FINAL PROJECT REPORT
WTFRC Project Number: #AH-05-506 (WSU Project #13C-3655-6325)

Project Title: Improving fruit finish and fruit quality in apples

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Total Project Funding: Year 1: 111,822 **Year 2:** 115,337 **Year 3:** 116,753

Budget History:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries	**$74,336**	**76,378**	**78,280**
Benefits	**22,586**	**23,859**	**23,273**
Wages			
Benefits			
Equipment	**0**	**0**	
Supplies	**8,500**	**8,500**	**8,500**
Travel	**3,400**	**3,500**	**3,500**
Miscellaneous[1]	**3,000**	**3,100**	**3,200**
Total	**$111,822**	**$115,337**	**$116,753**

[1]Funds transferred to Dr. Gordon Brown, Cooperator at Scientific Horticulture, Tasmania, Australia

Overall objective: To understand the factors that influence fruit finish of apples and develop and implement management practices that will lead to better fruit finish for growers and to investigate the impact heat-induced disorders have on fruit quality during cold storage. Specific objectives related to improving fruit finish and fruit quality are outlined below:

Specific objectives:

I. Examine postharvest internal fruit quality as affected by preharvest skin disorders such as sunburn, stain, "flecking" in Fuji apples, and russet in Golden Delicious. Firmness, soluble solids, and titratable acidity will be monitored in sunburned fruit over time in regular atmosphere cold storage.
II. Characterize pigment changes as they relate to color development and to several skin disorders (e.g. sunburn and Fuji stain) that detract from good fruit finish.
III. Investigate the causes of the disorders called "flecking" in Fuji and russet in Golden Delicious apples and study ways to prevent the incidence of both disorders.

Significant Findings:

OBJECTIVE I: Effects of skin disorders on postharvest fruit quality:

1. As sunburn severity increased, firmness and soluble solids (SSC) increased whereas titratable acidity (TA) decreased in all five apple cultivars studied (See Fig. 1). Decreasing TA in apples is thought to shorten storage life; thus, sunburn damage probably shortens storage life.
2. In contrast, as severity of "flecking" increased in Fuji apples, firmness, soluble solids, and titratable acidity generally increased (See Fig. 2).
3. As severity of russet increased in Golden Delicious apples, firmness, soluble solids, and titratable acidity also increased (See Fig. 3).

OBJECTIVE II: Pigment concentration changes as related to several apple skin disorders:

1. Chlorophyll a and b (green pigments) concentrations of sunburned apples were significantly lower than in non-sunburned apples. Changes shown for Fuji are representative (Fig. 4).
2. Anthocyanin (red pigment) concentrations in Fuji, Gala, and Delicious were significantly lower in sunburned apples as compared to non-sunburned apples. Changes shown for Fuji are representative (Fig. 5).
3. When carotenoids (yellow/orange pigments) in sunburned Fuji's were compared to non-sunburned apples, Beta-carotene and total xanthophylls (V+A) increased significantly in sunburned Fuji (Fig. 6).
4. Fuji, Gala, Delicious, Granny Smith, and Golden Delicious had significant increases in total quercetin glycosides (tan pigments) in sunburned apples as compared to non-sunburned apples. A representative response is shown for Fuji in Fig. 7 with Gal and Glu+Rut changing most.
5. In sunburned apples, decreased concentrations of green and red pigments allowed the yellow/orange carotenoids and tan quercetin glycosides to become more pronounced as yellow or brown spots on the sun-exposed side.

OBJECTIVE III: Fuji flecking and Golden Delicious russet:

1. Both disorders were induced before 7 weeks after full bloom (WAFB), but usually did not become visible until later. Percentage of fruit with Fuji flecking increased between 18 and 20 WAFB. Similarities between the two disorders suggest that Fuji flecking is a type of russet and appears to be associated with lenticels.
2. Microscopic observations showed that epidermal cells became phellogen with formation of phellem as early as 9 WAFB. Phellem was located around or near the lenticels. Phellem accumulated and penetrated through the epidermal cells to form flecking.

