

FINAL PROJECT REPORT
WTFRC Project #AH-03-321

WSU Project #13C-3655-7326

Project title: Fuji Stain—Causes and Prevention

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Overall objective: To understand the factors involved in the development of stain in ‘Fuji’ apples and search for ways to reduce it. Specific objectives are outlined below:

1. Investigate the effects of preharvest environmental stress on development of ‘Fuji’ stain.
2. Investigate the effects of MCP treatment on development of ‘Fuji’ stain.
3. Study the effect of various crop protectant formulations on development of ‘Fuji’ stain.
4. Determine whether blocking UV-B irradiation decreases development of ‘Fuji’ stain in cold storage.
5. Investigate whether nutrient imbalances enhance stain development.
6. Study the effects of drenching on development of ‘Fuji’ stain.
7. Investigate the effects of fumigation with methyl bromide.
8. Investigate the effects of grading (packingline) operations, such as waxing fruit, on development of ‘Fuji’ stain.
9. Search for ways to reduce ‘Fuji’ stain through use of additional techniques such as UV-B filters, orchard cooling, postharvest drenches and new grading practices.

Significant findings:

1. ‘Fuji’ stain, a skin disorder, appears only after cold storage (Fig. 1). Stain develops on the side of fruit previously exposed to sun.
2. Stain disorder affected only the epidermal layer (peel tissue) and was not found to extend into the flesh (Fig. 2).
3. Heat- and/or light-induced stain is directly related to the degree (grade) of sunburn on fruit as apples entered cold storage. No stain appeared in apples that had no sunburn, but stain incidence increased sharply as sunburn damage increased (Fig. 3).
4. Choice of rootstock (M.9 vs. M.26) had no significant effect on development of stain in ‘Fuji’ apples stored over four months (Table 2).
5. Postharvest treatment of ‘Fuji’ apples with MCP decreased stain during regular atmosphere cold storage (Fig. 4).
6. Preharvest applications of either ReTain or MCP reduced incidence of stain significantly (Fig. 5).
7. RAYNOX[®] applied three times during the 2003 season significantly reduced the appearance of ‘Fuji’ stain in treated apples that were held in cold storage over a period of four months (Fig. 6).
8. Apples sprayed four times during 2002 with RAYNOX[®] for sunburn protection developed less stain than control apples or apples sprayed four times with VaporGard[®] or Surround[®] (Fig.7).
9. Several mineral nutrients were higher in peel from stained fruit as compared to normal tissue (Table 3), but the significance of these changes is not known.

10. Brown in Tasmania studied effects of prefumigation drenching on the development of 'Fuji' stain. MCP and 2% ascorbic acid were the most effective treatments against stain (Fig. 8).
11. Evaporative cooling systems significantly reduced 'Fuji' stain when compared to controls. This suggests that high fruit surface temperature is a cause of 'Fuji' stain and that stain can be decreased in apples protected from extreme heat stress (Fig. 9).

Methods:

Objective 1: Apples were harvested from an orchard in the Yakima Valley with a history of high incidence of stain in trees grown on M.9 and M.26 rootstock. These apples were stored at 33°F and evaluated monthly for the appearance of stain over a four-month period of storage.

Objective 2: 'Fuji' apples were harvested from an orchard in the Yakima Valley that has a history of high incidence of stain. The apples were treated with 1 ppm MCP for 12 hours at 68°F, and following MCP treatment they were stored in a room at Stemilt at 33°F. The fruit were evaluated at monthly intervals to follow the appearance of stain over four months. During 2005, Gordon Brown in Tasmania applied MCP to 'Fuji' apples collected from four orchards. He then evaluated stain after cold storage. In 2004, several chemicals were sprayed on September 9 and/or 29. The details of treatments are shown in Table 1. Fruit were harvested on October 12, 2004, and put into cold storage the following day. Thirty apples were collected from each replicate. For replicate I, all the fruits were labeled and investigated at two-week intervals until mid-May, 2005. For replicates II to V, evaluation was made once a month and the stained fruit was marked once stain was observed. Duncan's multiple range test was used for statistical analysis.

Objective 3: In 2003, apples treated with RAYNOX[®] and untreated controls were harvested from the same orchard in the Yakima Valley. The degree of sunburn present at harvest was determined, and apples were separated into lots of thirty for each class of sunburn (according to the Schrader-McFerson classification of sunburn). These apples were placed in a regular atmosphere (RA) cold room at Stemilt and maintained at 33°F for four months. The fruit were evaluated at monthly intervals to follow the appearance of stain in these different lots of apples.

