2008 Apple Research Review
January 17-18
Red Lion Hotel
Yakima, WA
DAY ONE AGENDA

Time	Page	PI	Project Title	Funding period
8:00		T. Schmidt	Welcome and introduction	
8:15		McFerson	Technology Roadmap Update	
			Final Reports	
8:30	1	Yoder	Influence of temperature on pollen germination & tube growth	05-07
8:45	11	Elfving	Growth and crop load management in apple trees with bioregulators	05-07
9:00	16	Elfving	Sprayable 1-MCP for managing apple postharvest quality	07
9:15	19	Schrader	Improving fruit finish in apple	05-07
9:30	30	Cheng	High temperature stress on apple fruit peel: physiology and detection	06-07
9:45	39	Dandekar	Defining ethylene regulation of apple fruit quality traits	05-07
			Break	
Group #			**Continuing Projects Poster Session - 10:30 am-12:00**	
1	51	T. Schmidt	Chemical thinning of apple	internal
1	63	Hanrahan	Programs to suppress sunburn, russet and lenticel breakdown of apples	internal
1		Castillo	Collaborative WTFRC research programs	internal
2	70	Auvil	Apple rootstock and scion evaluation	internal
2	79	Fazio	Replant disease tolerance of Geneva rootstocks	06-08
2	87	Dhingra	Cultivar improvement via trait-targeted sport induction	07-08
3	92	van Nocker	Auxin and ethylene dynamics in the abscission zone	06-08
3	97	Aldwinckle	Functional genomics of flowering in apple	07-09
3	104	Dhingra	Establishing trait–gene relationships and gene discovery in apples	07-08
3	108	Zhu	Functional genomics and marker development for apple sensory qualities	07-09
			Break	
			Committee funding deliberations/lunch 12:00-1:30	
			Final Reports	
1:30	115	Mitcham	A new approach to understand and control bitter pit in apple	07
1:45	126	Klein/Riley	Testing of a sticker for ethylene release from apples	06-07
2:00	135	Andrews	Identifying disease prevention benefits of apple consumption Extension	05-06
2:15	146	Pitts	Estimating apple firmness using tensile mechanical properties Extension	06
2:30	159	Hirst	Flower bud development in apple	06-07
2:45	167	Hirst	Mechanism of apple fruit growth under Washington conditions	07
3:15	168	Peace	Adapting available genomics tools to enhance WA apple breeding	07
3:00	156	Ross	Sensory profiles and consumer acceptance of apple breeding selections	2007
3:30	169	Bliss	Consulting for the Washington apple breeding project	07

FINAL PROJECT REPORT
WTFRC Project Number: #439934

Project Title: Temperature Effect on Pollen Germination and Tube Growth in Apples

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David Carbaugh, Research Specialist, Va Tech AHS-AREC, Winchester, VA
Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA

Other funding Sources

Agency Name:
Amount awarded:
Notes:

Total Project Funding: **$**93,439.00

Budget History:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries	20,000	20,600	21,218
Benefits	7,350	8,845	9,110
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	1,000	1,316	1,000
Travel	0	0	0
Contractual services	1,000	1,000	1,000
	0	0	0
	0	0	0
Miscellaneous	0	0	0
Total	**29,350**	**31,761**	**32,328**

SIGNIFICANT FINDINGS

- Observation have been conducted on pollen tube growth in approximately 10,000 styles of more than 2000 hand-pollinated flowers.
- Our database includes the commercial cultivars Gala, Golden Delicious and Fuji and the pollinizing cultivars Gala and Golden Delicious as well as Manchurian and Snowdrift crabapples and 12 other crabapples (Figs. 1 & 2).
- Results have been combined to formulate pollen tube growth patterns suitable for development and testing of a computer-generated pollen tube growth model for Washington State growers.