3. The frequency of stomata per fruit in Fuji was significantly higher than in Gala. Most stomata became non-functional by 7 WAFB and lenticels appeared.
4. Induction of flecking and russet was enhanced by chemical thinners (Sevin +NAA, ATS, and lime sulfur). ATS applied at full bloom and Ethephon or Sevin + NAA applied at 1 WAFB significantly reduced the amount of epicuticular wax at 10, 11, and 12 WAFB. Incidence of the disorders was often highest at the ends of rows (more spray applied).
5. Fuji and Golden Delicious were bagged at intervals to protect young fruit from chemical injury. Incidence of flecking and russet increased significantly as date of bagging was delayed.
6. Epicuticular wax weight/fruit and cuticle thickness were significantly higher for Gala vs. Fuji.
7. Pubescence weight per fruit decreased with fruit development in Fuji, Gala, and Golden Delicious from full bloom until 7 WAFB with most pubescence disappearing by 7 WAFB. Pubescence weight per fruit in Gala was higher than that in Fuji and Golden Delicious.
8. Bagging Fuji with nylon mesh bags blocked some UV-A and UV-B light, and decreased the percentage of flecking by approximately 30%. This suggests that excess solar radiation may be another factor inducing flecking.
9. Augmenting epicuticular wax of the cuticles with three or four weekly applications of RAYNOX® after 2 WAFB significantly reduced the percentage of fruit with flecking in 2 years, but no response was seen in 2 different years.
10. Water content of the peel and outer cortex decreased as flecking severity increased (Fig. 8).

Methods:

Objective I on Postharvest internal fruit quality as affected by pre-harvest skin disorders:

Five apple cultivars (Gala, Jonagold, Granny Smith, Golden Delicious, and Fuji) were harvested at commercial harvest times and sorted into five grades of sunburn (NB = no sunburn, and SB-1 to SB-4 = increasing severity of sunburn browning). Firmness, SSC and TA were determined at harvest, and after 3 and 6 months in regular atmosphere cold storage. Fruit quality was also measured at harvest and after 1, 2, 3, and 4 months in cold storage for Fuji apples with flecking (grades g-0 to g-4) and Golden Delicious apples with russet (grades g-0 to g-4).

Objective II on Pigment Changes of Fruit with Stress-Induced Disorders:

Pigments were extracted from apple peel disks. Reverse-phase high performance liquid chromatography (HPLC) was used to analyze pigment compositions.

Objective III on the causes and prevention of Fuji "flecking" and Golden Delicious russet:

Fuji's were bagged with nylon mesh bags that blocked a percentage of UV-A and UV-B light at 14 WAFB. Fruit were evaluated for the development of flecking weekly and at harvest time.

To determine water content of peel and cortex, plugs 1 cm diameter X 1.5 cm long were taken with a cork borer and weighed before and after drying to determine water content in different grades of flecking. The occurrence and development of Fuji flecking was monitored weekly with a digital camera.

Results and Discussion:

OBJECTIVE I—Fruit Quality Analyses of Fruit with Heat and Light-Induced Disorders:

1. Sunburn—Fruit Quality of Gala, Golden Delicious, Jonagold, Granny Smith, and Fuji

a. Firmness. The five cultivars investigated in 2006 all increased in firmness as severity of sunburn increased (Fig. 1). The firmness of all fruit decreased over time in cold storage, but the trend of higher firmness with increasing severity of sunburn was retained (Fig. 1 for harvest and 3 months; data not shown for 6 months).

b. Soluble Solids Content (SSC). The SSC in fruit was generally higher in apples with increasing sunburn severity at harvest and after 3 months cold storage (Fig. 1). The same trend was observed after 6 months cold storage (data not shown). In contrast to firmness, SSC did not change appreciably during cold storage.

c. Titratable Acidity (TA). In contrast to firmness and SSC, TA decreased with increasing sunburn severity in all five cultivars (Fig. 1). Counter to SSC, TA for each Sunburn grade decreased sharply in cold storage. Granny Smith was two to three times higher in TA at harvest than the other cultivars.

