than to say that they are probably due to a multitude of factors including but not limited to differences in growing conditions and the individual physiologies of these cultivars.

The observations of idaein in the three red cultivars (i.e. 'Fuji', 'Delicious', and 'Gala') show a remarkably consistent trend of decreasing idaein concentration from NB, to halo, to OH, to SB-2. It was mentioned earlier that the idaein concentrations in 'Fuji' and 'Delicious' were similar and consistent with the results in chapter 2 for 'Fuji'. The similarity among all three red cultivars indicates a consistent decrease in idaein accumulation in response to light and heat stress across the red cultivars.

The trends observed in total quercetin glycosides and chlorogenic acid across the SB-2, halo, OH and NB peel types for all of the cultivars are remarkably similar to each other. However, the results of the epicatechin analysis showed no trends or consistency, with most differences being insignificant. Despite the lack of an increase in epicatechin in these results, it was found in considerable quantities and the argument laid out in chapter 2 concerning the phenolic compounds is supported by these data as well.

The green cultivars (i.e. 'Golden Delicious' and 'Granny Smith') had an additional blush peel type. The results of the blush peel type from both cultivars reveal several
interesting results. First, aside from having the highest anthocyanin concentration of the 'Golden Delicious' and 'Granny Smith' peel types, the characteristics of both cultivars’ blush peel types are quite different from each other. The chlorophyll and carotenoid concentrations observed in the 'Golden Delicious' blush peel were generally similar to the halo peel type, while concentrations in the 'Granny Smith' blush peel were generally similar to the OH and NB peel types. The total quercetin glycoside concentrations in the blush peels were generally similar to the OH peel and halo peel for 'Golden Delicious' and 'Granny Smith', respectively. Although it is not known why some apples of green cultivars develop a blush, the results indicate that the blush correlates with higher concentrations of anthocyanin and quercetin glycosides. The blush peels were the only peels observed in this study to have higher concentrations of both anthocyanins and quercetin glycosides than were found in the NB and OH peel types. This natural coincidental increase in anthocyanin and quercetin glycosides appears to be isolated to the green cultivars. This is supported by the fact that the NB peel (i.e. fully exposed and non-sunburn peel) of the red cultivars had similar quercetin glycoside concentrations to the OH peel.

The high correlations between the h° and the various pigments indicate their relatedness to the color change associated with sunburn and not all cultivars show high correlations for all the pigments. The color change of 'Gala' appears to be more related to the chlorophyll and idaein concentrations than to the carotenoids and quercetin glycosides, while the color change of 'Granny Smith' is more dependent on the quercetin glycosides and chlorophyll. The color change associated with sunburn of 'Fuji', 'Delicious', and 'Golden Delicious' appears to be related to all pigments that are found in consistently detectable quantities. These associations need to be interpreted with caution and are not meant to infer
that pigments that are poorly correlated with changes in h° do not affect the color or that
changes in these pigment concentrations would not result in color change. Poor correlations
only indicate that those pigments did not change consistently with the change in h° as a result
of sunburn in that cultivar. It should also be mentioned that these correlations are not a
method of determining pigment concentrations or ratios from h°, because a single h° can be
obtained from many combinations of these pigments.

In conclusion, comparisons among the different peel types reveal significant
differences in nearly all the compounds quantified in all of the cultivars. In general, the
changes in chlorophyll, chlorogenic acid, idaein and quercetin glycoside concentrations were
consistent among the five cultivars. However, in 'Granny Smith', β-carotene and lutein
increased as the distance from the sunburned area increased, but in 'Fuji' and 'Delicious', β-
carotene decreased and lutein did not change. The significance of the differential responses
of β-carotene and lutein among the cultivars is unknown. Further study may be warranted to
better understand the metabolic differences among the cultivars and to ascertain how these
metabolic differences relate to pigment changes associated with sunburn. As concluded in
chapter 2 the changes in concentrations of the green, yellow and red pigments associated
with sunburn result in different pigment ratios in the peel and in turn results in a change in
peel color that we see as sunburn.
Literature Cited


Plate 1. Separation of the sun-exposed side of sunburned apples into the sunburned area, the area outside the sunburned area (halo), and the area outside the halo (OH).
Table 1. 2005 and 2006 mean chlorophyll a/b ratios for SB-2, halo, OH, NB, and blush peel types. Means with the same letter in parenthesis are not significantly different (P ≤ 0.05). Comparisons were made within cultivars, but not between cultivars. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no sunburn.

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</tr>
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<td>Gala</td>
</tr>
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</tr>
<tr>
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<td>2.5 (a)</td>
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<td>2.3 (b)</td>
</tr>
<tr>
<td>NB</td>
<td>2.2 (d)</td>
<td>2.4 (ab)</td>
</tr>
<tr>
<td>Blush</td>
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<td>NA</td>
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</tbody>
</table>

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Table 2.  2006 colorimetric data for 'Fuji', 'Gala', 'Delicious', 'Golden Delicious', and 'Granny Smith' apple. Means with the same letter in parenthesis are not significantly different (P ≤ 0.05). L*, lightness factor; \( h^\circ \), hue angle; C*, chroma; SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no sunburn.