Objective 4: 'Fuji' apples to which RAYNOX[®], Surround WP[®] and VaporGard[®] had been applied four times during 2002 were harvested, stored, and evaluated at one-month intervals for stain. Schrader and Sun applied RAYNOX[®], a UV-B blocking material, to 'Fuji' apples during mid-September 2003 to see if decreased UV-B irradiation during later stages of development would decrease stain. Treated fruit were harvested along with untreated controls. All fruit were placed in cold storage at Stemilt and evaluated periodically to see if stain appearance was affected.

Objective 5: Nitrogen status of orchards was estimated by using a Minolta SPAD meter to estimate chlorophyll in leaf tissue to compare N status vs. stain incidence. Normal 'Fuji' apples were harvested from three orchards and put into cold storage. In January 2005, 26 stained and 15 normal fruit were selected and labeled for mineral analysis. Three types of peel samples were prepared: (1) stain peel (SP): stain area on fruit surface; (2) healthy peel (HP): healthy area near the stain patch; (3) normal peel (NP): healthy area of normal fruits. Three replicates were prepared for each group of peel samples. The typical area on each fruit of distinctive groups was peeled accordingly with a thickness of about 0.5 mm before the skin was put into a refrigerator at -7°C for one day. The peel samples were then freeze-dried. When completely dry, the peel was ground to a fine powder in a clean mortar. The concentrations of P, K, Ca, Mg, S, Fe, B, Mn, Cu, Zn, Na, Mo and Al were assessed by inductively coupled plasma emission spectroscopy (ICP-OES), and total nitrogen was assessed by Ion analysis by flow injection analysis (LCHAT QuikChem 8000) at the Soil & Plant Analysis Lab, University of Wisconsin-Madison.

Objective 6: Brown in Australia compared several prefumigation drenches (DPA, Stopit, and ascorbic acid) with 1-MCP to limit the subsequent appearance of stain.

Objective 7: Brown in Tasmania compared additional prefumigation drenches in 2004 (Ascorbyl palmitate and an experimental formulation) with studies done in 2003 to determine effect on occurrence of fumigation scald.

Objective 8: Brown in Tasmania did additional work during 2005 to study effects of packingline operations on ‘Fuji’ stain. He compared three finishing waxes and treated half the fruit with methyl bromide after fruit cleared the packingline. He also placed fruit in a controlled atmosphere chamber (heater, high CO₂, and low O₂ environment) to compare heating to methyl bromide fumigation as a temperature disinfestation of fruit bound for export to Japan.

Objective 9: Orchard cooling with overhead sprinklers was studied to determine its effect on reducing ‘Fuji’ stain. An artificial sensor developed in our lab controlled an evaporative cooling (EC) system that was activated in a ‘Fuji’ apple orchard before temperatures were high enough to cause sunburn damage. These apples were harvested at maturity, stored at 33°F for four months, monitored for the appearance of stain, and compared to untreated (no EC) apples.

Table 1. Preharvest treatments tested for stain reduction.

Treatment	Concentration	Application date	
ReTain2 (powder)	125 ppm	Sep 9	Sep 29
MCP2 (powder)	250 ppm	Sep 9	Sep 29
DPA (liquid)	1000 ppm	Sep 9	Sep 29
ReTain1 (powder)	125 ppm		Sep 29
MCP1 (powder)	250 ppm		Sep 29
Ethrel (liquid)	300 ppm		Sep 29
CK	Water		Sep 29

Results and discussion:

1. ***Fuji stain appearance during cold storage.*** Fuji stain does not appear at harvest but only after a few months in cold storage (Fig. 1). The symptoms of stain are diversified, and a great variation in color changes exists among individual fruit. The apple on the left was photographed in October as it was placed into cold storage. It was photographed periodically thereafter until it was removed from cold storage in April (photo on right).



Fig. 1. Appearance of stain as the period of cold storage was extended from October (left) to April (photo on right).

2. ***Comparisons of skin and flesh in controls vs. ‘Fuji’ apples with stain.*** In mid-March of 2005, typical stained and normal fruit were cut in half to compare the textures of peel and flesh. The stain disorder was limited to the epidermal layer (peel) and did not extend into the flesh (Fig. 2).