Sampled at Harvest	Sampled after 3 months in cold storage
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Fig. 1. Firmness, SSC, and TA in five cultivars of apples at harvest and at 3 months after harvest. Five classes of sunburn ranging from no sunburn (Sb-0) to severe sunburn browning (Sb-4) were compared for Gala, Jonagold, Golden Delicious, Granny Smith, and Fuji.

2. Fuji Flecking—Effects on Fruit Quality

Firmness, SSC, and TA generally increased in Fuji as severity of "flecking" increased (Fig. 2).

3. <u>Golden Delicious Russet—Effects on Fruit Quality</u>

Firmness, SSC, and TA usually increased in Golden Delicious as severity of russet increased (Fig. 3).

g-0 g-1 g-2 g-3 g-4

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Fig. 2. Firmness, SSC, and TA in Fuji apples after 1, 2, 3, and 4 months of storage. Five classes of flecking ranging from no flecking (g-0) to severe flecking (g-4) were compared.

g-0 g-1 g-2 g-3 g-4

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Fig. 3. Firmness, SSC, and TA in Golden Delicious apples at harvest and after 1, 2, 3, and 4 months of storage. Five classes of russet from no russet (g-0) to severe russet (g-4) were compared.

In summary, fruit that are lower in TA at harvest are known to have a shorter storage life. Thus, apples damaged by sunburn and which had lower TA would be expected to have a shorter storage life. In contrast, neither Fuji flecking nor Golden Delicious russet caused a decline in TA as severity of russet increased. Therefore, the effects of sunburn are more than skin deep. The effect of these skin disorders on taste and attributes other than firmness, SSC and TA have not been studied.

OBJECTIVE II: Pigment concentration changes as related to several apple skin disorders:
a. Chlorophyll a and b (green pigments) concentrations of sunburned apples were significantly lower than in non-sunburned apples. Changes shown for Fuji are representative (Fig. 4).