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<th>( h^\circ )</th>
<th>C*</th>
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Table 3. Linear equations for the linear regression of hue angle against total chlorophylls, total carotenoids, idaein, and total quercetin glycosides.

<table>
<thead>
<tr>
<th></th>
<th>Fuji</th>
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† Hue angle = a + b (concentration)

ND, idaein was not consistently detectable in 'Golden Delicious' and 'Granny Smith'
Fig. 1A. Chlorophyll a (chl a) and chlorophyll b (chl b) concentrations in µg·g⁻¹ fresh wt. for 'Fuji' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 1B. Chlorophyll a (chl a) and chlorophyll b (chl b) concentrations in µg·g⁻¹ fresh wt. for 'Gala' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 1C. Chlorophyll a (chl a) and chlorophyll b (chl b) concentrations in µg·g⁻¹ fresh wt. for 'Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 1D. Chlorophyll a (chl a) and chlorophyll b (chl b) concentrations in µg·g⁻¹ fresh wt. for 'Golden Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 1E. Chlorophyll a (chl a) and chlorophyll b (chl b) concentrations in $\mu$g·g$^{-1}$ fresh wt. for 'Granny Smith' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P $\leq$ 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 2A. 2005 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in μg·g⁻¹ fresh wt. for 'Fuji' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 2B. 2006 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in µg·g⁻¹ fresh wt. for 'Fuji' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 2C. 2005 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in µg·g⁻¹ fresh wt. for 'Gala' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
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Fig. 2F. 2006 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in μg·g⁻¹ fresh wt. 'Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 2G. 2005 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in μg·g⁻¹ fresh wt. 'Golden Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 2H. 2006 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in µg·g⁻¹ fresh wt. 'Golden Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 2I. 2005 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in μg·g⁻¹ fresh wt. 'Granny Smith' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 2J. 2006 β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in µg·g⁻¹ fresh wt. 'Granny Smith' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 3A. 2005 and 2006 idaein concentrations in mg·g⁻¹ fresh wt. for 'Fuji' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 3B. 2005 and 2006 idaein concentrations in mg·g⁻¹ fresh wt. for 'Gala' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 3C. 2005 and 2006 idaein concentrations in mg·g⁻¹ fresh wt. for 'Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn.
Fig. 3D. 2005 and 2006 idaein concentrations in $\mu$g·g$^{-1}$ fresh wt. for 'Golden Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different ($P \leq 0.05$). Mean comparisons were made within years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 3E. 2005 and 2006 idaein concentrations in \( \mu g\cdot g^{-1} \) fresh wt. for 'Granny Smith' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (\( P \leq 0.05 \)). Mean comparisons were made within years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Fig. 4A. 2005 total quercetin glycoside concentrations in mg g⁻¹ fresh wt. for the applicable peel types of 'Fuji', 'Gala', 'Delicious', 'Golden Delicious', and 'Granny Smith' apples. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within cultivars. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no sunburn.
Fig. 4B. 2006 total quercetin glycoside concentrations in mg·g⁻¹ fresh wt. for the applicable peel types of 'Fuji', 'Gala', 'Delicious', 'Golden Delicious', and 'Granny Smith' apples. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within cultivars. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no sunburn.
Fig. 5A. 2005 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Fuji' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 5B. 2006 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Fuji' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 5C. 2005 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Gala' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

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Fig. 5D. 2006 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Gala' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

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Fig. 5E. 2005 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 5F. 2006 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 5G. 2005 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Golden Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 5H. 2006 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Golden Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 5. 2005 concentrations of individual quercetin glycosides in mg·g\(^{-1}\) fresh wt. for 'Granny Smith' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

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Fig. 5J. 2006 concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Granny Smith' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 6A. 2005 and 2006 chlorogenic acid and epicatechin concentrations in mg·g⁻¹ fresh wt. for 'Fuji' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; CA, chlorogenic acid.
Fig. 6B. 2005 and 2006 chlorogenic acid and epicatechin concentrations in mg·g⁻¹ fresh wt. for 'Gala' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; CA, chlorogenic acid.
Fig. 6C. 2005 and 2006 chlorogenic acid and epicatechin concentrations in mg·g⁻¹ fresh wt. for 'Delicious' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; NB, no sunburn; CA, chlorogenic acid.
Fig. 6D. 2005 and 2006 chlorogenic acid and epicatechin concentrations in mg·g⁻¹ fresh wt. for ‘Golden Delicious’ SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds in individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn; CA, chlorogenic acid.
Fig. 6E. 2005 and 2006 chlorogenic acid and epicatechin concentrations in mg·g⁻¹ fresh wt. for 'Granny Smith' SB-2, halo, OH, and NB peel types. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual years. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, outside the halo; Blush, some red color and no sunburn; NB, no red color and no sunburn.
Changes in Pigment Concentrations Associated with 'Fuji' Stain

ADDITIONAL INDEX WORDS. *Malus domestica*, apple, chlorophyll, carotenoids, anthocyanin, quercetin, discoloration