Highlights of Year 1 – (2005)

- Pollen tube growth to base of styles on Gala pistils occurred in less than 96 hours after pollination at alternating 55ºF 12-hr light/35ºF 12-hr dark (55/35º lt/dk) with all pollinizers tested.
- Pollen tube growth to base of styles on Gala pistils in tests at 65/40º lt/dk and 75/45º lt/dk showed all with pollen tube growth to end of styles 48 hours after pollination.
- Pollen tubes reached the end of some styles in less than 48 hours after pollination with Manchurian and Snowdrift pollen at 65/40º lt/dk and 75/45º lt/dk test trials. At 55/35º lt/dk pollen tubes reached the end of some Gala styles in less than 96 hours after pollination with Manchurian and Snowdrift pollen (Figs. 3 & 4).
- There were no evident pollen tubes at stylar bases of Golden Delicious pistils pollinated with Golden Delicious pollen in any test.
- Some Golden Delicious pollen tubes growing on Golden Delicious stigmas and in styles developed bulbous ends which appeared to impede growth of tubes. This was not observed with Manchurian and Snowdrift pollen and may be related to pollen incompatibility.
- Stigma receptivity to pollen germination continued to 7 days after full bloom and pollen tubes grew to the end of styles after 96 hours at 55/35º lt/dk. Optimum receptivity occurred 3 days after full bloom with tube growth to the bases in 100% of the styles, 45% after 5 days, and 30% after 7 days.
- Crabapple pollinizers are cultivar/temperature sensitive.

Highlights of Year 2 – (2006)

- In-orchard applications of Liquid Lime Sulfur + Crocker's Fish Oil (LLS+CFO), based on predicted fertilization timing, were shown to be effective for preventing fruit set (Fig. 5).
- Growth progress of pollen tubes in styles affects the success/failure of application of bloom thinner (LLS+CFO, Fig. 6).
- LLS+CFO applied 4 hours (72°F) after hand pollination of 'Golden Delicious' pistils on-tree stopped all growth of pollen tubes into styles from stigmas. Mean temperature for 24-hr period after application was 63°F.
- LLS+CFO applied 24 hours (72°F) after pollination stopped all growth of pollen tubes to base of styles from stigmas. Average temperature for 24-hr period after application was 67°F and it was predicted that ovule fertilization had not yet occurred.
- LLS+CFO applied 48 hours (68°F) after pollination of 'Golden Delicious' pistils in orchard had little or no effect on growth of pollen tubes into styles from stigmas. Mean temperature for 24-hr period after application was 57°F and ovule fertilization had been predicted.
- Mean number of pollen tubes reaching base of 'Golden Delicious' styles sprayed 48 hours after pollination was similar to styles that received no spray treatment.
- LLS+CFO applied 4 hours after pollination of 'Fuji' pistils on detached spurs and placed in 75/45°F (lt/dk) rotation for 48 hours stopped all pollen tube growth from stigmas into styles.
- Controlled temperature/light tests on hand-pollinated 'Gala' and 'Golden Delicious' pistils confirm '06 data.

- Model predictions using 2006 full bloom (FB) and temperature data from three locations in Washington State show a range required for ovule fertilization after pollination: 30 hours (full bloom April 28) at Wenatchee to 102 hours at Omak/Pogue Flats (full bloom April 30, Fig. 7&8).

Highlights of Year 3 – (2007)

- Comparing in-orchard growth of Snowdrift pollen tubes into 7 cultivars, Golden Delicious flowers favored the longest pollen tube growth (6.4 mm) after 24 hours (Fig 9).
- Snowdrift pollen tube growth Red Delicious flowers was 2.0 mm in 24 hours.
- Snowdrift pollen tube growth was intermediate in Braeburn, Pink Lady, Gala, Honeycrisp, and Fuji after 24 hours.
- Pollen tube growth was slower in older flowers, decreasing 50% in flowers pollinated 3, 4, and 5 days after opening compared to those pollinated in the first 2 days later (Fig. 10).
- Thunderchild ranked higher than Snowdrift and Selkirk for number of pollen tubes penetrating Gala stigma bases; a commonly used commercial pollinizer, Manchurian, was low.
- Comparison of mean length of longest pollen tubes in Gala vs Fuji 96 hours after pollination showed equivalent pollen tube growth at 65/40ºF lt/dk and 75/45ºF lt/dk.
- Pollen tubes had reached the base of all Gala and Fuji styles tested at 65/40ºF lt/dk and 75/45ºF lt/dk at 72 hours after pollination.
- There were twice as many pollen tubes reaching base of Gala styles compared to Fuji.