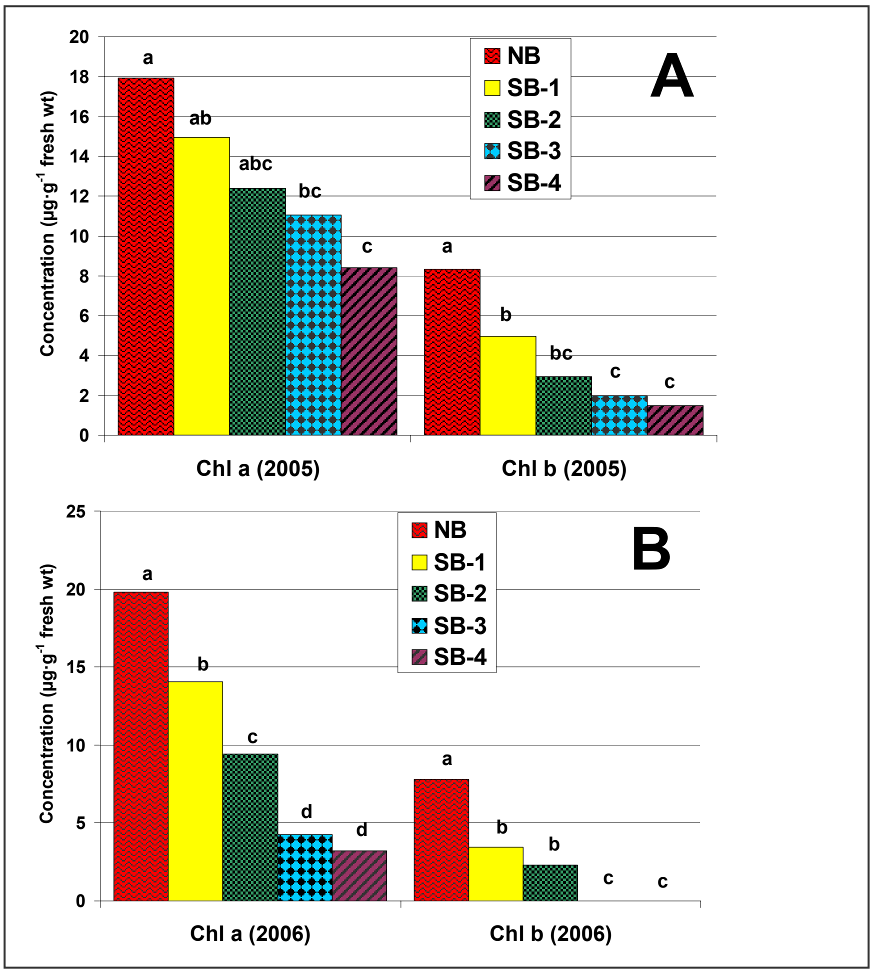

Fig. 4. Changes in chlorophyll a and b concentrations in Fuji apples with different degrees of sunburn damage. Fig. A is for 2005 and Fig. B is for 2006.

b. Anthocyanin (red pigment) concentrations in Fuji, Gala, and Delicious were significantly lower in sunburned apples as compared to non-sunburned apples. Changes shown for Fuji are representative (Fig. 5). No anthocyanin was detected in non-sunburned Golden Delicious or Granny Smith without blush.

c. When carotenoids (yellow/orange pigments) in sunburned Fuji's were compared to non-sunburned apples, Beta-carotene and total xanthophylls (V+A) increased significantly in sunburned Fuji (Fig. 6). In another study (data not shown), these pigments increased in both sunburned Delicious and Fuji, but Beta-carotene decreased in sunburned Granny Smith and remained unchanged in Gala and Golden Delicious.

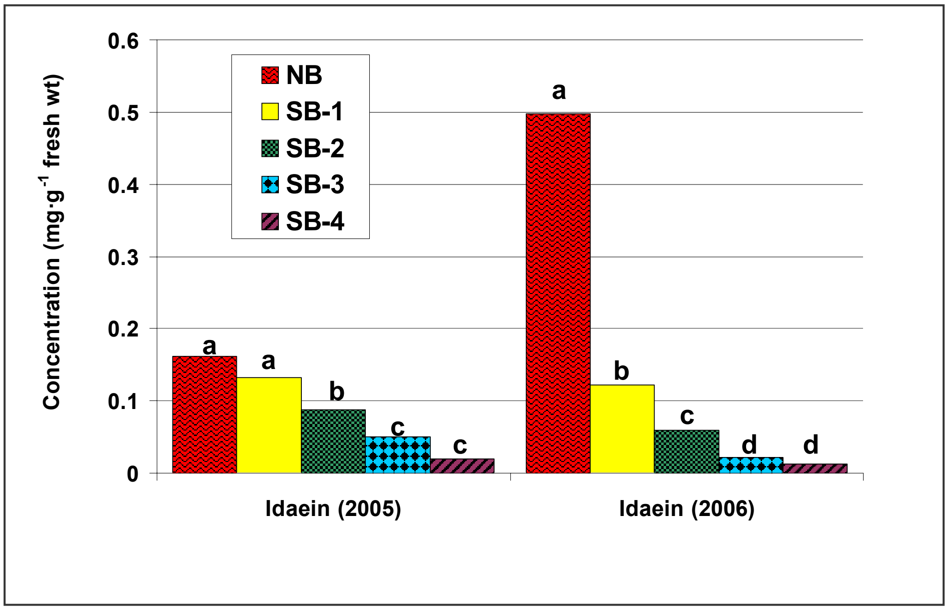

Fig. 5. Changes in Anthocyanin (idaein) concentrations in Fuji apples with different degrees of sunburn damage in 2005 and in 2006.

d. Fuji, Gala, Delicious, Granny Smith, and Golden Delicious had significant increases in total quercetin glycosides (tan pigments) in sunburned apples as compared to non-sunburned apples. A representative response is shown for Fuji in Fig. 7 with Gal and Glu+Rut changing most.

These pigment changes help explain why sunburned apples become yellow or brown. As the green and red pigments disappear in sunburned apples, the yellow/orange and tan pigments become more prominent and make the sunburned spots appear yellow or brown, depending on the severity of sunburn.