ABSTRACT. 'Fuji' apples (*Malus domestica* Borkh cv. 'Fuji') that develop stain during cold storage are down graded or even culled depending on the severity. This post-harvest disorder is unique to ‘Fuji’ and is a discoloration of the peel on the sun-exposed side of apples. Because this disorder is a peel discoloration, peel pigments were hypothesized to be involved. For this reason the concentrations of chlorophylls, carotenoids, anthocyanins, and other phenolic compounds in peel disks taken from areas exhibiting stain were compared to the concentrations observed in other 'Fuji' apple peel types that did not exhibit stain discoloration. In the first experiment stained peel from sunburned apples exhibiting 'Fuji' stain was compared to peel from three areas of sunburned apples not exhibiting stain (i.e. sunburned peel, the area around the sunburned peel (halo), and the area around the halo (OH)). Additionally, the stained peel was compared to the sun-exposed side of non-stained non-sunburned apples (NSNB). The second experiment compared stained peel to NSNB peel and the area outside the stained area (OS) on those fruit with stain. The chlorophyll and \(\beta\)-carotene results were not consistent between the two experiments. The concentrations of lutein, violaxanthin, and antheraxanthin were consistently higher in the stained peel but were generally insignificant. The concentrations of idaein, quercetin glycosides, and epicatechin were consistently low in the stained peel of both experiments. This is in contrast to our earlier studies in which quercetin glycosides and epicatechin were higher in sunburned peel
of ‘Fuji’ and inversely related to idaein. This consistent and unique characteristic of stained peel indicates an association of these compounds with the incidence of stain.

The reduced idaein and quercetin glycoside concentrations help explain the discoloration associated with 'Fuji' stain, as lower concentrations would allow the chlorophylls and carotenoids to be more apparent. Thus even though we were unable to determine consistent and significant concentration changes in chlorophylls and carotenoids, their presence contributes to the overall discoloration associated with stain.
One of the main factors affecting marketability of apples is their appearance. As such, any blemishes or discolorations reduce their value. One such discoloration that occurs on ‘Fuji’ apples is stain. 'Fuji' stain is an erratic post-harvest storage disorder that is neither well researched nor well understood. It appears to be unique to 'Fuji' as we have not observed this disorder in several other cultivars stored under the same conditions. Typically, the discoloration first appears after one or more months of storage with longer storage times resulting in increased discoloration (i.e. more surface area and darker color) and increased incidence (i.e. more apples affected) (Schrader et al., 2004). The color of 'Fuji' stain is usually referred to as greenish grey, dark brown, purple or muddy (Plate 1).

Gong and Mattheis (2003) reported that stain was induced by exposure to supplemental ultraviolet-B (UV-B) radiation and that it was accompanied by enhanced NADH oxidase and phenylalanine ammonia-lyase (PAL) activities. 'Fuji' stain has also been reported to be associated with apple sunburn where it developed in the area surrounding the sunburned area or the halo area (Schrader et al., 2003). The incidence of stain was much higher in fruit that had more serious sunburn. Schrader et al. (2003) suggest that stain appearing in the halo area may be induced by high fruit surface temperatures and that there may be other types of stain as it was not always found in the halo area. Schrader et al. (2004) later reported that the use of RAYNOX®, a lipophilic UV-B blocking sunburn protectant, to decrease sunburn also reduced the incidence of ‘Fuji’ stain during cold storage by more than 50%. This implies that decreasing UV-B absorption in apples sprayed with RAYNOX® decreased' Fuji' stain, and is consistent with the earlier report of Gong and Mattheis (2003).

The focus of the work presented here is on naturally occurring 'Fuji' stain. The intent of this study was to determine if there are any changes in concentrations of chlorophylls,
carotenoids, anthocyanins, and other related compounds in the apple peel that can be associated with the discoloration associated with 'Fuji' stain.

Materials and Methods

The first experiment was conducted on 'Fuji' apples grown in the 2005 growing season. 1200 apples were harvested into 42 lb. boxes from an orchard located at the Washington State University – Tree Fruit Research and Extension Center (WSU-TFREC) in Wenatchee, Wash. The apples were sorted into sunburned and non-sunburned lots and then stored in regular atmosphere cold storage for 4 months at 0.5 °C. After 4 months of storage, stained apples were sorted out and peel disks (16 mm diameter, 1mm thick) of the sunburn (SB-2), halo, outside the halo (OH), and non-stained non-sunburned (NSNB) areas were taken as described in chapter 3. Samples were taken from 10 apples and analyzed individually. Due to the small amount of stained area on each apple it was not possible to harvest the desired four peel disks from each apple. As a result 20 apples were used and two disks from each of the 10 stained apples were used for chlorophyll and carotenoid analysis, and two disks from each of the other 10 stained apples were used for the phenolic analyses.