METHODS

Pollen collection: Golden Delicious trees grown in root bags were removed from the orchard in early March and placed in cold rooms to delay flowering, then placed in a greenhouse to induce flowering for harvesting pollen. Branches of Manchurian and Snowdrift crabapples were collected from the field and forced to produce pollen in the greenhouse. Some pollen was also collected from trees in the field. Balloon stage flowers with anthers that had not yet dehisced were harvested for pollen. Anthers were removed from stamens of harvested flowers and allowed to dry overnight at room temperature, then pollen was screened, placed in glass vials and stored at 0°C in a larger jar containing Drierite. Pollen viability was checked on an agar/sucrose/boric acid mixture by incubating it at room temperature for 1 or 2 hours before scoring for germination under the microscope.

General procedures, growth chamber pollination studies: Gala, Fuji or Golden Delicious trees grown in root bags were removed from the orchard row early in March and held in a cold room to delay onset of bloom, then forced in a greenhouse to induce bloom. At late balloon stage, 12 flowers /treatment were selected for the pollination experiment. One day before hand pollination of test flowers, all anthers were removed from test flowers to prevent self-pollination. All other flowers on test trees were removed to prevent cross-pollination and to balance the test blossom distribution. Selected flowers were tagged and pollen was applied to stigmas with a #2 brush. Trees were then placed in temperature-controlled rooms under HPS 1000 watt lamp (approx. 600 $\mu mols \cdot m^{-2}s^{-1}$ at the tree upper canopy) for indicated lengths of time, temperature, and lighting. Flowers were removed from trees at indicated times, placed in a solution of 5% sodium sulfite in labeled glass containers, boiled for 15 min., then refrigerated until microscopic examination. Five styles from each of three flowers were detached from the ovary, dipped in fluorescence solution, squashed between microscopic slides, and allowed to incubate 24 hrs before examination with epi-UV light using a Zeiss HBO-50 high pressure mercury vapor light source at 100X. Collected data included abundance of pollen germination/tube growth (0-10) on the stigma surface (rating scale), number of tubes penetrating the stigma base, mean length of the longest pollen tube, mean style length, and number of pollen tubes reaching the base of the style.

General procedures, field pollination studies: Flowers on orchard trees were selected at late balloon stage for field pollination test conducted to examine the effect of a bloom thinning treatments using Liquid Lime Sulfur + Crocker's Fish Oil (LLS+CFO), applied at selected intervals after pollination, on pollen germination and tube growth. Golden Delicious/M.27 root-bagged trees in the field were used for this experiment. Trees were selected for uniformity and divided into four groups. Flowers at full bloom were hand-pollinated with Snowdrift pollen and any unused flowers were removed. Trees were covered with white insect netting until spray applications to prevent pollination from natural sources. Treatments were applied at 4, 24, and 48 hours after pollination. LLS+CFO was applied only once per treatment. Flowers, collected 48 hours after the treatment was applied, were processed and evaluated as indicated above or same for all field experiments. Hourly temperature for the period from application to sample harvest was recorded.