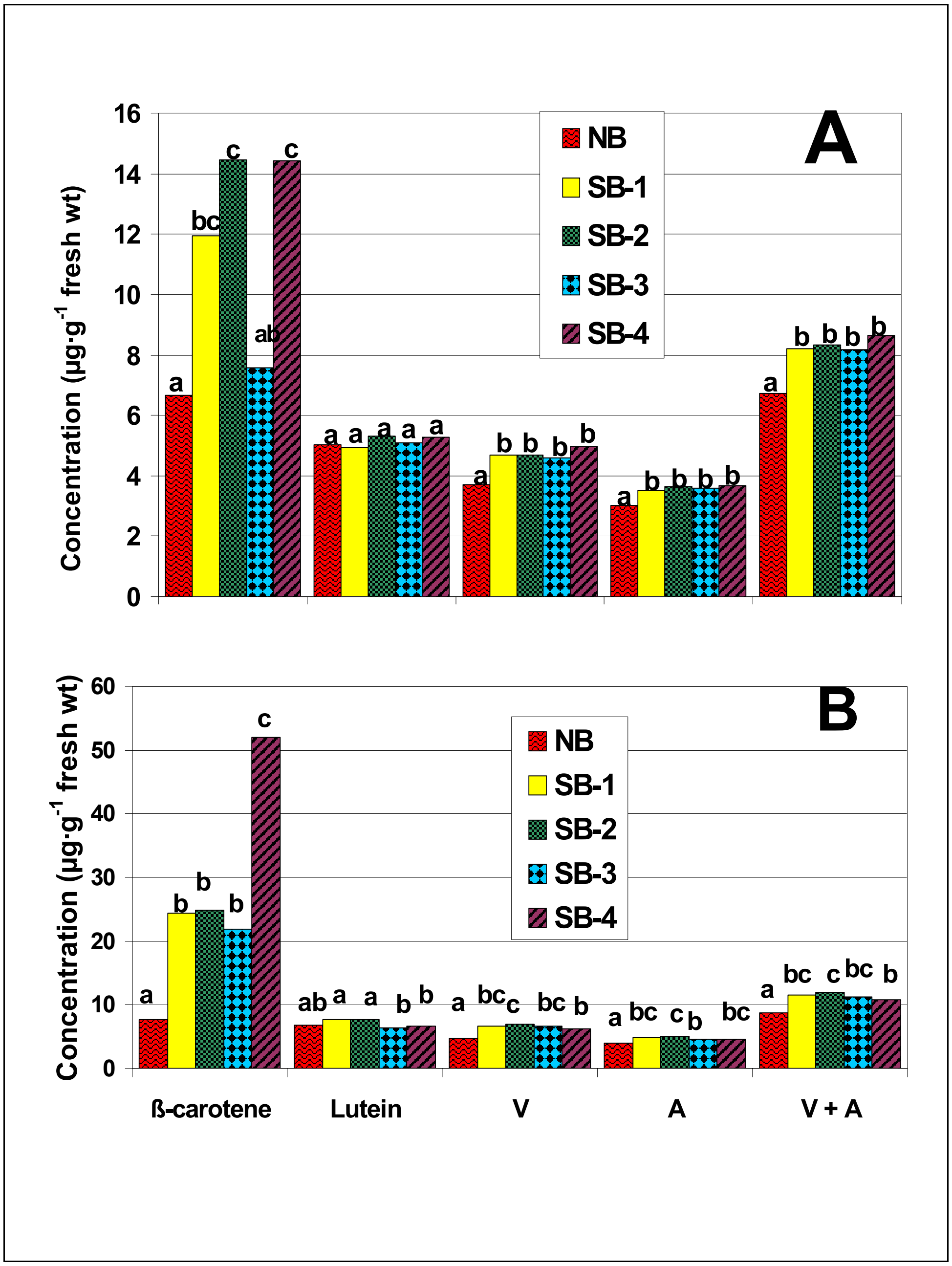

Fig. 6. Carotenoid concentrations for 2005 (A) and 2006 (B) peel samples.
V = Violaxanthin; A = Antheraxanthin