Due to the low incidence of stain in the orchard used in the first experiment and the general unpredictability of stain, the second experiment was conducted by collecting apples from a packing house once stain had developed. Additionally, it was decided to pool samples. Samples consisted of peel disks (12 mm diameter, 1 mm thick) taken from the stained area of stained apples, the area outside the stained area (OS), and the sun-exposed side of non-stained non-sunburned apples (NSNB) after 2 months of regular atmosphere cold storage at 0.5 °C. Twenty apples were used and two peel disks were taken from each apple. Four disks from four different apples were pooled to make one sample, making sure that one
disk from each apple was analyzed for chlorophylls and carotenoids and the other disk was analyzed for phenolics. All peel disks were flash frozen in liquid nitrogen immediately after removal from the apple and stored at -80 °C until analysis.

**Extractions and Pigment Analyses.** The extraction and pigment analysis methods used were the same as described in chapter 2.

**Peel Color Analyses.** In 2006, before peel disk samples were taken, the color of the stain, OS, and NSNB of each apple to be sampled was determined using a colorimeter (CR-300 Chroma Meter, Minolta Corp., Osaka, Japan). The CIE L*a*b* (L*, lightness factor) color space was used and the hue angle (h°, \(\tan^{-1}(b*/a*)\)) and chroma (C*, \(\sqrt{(a*)^2 + (b*)^2}\)) were calculated (McGuire, 1992).

**Statistical Analyses.** One-way analysis of variance (ANOVA) was performed to determine if significant differences existed among the pigment concentrations of the peel types. Fisher’s least significant differences (LSD) were calculated when appropriate to determine which means were statistically different (P < 0.05; Proc GLM; Means/LSD; SAS Institute Inc.).

**Results**

**Chlorophyll Analyses.** In the first experiment there was a general decrease in chl a and chl b concentrations moving from the NSNB to the SB-2 peel with no significant difference between chl a concentrations in the NSNB, halo and OH peel types but each being significantly higher than in SB-2 peel (Fig. 1A). For chl b, SB-2 and halo had significantly lower concentrations than OH, stain, and NSNB. The chl a and chl b concentrations of the stained area were significantly higher than in all the other peel types. The chlorophyll analyses of the second experiment revealed no differences in chl a and chl b concentrations
between the stained area and OS (Fig. 1B). A significantly lower concentration of chl b was found in the NSNB peel than was found in the stain and OS peels. The chlorophyll a/b ratio (chl a/b) of the stained peel of the first experiment was intermediate to the halo and OH peel types and slightly higher than the NSNB peel, but lower than SB-2 peel (Table 1). In the second experiment, the chl a/b of the stained peel was no different from the OS peel and was slightly lower than the NSNB peel (Table 1).

**CAROTENOID ANALYSES.** The β-carotene analyses in the first experiment showed increasing concentrations from the OH to the SB-2 peel, but the difference between the SB-2 and halo samples was not significant (Fig. 2A). The β-carotene concentration of the stained peel was no different from that in any of the other peel types (Fig. 2A). In the second experiment the β-carotene concentration was significantly higher in the stained peel than in the OS or NSNB peels (Fig. 2B).

In the first experiment, lutein, violaxanthin (V), and antheraxanthin (A) concentrations showed a general increase from SB-2 to halo to OH to NSNB to stain (Fig. 2A). The SB-2 concentration of lutein was significantly lower than in the other peel types and the concentration of lutein in the stain peel was significantly higher than all peel types except NSNB (Fig. 2A). The concentration of V was significantly higher in the stain peel than in SB-2. The concentration of A in the stain peel was significantly higher than in SB-2, halo, and OH. In the second experiment, very few differences in lutein, V, and A concentrations were observed among the different peel types (Fig. 2B). Only the concentrations of A in the NSNB and stain peels were significantly higher than the concentration in OS. Zeaxanthin was not detected in either study, likely because the apples were cold stored in the dark over night before peel disks were collected.
**Phenolic Analyses.** In the first experiment, there was a trend of decreasing idaein concentration from NSNB to OH to halo to SB-2 peel types, with an insignificant difference observed between the halo and OH peels (Fig. 3A). The idaein concentration of the stained peel and SB-2 peel was significantly lower than that in the halo, OH, and NSNB peels. The idaein concentration of the stained peel in the second experiment was significantly lower than OS peel and NSNB peel (Fig. 3B). The concentration of idaein in the OS peel was significantly lower than in the NSNB peel.

In the first experiment, quercetin glycoside concentrations generally decreased significantly from SB-2 to halo to OH (Fig. 4A). Quercetin glycoside concentrations in the stained area were comparable to but lower than the concentrations observed in the NSNB and OH peels (Fig. 4A). Quercetin 3-xyloside (Xyl) and quercetin 3-arabinopyranoside (Arap) were the only quercetin glycosides whose concentration in the stain peel was significantly lower than that observed in the NSNB peel. In the second experiment, lower concentrations of the individual quercetin glycosides were observed in the stained area as compared to the NSNB and OS peels (Fig. 4B). The differences between the stained peel and the NSNB peel were always significant, but the differences between the stained peel and the OS peel were not (Fig. 4B).