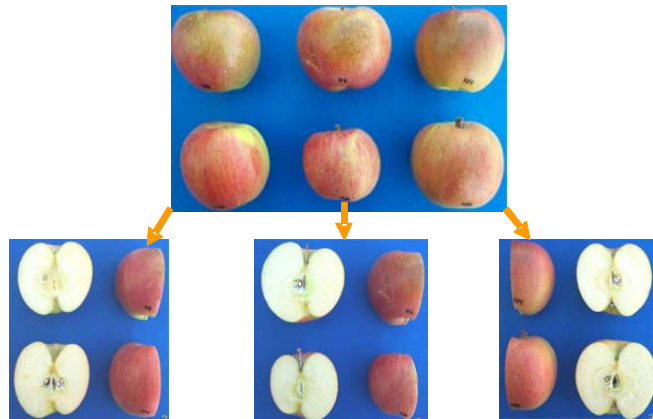


Fig. 2. Observations of flesh in apples with 'Fuji' stain.

3. **Effects of preharvest environmental stress on later development of 'Fuji' stain.** Apples that had been heat stressed to the extent that they were sunburned had a much higher incidence of stain during cold storage as compared to non-sunburned apples. 'Fuji' apples from an orchard with a history of high incidence of stain were sorted and stored based on grade of sunburn and then evaluated monthly during cold storage for stain development. Stain increased markedly after four months of cold storage in those apples that had more severe sunburn (Fig. 3).

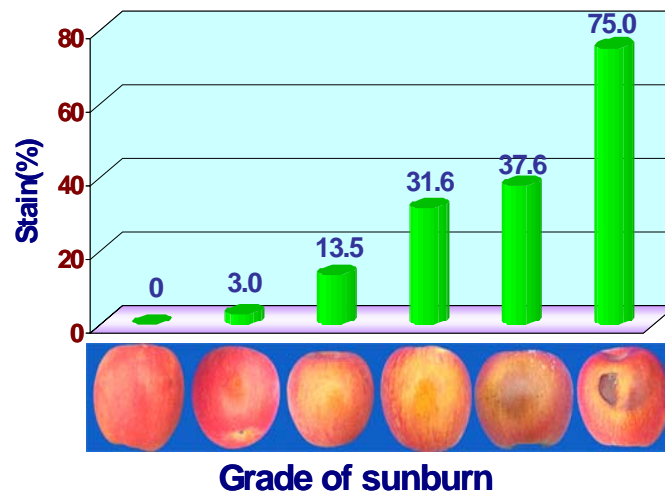


Fig. 3. Effect of grade of sunburn on 'Fuji' stain during cold storage.

4. **Effect of rootstock on incidence of 'Fuji' stain.** 'Fuji' apples grown on M.9 or M.26 rootstocks showed no significant differences in appearance of stain during cold storage (Table 2).

Table 2. Effect of rootstock and sunburn incidence on 'Fuji' staining during cold storage.

<u>Rootstock</u>	<u>Staining with sunburn</u>	<u>Staining without sunburn</u>
'Fuji'/M.9	43.2	4
'Fuji'/M.26	56.9	5.2

5. **Effects of MCP and other treatments on ‘Fuji’ staining during cold storage.** MCP had no significant effect on the appearance of stain in treated vs. untreated fruit after one month in storage. However, after 2, 3, and 4 months of storage, the untreated controls had significantly more ($P < 0.05$, 0.01 and 0.01 , respectively) stain than MCP-treated fruit. The incidence of stain increased over the period of storage in both treated and untreated fruit. Stain in untreated fruit after four months of cold storage increased to 32.6% vs. 17.2% in fruit treated with MCP (Fig. 4).

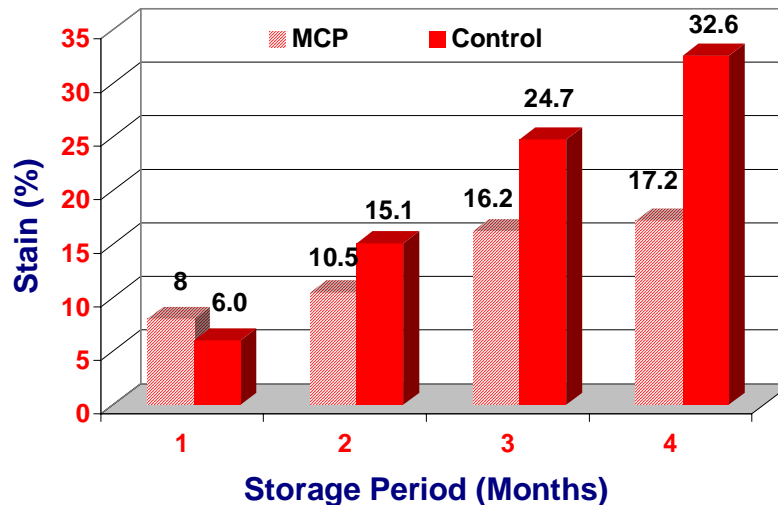


Fig. 4. Effect of postharvest application of MCP on ‘Fuji’ staining during cold storage.