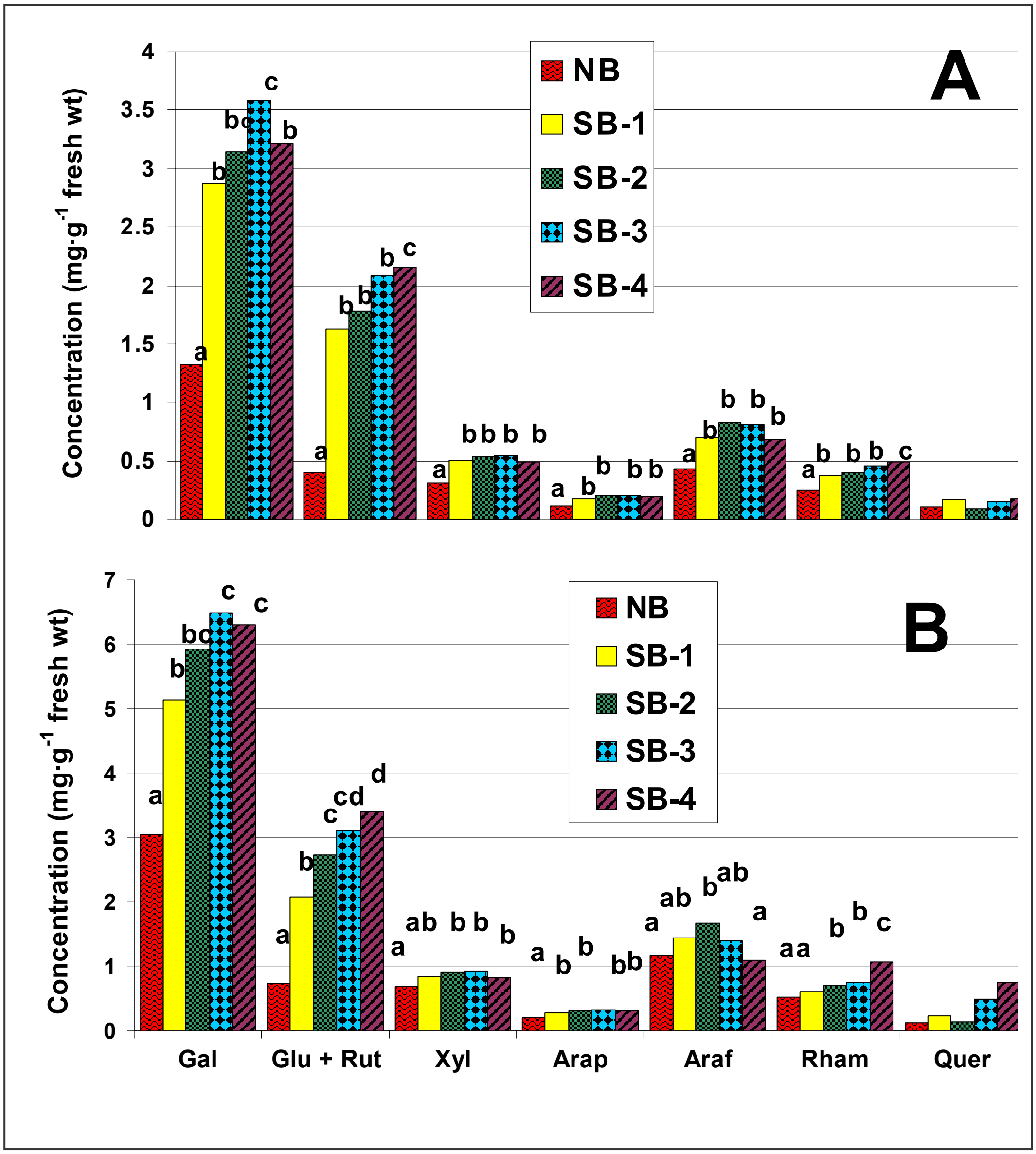

Fig. 7. Quercetin glycoside concentrations for 2005 (A) and 2006 (B) peel samples. Gal = quercetin galactoside; Glu + Rut = quercetin glucoside and quercetin rutinoside; Xyl = quercetin xyloside; Arap= quercetin arabinopyranoside; Araf = quercetin arabinofuranoside; Rham = quercetin rhamnoside; Quer = quercetin