In the first experiment, the chlorogenic acid concentration in the SB-2 peel was significantly higher than that found in the halo, OH, and stain peels (Fig. 3A). Additionally, there was a general trend of decreasing concentration from SB-2 to halo to OH. In the second experiment chlorogenic acid concentrations were significantly higher in the stained peel than in the NSNB and OS peels (Fig. 3B).
In the first experiment, epicatechin concentrations showed a general decrease from SB-2 to halo to OH, with SB-2 and halo epicatechin concentration being significantly higher than in OH (Fig. 3A). The epicatechin concentrations in the NSNB, SB-2, and halo peel types were statistically the same. The epicatechin concentration of the stained area in the second experiment was significantly lower than OS but not NSNB (Fig. 3B).

**Colorimetric Data.** The colorimetric data indicate that the L* of the stain and NSNB peels are no different from each other, but both are significantly lower than that of the OS peel (Table 2). The h° decreased significantly from stain to OS to NSNB while the C* increased significantly.

**Discussion**

We believe stain to be unique to 'Fuji' apples as we have not observed this disorder in other cultivars stored under similar conditions. There are reports from Japan that the cultivar ‘Hokuto’ develops a disorder that is also called “stain”. Noro et al. (1996; 1998) suggested that stain in 'Hokuto' apples may be related to apple scald as they were able to induce stain in 'Hokuto' and scald in ‘Mutsu’ apples using trans-2-hexenal. Given that 'Hokuto' is the offspring of 'Fuji' and ‘Mutsu’, the reaction of 'Hokuto' to trans-2-hexenal could be due to scald susceptibility acquired from its ‘Mutsu’ parentage and thus not related to 'Fuji' stain. Additionally, the stain we observe in ‘Fuji’ apples is associated with sunburn and is found on the sun-exposed side of the apple while scald is usually associated with the shaded side of susceptible apple cultivars. To our knowledge the induction of 'Fuji' stain using trans-2-hexenal has not been reported. Given that 'Hokuto' stain is linked to scald development while 'Fuji' stain is not, we believe that the two disorders are different despite their similar names.
'Fuji' stain was the focus of this study and the intent was to determine if the discoloration associated with 'Fuji' stain was due to different concentrations of various pigments in the cells of the affected tissue. The first experiment compared the stained peel of stained apples with different types of peel from non-stained sunburned apples (i.e. SB-2, halo, OH) as well as non-stained non-sunburned (i.e. NSNB) peel from non-stained non-sunburned apples. The premise was that stain is typically associated with sunburn and often appears outside the sunburned area (i.e. in the halo or OH areas). Hence, a comparison between the stained peel and the OH and halo peels should be valid as they would have experienced the most similar environmental conditions. The premise of the second experiment was much simpler. Measure the pigment concentrations of two different colored peel types that are side by side on the apple (e.g. stain and OS) and see which pigments differ. The results of the two experiments show that there are indeed differences between the stained peel and the various peel types. But what does this mean?

The results of the first experiment showing significantly higher chl a and chl b concentrations in the stained peel indicate that chlorophylls may be related to the stain discoloration. However, the second experiment showed no differences in chlorophyll concentrations between the stained and OS peels. Additionally, the chl a/b ratio of the stained peel in the first experiment was non-significantly higher than in the NSNB, but in the second experiment the chl a/b ratio of the stained peel was significantly lower than that of the NSNB peel. The discrepancy between the two experiments makes it difficult to determine how chlorophylls change in relation to stain. The apples in the two experiments were from different orchards and thus many factors were different. Differences between the orchards could have directly caused the differences in chlorophyll concentrations or could have
resulted in the differential expression of stain in each orchard. Thus, despite being inconsistent, changes in chlorophylls cannot be ruled out as a characteristic of 'Fuji' stain.

The $\beta$-carotene results of the second experiment which showed higher concentrations in the stained area seemed to indicate that it may play a role. However, the first experiment showed that $\beta$-carotene concentrations in the stained area were only higher than in the OH peel, and even then this difference was not significant. As was discussed with regard to the chlorophylls the discrepancy between the two experiments may be the result of the differential expression of 'Fuji' stain in different orchards.

Changes in lutein concentration might be related to the discoloration. In both years the stain peel had the highest concentrations; however, the lack of significance among the peel types in the second experiment makes it difficult to discern whether lutein is changing. Violaxanthin and antheraxanthin may also be related as they were found in higher concentrations in the stained peel than in the other peels in the first experiment and in higher concentrations as compared to the OS peel of the second experiment. However, just as with lutein, their relationship to stain is clouded by the general lack of significant differences observed between the stain peel and the other peel types.

The most intriguing results are those of the idaein and quercetin glycoside analyses. The results from both years showed that in comparison to the other peel types the stain peel had low concentrations of both idaein and quercetin glycosides. Although the concentrations were not necessarily significantly lower than all the peel types, the relatively low concentrations of idaein and quercetin glycosides found in concert has not been seen in the sun-exposed peel of 'Fuji' apples or in other cultivars that we have studied. Our previous work on apple sunburn showed a decrease in idaein and a concomitant increase in quercetin
glycoside concentrations (chapter 2 and 3). The only exception to this was the blushed peel of 'Granny Smith' which showed increased levels of both idaein and the quercetin glycosides (Chapter 3). This characteristic of stained peel distinguishes it from the other sun-exposed peel types and indicates that the differences observed in the stained peel are not changes that have been associated with sunburn. This is not to say that sunburn is not related to the pigment changes in stained tissue, but that the effects are secondary at best.