During 2005, Gordon Brown in Tasmania applied MCP to apples from four orchards and evaluated stain after cold storage. No stain developed in any of the treatments (including the untreated controls) even after fruit were also fumigated with methyl bromide to encourage ‘Fuji’ stain development (data not shown). Preliminary experiments during 2005 with controlled atmosphere and temperature (CATTs) disinfestation of fruit bound for export to Japan indicated that this CATTs treatment enhances the appearance of stain to a greater extent than methyl bromide.

During 2004, we applied sprayable MCP, DPA, Ethrel and ReTain on September 9 and/or Sept. 29 (see Table 1 for details). The final stain rates of either ReTain2 or MCP2 were significantly lower than for Ethrel and the control, with no significant difference between any additional treatments and the control, suggesting that spraying with 125 ppm ReTain or 250 ppm MCP twice before harvest had a beneficial effect on prevention of stain incidence (Fig. 5).

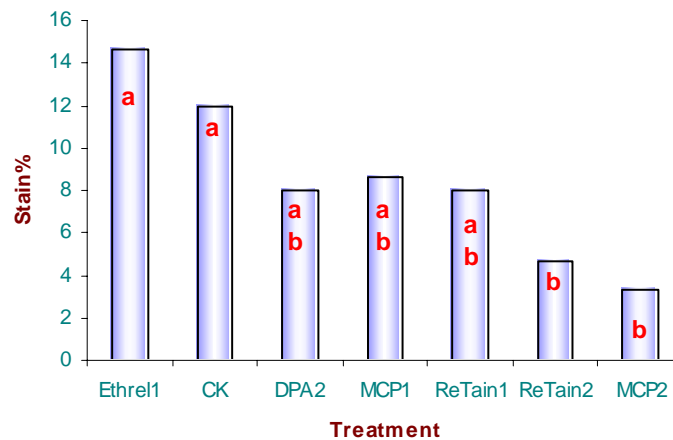


Fig. 5. Effect of preharvest treatments of Ethrel, DPA, MCP, or Retain on 'Fuji' stain.

6. **Effects of RAYNOX[®] on 'Fuji' staining during storage.** RAYNOX[®]-treated 'Fuji' apples had a lower incidence of stain than untreated apples after four months of storage at 33°F in a regular atmosphere (RA) cold room. The appearance of stain increased over the period of storage, and untreated controls developed stain at an increasing rate compared to rate of stain development in RAYNOX[®]-treated fruit (Fig. 6).

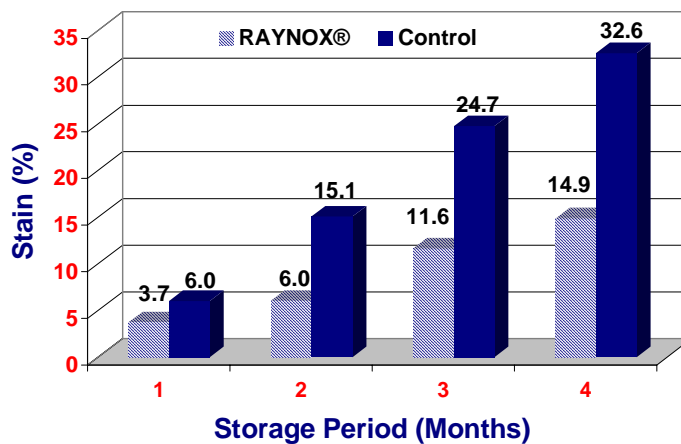


Fig. 6. Effect of RAYNOX[®] on 'Fuji' staining during cold storage (2003 apples).