OBJECTIVE III: Fuji flecking and Golden Delicious russet:
We reported extensively on this objective in the two previous reports (2006 and 2007), so only a summary with little new data are presented herein.
a) We observed that both disorders were induced before 7 weeks after full bloom (WAFB), but usually did not become visible until later. Percentage of fruit with Fuji flecking increased between 18 and 20 WAFB. Similarities between the two disorders suggest that Fuji flecking is a type of russet and appears to be associated with lenticels.

b) The frequency of stomata per fruit in Fuji was significantly higher than in Gala. Most stomata became non-functional by 7 WAFB and lenticels appeared. Microscopic observations showed that epidermal cells became phellogen with formation of phellem as early as 9 WAFB. Phellem was located around or near the lenticels. Phellem accumulated and penetrated through the epidermal cells to form flecking.
c) Epicuticular wax weight/fruit and cuticle thickness were significantly higher for Gala than for Fuji, and may be one of the factors that make Fuji's more susceptible. Pubescence weight decreased with fruit development in Fuji, Gala, and Golden Delicious from full bloom until 7 WAFB with most pubescence disappearing by 7 WAFB. However, pubescence weight in Gala was higher than that in Fuji and Golden Delicious, and may be another factor making Fuji and Golden Delicious more susceptible. In a commercial orchard, flecking was less severe in a block of Fuji's that had more pubescence than in an adjacent block with less pubescence.
d) Induction of flecking and russet was enhanced by chemical thinners (Sevin +NAA, ATS, and lime sulfur. ATS applied at full bloom and Ethephon or Sevin + NAA applied at 1 WAFB significantly reduced the amount of epicuticular wax at 10, 11, and 12 WAFB. Incidence of the disorders was often highest at the ends of rows (more spray applied). When Fuji and Golden Delicious were bagged at intervals to protect young fruit from chemical injury, less flecking and russet appeared. The incidence of flecking and russet increased significantly as the date of bagging was delayed, suggesting that fruit are most susceptible at an early stage of development.
e) Bagging Fuji with nylon mesh bags blocked some UV-A and UV-B light, and decreased the percentage of flecking by approximately 30%. This suggests that excess solar radiation may be another factor inducing flecking.
f) Because Fuji has less epicuticular wax, we reasoned that augmenting epicuticular wax of the cuticles with three or four weekly applications of RAYNOX® in early stages of development would be beneficial in reducing flecking. In 4 years of testing with applications beginning at 2 WAFB, the percentage of fruit with flecking was significantly reduced in 2 years, but no response was seen in the other 2 years. We cannot explain the inconsistency in these results, but it may be due to environmental differences among years.
g) A recent study showed that water content of the peel and outer cortex decreased as flecking severity increased (Fig. 8). Several "plugs" were removed with a cork borer from apples with different grades of flecking (g-0 to g-4). Fresh weights were measured, the samples were dried, and then dry weights were determined. The percent water content was calculated and plotted. It can be seen that water content decreased as the severity of flecking increased (Fig. 8).

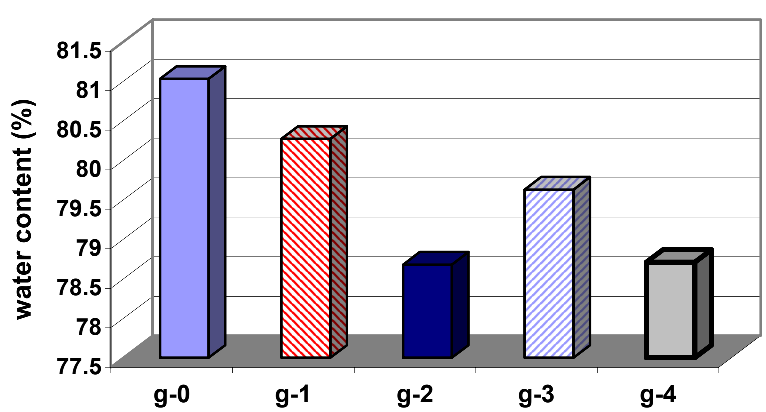

Fig. 8. Water content of Fuji apples with different grades of flecking.