Because epicatechin and chlorogenic acid do not absorb visible light, changes in their concentrations cannot be directly linked to the discoloration of stained peel. However, changes in their concentrations provide some helpful information because they share many biosynthetic steps with idaein and the quercetin glycosides. Idaein, quercetin glycosides, epicatechin, and chlorogenic acid are formed via a common pathway. All are formed from p-Coumaroyl-CoA which is formed via the phenylpropanoid pathway (shikimic acid pathway). However, idaein, quercetin glycosides, and epicatechin require the additional step of fusing orsellinic acid (product from the polyketide pathway) to p-Coumaroyl-CoA (product of the phenylpropanoid pathway) to make a chalcone.

The concentration of epicatechin in the stain peel was low in both years while the chlorogenic acid concentrations were inconsistent between the 2 years. The fact that idaein, quercetin glycosides, and epicatechin were found in lower concentrations while chlorogenic acid was not suggests a reduction in the portion of the biosynthetic pathway that is not shared by chlorogenic acid. It is also possible that there is selective degradation of idaein, the quercetin glycosides, and epicatechin.

The color of 'Fuji' stain is usually referred to as greenish grey, dark brown, purple or muddy. Using the L*, h°, and C* values objectively describes the color of the 'Fuji' stain in
this study and allows readers to reproduce the color (i.e. on a computer monitor). Using the values for stain given in table 2 a muddy brownish grey color is achieved. These values can also be used to objectively define color changes between the different peels. Doing so shows that the $h^\circ$ increased from NSNB to OS to stain which indicates decreased redness and is consistent with the reduced idaein (i.e. red anthocyanin) concentrations.

In conclusion, the simultaneously low concentrations of idaein, quercetin glycosides, and epicatechin have not been observed in our earlier research on sun-exposed apple peel and appear to be defining characteristics of 'Fuji' stain. Even though consistent trends were not observed in chlorophyll and $\beta$-carotene between the two experiments their presence does affect the overall appearance and final color of the stained area. Their continued presence along with reduced concentrations of idaein accounts for the higher $h^\circ$ of stained peel which not only indicates that the stained peel is less red but also more yellow and green.
Literature Cited


Plate 1. 'Fuji' apples with 'Fuji' stain in the periphery of sunburn browning.
Table 1. Chlorophyll a/b ratios for experiments 1 and 2. Means with the same letter in parenthesis are not significantly different (P ≤ 0.05). SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; OS, outside stained area; Stain, stained peel.

<table>
<thead>
<tr>
<th>Exp. 1</th>
<th>Chl a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB-2</td>
<td>2.5 (a)</td>
</tr>
<tr>
<td>Halo</td>
<td>2.5 (a)</td>
</tr>
<tr>
<td>OH</td>
<td>1.9 (b)</td>
</tr>
<tr>
<td>NSNB</td>
<td>2.0 (b)</td>
</tr>
<tr>
<td>Stain</td>
<td>2.3 (ab)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Exp. 2</th>
<th>Chl a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSNB</td>
<td>2.7 (a)</td>
</tr>
<tr>
<td>OS</td>
<td>2.5 (b)</td>
</tr>
<tr>
<td>Stain</td>
<td>2.5 (b)</td>
</tr>
</tbody>
</table>

Table 2. 2006 colorimetric data. Means with the same letter in parenthesis are not significantly different (P ≤ 0.05). L*, lightness factor; h°, hue angle; C*, chroma; NSNB, no stain and no sunburn apple; OS, outside stained area; Stain, stained peel.