7. **Effects of preharvest applications of different formulations on 'Fuji' staining during cold storage.** Fuji apples sprayed during the 2002 growing season with four applications of RAYNOX[®], Surround WP[®] or VaporGard[®] were placed in cold storage and evaluated periodically for stain. Stain increased with time in all treatments, but the increase was slower in apples that had been sprayed with RAYNOX[®] for sunburn protection (Fig. 7). This suggests that blocking some of the damaging UV-B radiation with RAYNOX[®] during the growing season is beneficial in decreasing stain.

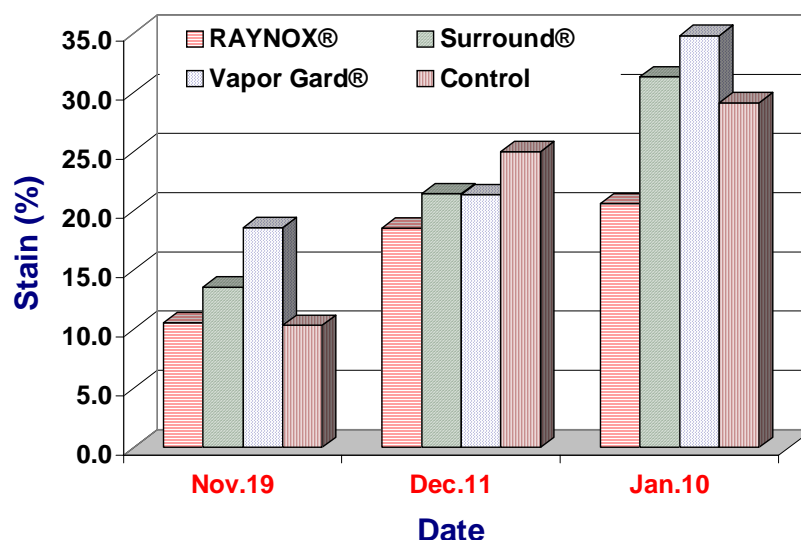


Fig. 7. Effect of different formulations on 'Fuji' stain during cold storage.

8. **'Fuji' stain in relation to mineral nutrient imbalance.** The concentrations of N, P, Mg, S, B, and Mn in stained peel (SP) were significantly higher than those in normal peel (NP) from other apples with no stain and were often higher in SP than in healthy tissue (HP) outside the stained area of the same fruit (Table 3). These assays were done in mid-January after nearly 3 months of cold storage. The relevance of this increase in certain minerals is not known at this time, but it appears to be a result of staining rather than a cause of staining.

Table 3. Mineral analyses of the peel from 'Fuji' apples with and without stain.

Type	N	P	S	Mg	B	Mn	Na
	%				ppm		
SP*	0.43 a	0.06 a	0.05 a	0.13 a	37.56 a	7.76 a	36.45 b
HP	0.40 a	0.05 b	0.04 b	0.08 b	31.49 ab	5.57 b	35.72 b
NP	0.33 b	0.04 c	0.03 c	0.07 b	26.87 b	5.16 b	61.29 a

*SP is apple peel from a stained area on the fruit.

HP is healthy peel from outside the stain area but from the same fruit.

NP is healthy peel from another apple that showed no stain.

9. **Effect of drenching on development of stain during storage (work from Tasmania).** 'Fuji' stain was not as severe in the 2003 season, with less than 10% of the fruit displaying symptoms. In contrast, up to 40% of fruit were affected in 200 so it is important to develop methods of reducing this disorder to guarantee reliable supplies of fruit with excellent skin finish. There was no significant grower and treatment interaction in the analysis of stain during 2002. Therefore, the data were averaged across all the growers. MCP and 2% ascorbic acid were the most effective treatments against stain, while none of the other treatments reduced this disorder compared to the untreated control (Fig. 8).

***Legend for Figure 8**

UTC = untreated control 2% SI = 2% Stopit®
 DPA = Diphenylamine 1% AA = 1% ascorbic acid
 1% SI = 1% Stopit® 2% AA = 2% ascorbic acid
 MCP = 1-MCP (SmartFresh™)