Publications from this research:

- David A Felicetti. 2003. Photooxidative Sunburn of Apples: Characterization of a Third Type of Apple Sunburn. Master of Science thesis. Wash. State Univ. (Major Professor: L. E. Schrader)
- Larry Schrader, Jianshe Sun, David Felicetti, Jeong-Hak Seo, Leo Jedlow, and Jianguang Zhang. 2004. Stress-Induced Disorders: Effects on Apple Fruit Quality. Proc. 99th Wash. State Hort. Assoc. Meetings. p. 116-119. See also http://postharvest.tfrec.wsu.edu/PC2003A.pdf
- Larry Schrader, J. Sun, J. Zhang, J.H. Seo, L. Jedlow, and D. Felicetti**.** 2005. Fruit Skin Disorders of Apples. Proc. 100th Wash. State Hort. Assoc. Meetings. 4 pp. http://postharvest.tfrec.wsu.edu/PC2004E.pdf
- Larry Schrader, J. Sun, J. Zhang, and D. Felicetti. 2006. Stress Management in Tree Fruit Production. Proc. 101st Annual Meeting, Wash. State Hort. Assoc. 3pp.
- David A. Felicetti. 2007. Apple (*Malus Domestica* Borkh.) Fruit Skin Disorders And Changes In Pigment Concentrations Associated With The Disorders. Ph.D. Dissertation, Washington State University. (Major Professor: L. E. Schrader), 148 pp.
- David A. Felicetti and Larry Schrader. (2008). Changes in Pigment Concentrations Associated with the Degree of Sunburn Browning of 'Fuji' Apple. J. Amer. Soc. Hort. Sci. 133(1):1-8.
- Larry Schrader, J. Sun, J. Zhang, D. Felicetti, and J. Tian. 2008. Heat and Light-Induced Apple Skin Disorders: Causes and Prevention. Acta Hort. (in press).

Significance to Industry and Potential Economic Impact:

The first aspect of this research combines pre-harvest and post harvest physiology, as little is known about the effects of pre-harvest-induced disorders (e.g. sunburn and stain) on internal fruit quality of apples. This research is providing a better understanding of how fruit quality and storability are impacted by sunburn and stain. This research should provide packers with important science-based guidelines about the effects of these pre-harvest stresses and disorders. In the past, sunburn has been thought to affect only the appearance of the fruit, but our results indicate that firmness, soluble solids (SSC), and titratable acidity (TA) are all affected by these disorders. Both firmness and SSC increase as the severity of sunburn increases, but TA decreases. Because decreasing TA shortens the storage life of fruit, it can be assumed that sunburn damage on apple will decrease the storage life of damaged apples. An industry-wide system for relating internal fruit quality to these skin disorders could lead to more equitable pricing to growers who sell fruit with these skin disorders.

The second project involves elucidating the pigment changes that occur when apples of various cultivars become sunburned. Identifying these pigment changes will help understand whether beneficial pigments (e.g. antioxidants) change with sunburn. This knowledge also may allow for genetic manipulation or selection to improve the apples' ability to withstand stress conditions that cause sunburn or 'Fuji' stain. The results indicate that chlorophylls (green pigments) and anthocyanins (red pigments) decrease with increasing sunburn, but that certain carotenoids actually increase with sunburn. Quercetin glycosides also increase with sunburn. Some of these carotenoids and quercetin are antioxidants and may impart some beneficial nutritional effects to sunburned fruit.

Fuji flecking is a serious problem in many orchards, and is having a serious economic impact on some growers. This disorder appears as a skin disorder on as many as 70% of the 'Fuji' apples in some orchards. We have identified some of the factors that induce Fuji flecking and russet in Golden Delicious, and also factors that enhance the expression of these disorders. We are still searching for treatments that will consistently reduce the amount of flecking and russet.