<table>
<thead>
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<th></th>
<th>L*</th>
<th>h°</th>
<th>C*</th>
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<td>Stain</td>
<td>44.1 (b)</td>
<td>64.7 (a)</td>
<td>13.7 (c)</td>
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<tr>
<td>OS</td>
<td>49.7 (a)</td>
<td>49.6 (b)</td>
<td>19.4 (b)</td>
</tr>
<tr>
<td>NSNB</td>
<td>43.9 (b)</td>
<td>30.9 (c)</td>
<td>29.3 (a)</td>
</tr>
</tbody>
</table>
Fig. 1A. Chlorophyll a and chlorophyll b concentrations in $\mu$g·g$^{-1}$ fresh wt. for 'Fuji' SB-2, Halo, OH, NSNB, and Stain peel types after 4 months of regular atmosphere storage. Bars that have the same letter above them are not significantly different ($P \leq 0.05$). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; Stain, stained peel.
Fig. 1B. Chlorophyll a and chlorophyll b concentrations in μg·g⁻¹ fresh wt. for 'Fuji' NSNB, OS, and Stain peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. NSNB, no stain no sunburn apple; OS, area outside the stained area; Stain, stained area.
Fig. 2A. β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in µg·g⁻¹ fresh wt. for 'Fuji' SB-2, Halo, OH, NSNB, and Stain peel types after 4 months of regular atmosphere storage. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; Stain, stained peel.
Fig. 2B. β-carotene, lutein, violaxanthin (V), and antheraxanthin (A) concentrations in µg·g⁻¹ fresh wt. for 'Fuji' NSNB, OS, and Stain peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. NSNB, no stain no sunburn; OS, area outside the stained area; Stain, stained area.
Fig. 3A. Idaein, chlorogenic acid, and epicatechin concentrations in mg·g⁻¹ fresh wt. for 'Fuji' SB-2, Halo, OH, NSNB, and Stain peel types after 4 months of regular atmosphere storage. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; Stain, stained peel.
Fig. 3B. Idaein, chlorogenic acid, and epicatechin concentrations in mg·g⁻¹ fresh wt. for 'Fuji' NSNB, OS, and Stain peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. NSNB, no stain no sunburn apple; OS, area outside the stained area; Stain, stained area.
Fig. 4A. Concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Fuji' SB-2, Halo, OH, NSNB and Stain peel types 4 months of regular atmosphere storage. Bars that have the same letter above them are not significantly different (P < 0.05). Mean comparisons were made within individual compounds. SB-2, sunburn degree of 2; Halo, area immediately surrounding sunburn area; OH, area outside the halo; NSNB, no stain and no sunburn apple; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnoside; Quer, quercetin.

* denotes tentative identification
Fig. 4B. Concentrations of individual quercetin glycosides in mg·g⁻¹ fresh wt. for 'Fuji' NSNB, OS, and Stain peel types. Bars that have the same letter above them are not significantly different (P ≤ 0.05). Mean comparisons were made within individual compounds. NSNB, no stain no sunburn apple; OS, area outside the stained area; Stain, stained area; Gal, quercetin 3-galactoside; Glu/Rut, quercetin 3-glucoside and quercetin 3-rutinoside; Xyl, quercetin 3-xyloside; Arap, quercetin 3-arabinopyranoside; Araf, quercetin 3-arabinofuranoside; Rham, quercetin 3-rhamnose; Quer, quercetin.

* denotes tentative identification
OVERALL SUMMARY AND CONCLUSIONS

The economic losses to the apple industry resulting from sunburn and 'Fuji' stain are great and these losses are a direct result of the discoloration associated with these disorders. As such, an in-depth analysis of the compounds involved in apple color development and how changes in concentrations of these compounds are associated with sunburn and stain is an important step to better understand these disorders.

In chapter 2, 'Fuji' apples were divided into classes or degrees of sunburn and the pigments and associated compounds characterized at harvest maturity. It was determined that the concentrations of chlorophylls a (chl a), chlorophyll b (chl b), and the anthocyanin, idaein, decreased with increased sunburn severity. The quercetin glycosides and the xanthophylls, violaxanthin (V) and antheraxanthin (A) increased sharply from non-sunburned apples (NB) to the lowest severity of sunburn (SB-1). Differences in quercetin glycoside concentrations among apples with sunburn (SB-1 to SB-4) were more moderate while differences in V and A were generally insignificant. Dramatic increases in the chl a/b ratios with increasing sunburn severity indicated a reduction in the amount of light harvesting complex II where most of the chlorophyll b is located. The h° was linearly correlated to the concentrations of total chlorophylls, total quercetin glycosides, and idaein, while it was not correlated to the concentrations of total carotenoids.

In chapter 3, pigment concentrations across the sun-exposed side of sunburned apples were compared to each other and also to the sun-exposed side of non-sunburned apples (NB). The sun-exposed side of sunburned apples was divided into three areas: the sunburned area (SB-2), the area immediately surrounding the sunburned area (halo) and the area immediately surrounding the halo (OH). Additionally, five cultivars (e.g. 'Fuji', 'Gala', ‘Delicious’,
'Golden Delicious', and 'Granny Smith') were compared. It was determined that the chlorophyll concentrations decreased and the quercetin glycoside concentrations increased from OH to halo to SB-2 for all five cultivars. Differences in chlorophyll and quercetin glycoside concentrations between the NB and OH were generally insignificant for all five cultivars. The trends in anthocyanin (e.g. idaein) concentrations were consistent among the red cultivars (i.e. 'Fuji', 'Gala', and ‘Delicious’) where it decreased from NB to OH to halo to SB-2. Differences in the carotenoids were cultivar dependent. The concentrations of the β-carotene and lutein decreased from OH to halo to SB-2 in 'Granny Smith'. In 'Fuji' and ‘Delicious’ the concentration of β-carotene increased from OH to halo to SB-2 with generally no change in lutein concentration. The carotenoids in 'Gala' and 'Golden Delicious' had inconsistent responses from year to year. The chl a/b ratios increased dramatically in 'Fuji' from NB to OH to halo to SB-2. The chl a/b ratios in the other cultivars did not increase dramatically as observed in 'Fuji' and in many instances, the chl a/b ratio in the SB-2 peel was not the highest. In general the h° was highly correlated to the concentrations of total chlorophylls and total quercetin glycosides. Additionally, the h° was well correlated to the idaein concentrations in the red cultivars (i.e. 'Fuji', 'Gala', and 'Delicious'). The h° was highly correlated to the concentration of total carotenoids for 'Fuji', 'Delicious', and 'Golden Delicious'.