RESULTS & DISCUSSION

Research on the influence of temperature on pollen germination, fertilization, and pollen tube growth on apple cultivars over the past 40 years has been limited. The search for a better understanding of the biological process of pollination and the effect of temperature on pollen tube growth and fertilization was the basis for our research project covering the last 3 years. Previous research (Williams, 1965) on the Effective Pollination Period (EPP) indicated that fertilization would normally take from 5-7 days after pollination for pollen tubes to fertilize the ovule. Our research under controlled temperature and light conditions and in-orchard under normal growing conditions showed the process of flower fertilization occurring in as little as 24 hours after pollen is applied to the stigmatic surface under optimum growing conditions. Under suboptimal growing conditions the same result of flower fertilization can occur in 48 hours or less. These factors are critical to a grower who may be applying a bloom thinner with a mode of action of stopping pollen tubes from growing into the style. Under these parameters, a grower who waits 48 hours after bloom to apply the bloom thinner would be wasting time, money, and material to stop bloom set due to faster than anticipated tube growth and callose plugging that occurs during tube growth down the style. A pollen growth model showing the growth pattern once it is attached to the stigma would give the grower an essential tool to help determine the time frame for application of bloom-thinners. Our research has yielded the data necessary for the development of such a model.

From its inception, this research project has conducted over 20 individual experiments on the effect of temperature on pollen tube growth on the stigma surface, growth into the style, and culminating in the pollen tubes reaching the ovules. We have conducted tests using commercially important cultivars such as Gala, Fuji, and Golden Delicious. We have prepared for hand-pollinated over 2000 flowers. Approximately 10,000 styles have been dissected and evaluated to determine the growth rate of pollen tubes at various times after pollination. We have used as pollen sources for our testing Manchurian and Snowdrift crabapple pollen. We have conducted preliminary studies using other crabapple pollens for tests and some of these pollinizers show promise but will require additional testing before they can be recommended to growers as alternatives to standard pollinizers presently in use in orchards. Our research results are now ready to be implemented into a computer-generated model to project pollen tube growth under actual growing conditions in Washington State orchards. The yearly summary of our project that follows gives a breakdown of some of the more important findings.

In 2004, we found that pollen tubes in "on-tree" flowers grew more rapidly to the base of the style under continuous light in growth chambers than in flowers that were detached in either the dark or light. Based on these results, we believe that reserves mobilized to the flowers by the tree and additional ongoing photosynthesis are important influences in determining the rate of pollen tube growth at various temperatures. These findings led us to believe that in most cases these tests should be conducted on-tree for more reliable and conclusive results.

Our experiments in 2005, conducted on trees under controlled light/dark temperature regimes continued to build on findings in 2004, yielding significant information on the effects of temperature and light on pollen germination, fertilization, and pollen tube growth on Golden Delicious and Gala. Data from these experiments and subsequent tests have been used to develop a model to predict pollen tube growth and fertilization. Our tests involving several pollinizers and fruiting cultivars show that we cannot generalize pollen germination/tube growth rates to all pollinizer/cultivar combinations. Additional tests under in-orchard field conditions are needed to test the robustness of that modeling program so that growers can use in practice. Any modeling program must be cultivar/pollinizer specific, not an all-encompassing model of one size fits all program.

2006 experiments conducted in temperature and light-controlled growth chambers and in the orchard yielded more useful information. Temperature effect or growth rate of pollen tubes after pollination has a significant impact on optimal timing of bloom thinner applications. Knowledge of growth rate of tubes into styles after pollination is critical for successful bloom-thinning practices. Our tests have shown that delaying applications by one day can result in the bloom thinner LLS+CFO having little or no effect on flower fertilization on earlier pollinated flowers but inhibits the more recently pollinated ones. By understanding pollen tube growth rate after pollination in relation to temperatures, growers may save time and money by reducing sprays or by applications of sprays at optimum thinning times. By developing temperature-based pollen tube growth models, we can follow the development of pollen tubes from pollination of stigmas to fertilization of ovules. These models could take into consideration the mode of action of the type of thinners used.