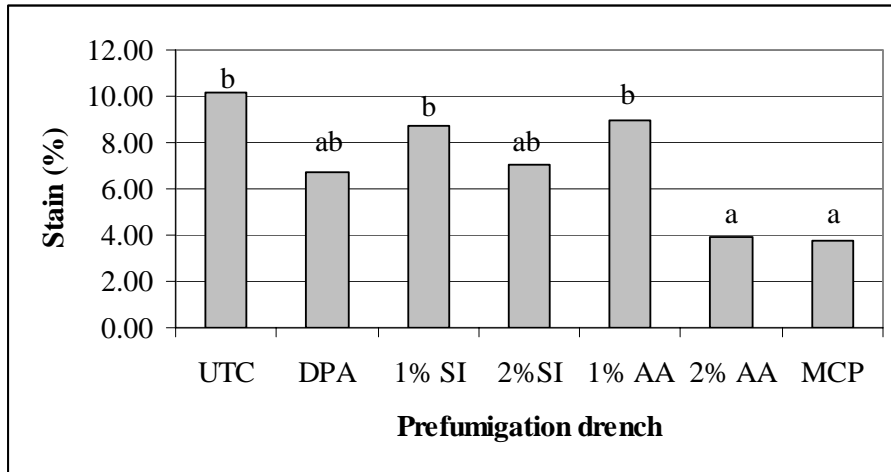


Fig. 8. Effect of drenching on development of stain during storage.

10. *Effect of evaporative cooling on ‘Fuji’ staining during cold storage.* Evaporative cooling was studied to determine its effect on the reduction of ‘Fuji’ stain during storage. A new fruit surface temperature sensor developed in our lab was used to control an evaporative cooling system that was activated before the fruit surface temperatures were high enough to cause sunburn damage. After four months of storage at 34°F, fruit with EC during the growing season had no staining as compared to control (without EC) (Fig. 5). This suggests that stain is a heat-induced disorder that can be decreased by protecting fruit from high fruit surface temperatures.

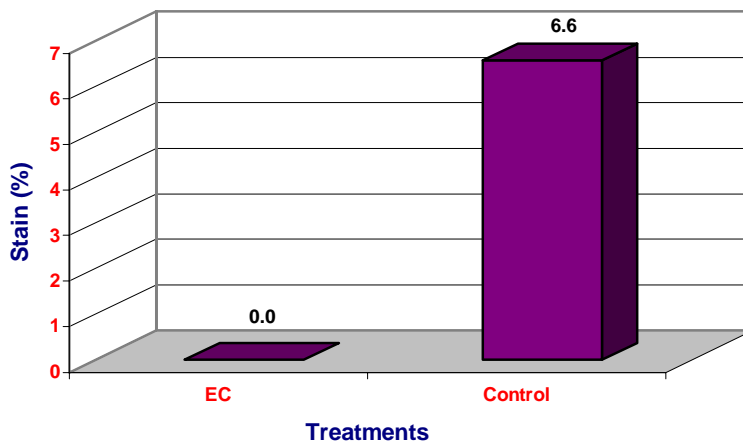


Fig. 9. Effect of evaporative cooling (EC) on ‘Fuji’ stain.

Budget:**Project title:** Fuji Stain-Causes and Prevention**PI:** Larry Schrader**Project duration:** 3 years (2003-2005)**Current year:** 2005**Project total (3 years):** \$142,818

Year	Year 1 (2003)	Year 2 (2004)	Year 3 (2005)
Total	\$42,120	\$48,112	\$52,586

Current year breakdown

Item	Year 1 (2003)	Year 2 (2004)	Year 3 (2005)
Salaries ¹	\$25,000	\$28,980	\$30,139
Benefits (40% - yr 1; 38% - yr 2; 42% - yr 3)	10,000	11,012	12,537
Wages ²	2,000	2,000	2,000
Benefits (16% - yrs 1 and 2; 10% yr 3)	320	320	200
Equipment			
Supplies ³	2,000	2,000	3,000
Travel ⁴	800	800	1,000
Miscellaneous ⁵	2,000	3,000	4,000
Total	\$42,120	\$48,112	\$52,586

¹ Salary for Research Associate to work with Schrader (Dr. J. Zhang returned in 2004 to work on 'Fuji' stain).

² Hourly help to assist with setting up experimental apparatus, collection and analysis of data.

³ Supplies include chemicals, materials for fabrication and maintenance of equipment, laboratory supplies, crop destruct payments, and cell phone charges.

⁴ Travel to experimental plots to evaluate and harvest fruit.

⁵ Collaboration continued with Dr. Gordon Brown in Tasmania, Australia. Schrader and Brown had many common interests in fruit finish (including stain and color development) and collaborated on the proposed stain experiments. \$4,000 was requested for Dr. Brown's efforts in year 3. He sought most of his support and gets matching funds from the Australian apple growers through Horticulture Australia.

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