The study in chapter 4 looked at 'Fuji' stain and used two different experimental approaches to explore the possibility that the discoloration was the result of changes in pigment concentrations. The first approach compared stained peel to various types of non-stained peel (e.g. SB-2, halo, and OH on one apple, and non-stained non-sunburned peel (NSNB) from a different apple). The rationale for this was that 'Fuji' stain, which occurs on
the sun-exposed side of ‘Fuji’ apples, is associated with sunburn and typically occurs in the halo and OH areas. The second approach compared the pigment concentrations in stained peel to those of the peel immediately outside the stained peel (OS) and to another NSNB peel. The results of the two experiments determined that the stained peel consistently contained low concentrations of quercetin glycosides and anthocyanins. This is very different from the results presented in chapters 2 and 3 which demonstrated that in sun-exposed peels (i.e. SB-1 to 4, halo, OH, and NB) of 'Fuji' apples, quercetin glycoside concentrations were high when idaein concentrations were low, and vice versa. This indicates that the occurrence of simultaneously low concentrations of quercetin glycosides and idaein is a unique characteristic of 'Fuji' stain.

In general, the results presented in chapters 2 and 3 pertaining to 'Fuji' apples are consistent. As discussed in chapter 3 some minor inconsistencies indicate that the concentrations of carotenoids and quercetin glycosides in the halo and OH peels may be influenced indirectly by stress factors (i.e. heat and light) that are directly affecting the SB-2 area. This helps explain why the h° was not highly correlated to total carotenoids with respect to the degree of sunburn (chapter 2) but it was correlated with respect to SB-2, halo, OH, and NB peel types (chapter 3).

The results presented in chapter 3 demonstrate that all apple cultivars do not respond similarly to stress. The differential responses observed among the cultivars are likely due to a wide range of factors. The effects that genetics and gene expression have on the phenotype and the physiology of apples appear to be important factors. The simplest example is the different trends in carotenoids observed between 'Granny Smith' (green apple) and 'Delicious' (red apple).
All three of the studies presented in this dissertation deal with the color of apples. In each study changes in the h° corresponded to changes in concentration of at least one pigment. The studies in chapters 2 and 3 showed high correlations between the h° and the concentrations of several pigments. In chapter 4, no correlation analysis was performed but the change in h° indicated a loss of red color which corresponded with the decreased idaein concentration. The results indicate that changes in pigment concentrations are involved in the color changes associated with sunburn and 'Fuji' stain. The effect that changes in pigment concentrations have on the overall color of the apple can be thought of as color mixing, where the addition or subtraction of one or more colors results in a different color. However, the final color is determined by the pigments that remain whether they changed or not. Color mixing not only explains the differences in color between peel types of a single cultivar (e.g. 'Fuji' SB-2 is a different color than 'Fuji' NB) but also explains differences in color among the same peel types of the different cultivars (e.g. 'Granny Smith' SB-2 is not the same color as 'Fuji' SB-2).

Although a great deal has been learned about apple sunburn and 'Fuji' stain and how differences in pigment concentrations are associated with them, some very important questions have not been answered. Is light or photo-oxidative stress responsible for the differences in concentrations of chlorophylls a and b between peel types? How is anthocyanin accumulation reduced while quercetin glycoside concentrations increase? Why does the β-carotene concentration in 'Granny Smith' trend differently than 'Fuji' and ‘Delicious’? Does the β-carotene concentration in 'Granny Smith' trend differently than 'Fuji' with respect to sunburn severity? These are just a few of the questions that have arisen from the results of these studies.
To answer some of these questions subsequent research is needed to investigate possible correlations between changes in pigment concentrations and changes in antioxidant system metabolites and enzymes. Changes in the metabolites, ascorbic acid and glutathione, and the enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAHR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and catalase (CAT), could provide information on the levels of oxidative stress within each of the peel types. The relative levels of oxidative stress could be compared to changes in pigment concentrations to determine if a relationship exists. Doing so would help determine if the changes observed here were the results of differences in light level or the result of the oxidative stress.

In conclusion, the results presented in chapters 2 and 3 indicate that there are indeed differences in pigment concentrations that are associated with sunburn. Chlorophyll, idaein and quercetin glycoside concentrations trend similarly among the five cultivars. However, carotenoid concentrations do not trend similarly among the five cultivars and demonstrate the need to conduct research on individual cultivars due to their unique physiologies and horticultural requirements. The results presented in chapter 4 indicate that the stained peel of 'Fuji' differs from the OS, SB-2, halo, OH, and NSNB peel types. Additionally, the changes in color that are associated with sunburn and stain and their associations with changes in pigment concentrations demonstrate the important role that pigments play in the development of the discolorations.