In 2007 tests comparing Gala vs Fuji, pollen tube growth rate was not significantly different at evaluated temperature and times. Pollen tube growth rate decreased significantly in flowers pollinated 3 or more days after first opening. Average length of pollen tubes growing into styles was approximately 50% less in flowers pollinated 3 days after first opened compared to those pollinated in the first two days after opening. Our tests on flowers pollinated in-orchard showed marked differences in pollen tube growth in the varieties tested. Compatibility and viability tests of several crabapple pollinizers showed significant variability in numbers of pollen tubes growing into styles, which might result in reduced fruit set. Our continued studies in 2007 on the effects of alternating temperatures (35ºF to 75ºF) and light on pollen germination and tube growth have been combined with previous years data to formulate a growth pattern used to calculate pollen tube growth in selected Golden Delicious, Gala, and Fuji apples. At present we are collaborating with the Washington Tree Fruit Research Commission and Vincent Jones of Washington State University Tree Fruit Research and Extension Center in the development of a computer-generated pollen tube growth model for Washington State growers.

Fig. 1

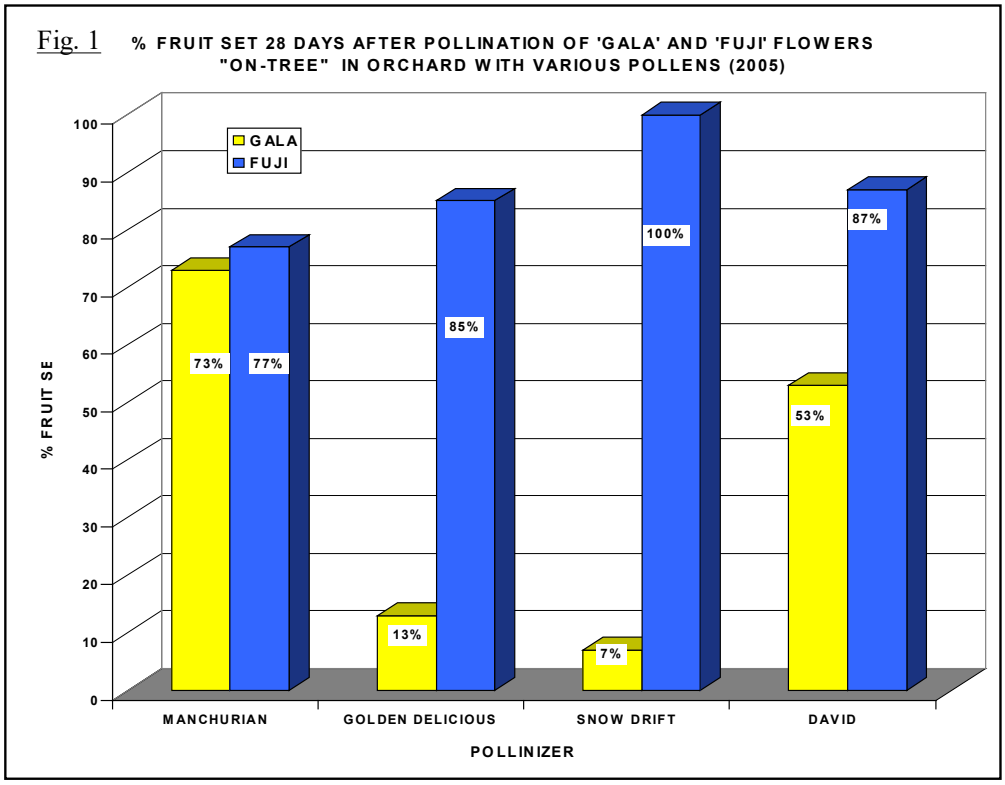

% FRUIT SET 28 DAYS AFTER POLLINATION OF 'GALA' AND 'FUJI' FLOWERS "ON-TREE" IN ORCHARD WITH VARIOUS POLLENS (2005)
GALA
FUJI
% FRUIT SE
100
90
80
70
60
50
40
30
20
10
0
73%
77%
13%
85%
7%
100%
53%
87%
MANCHURIAN
GOLDEN DELICIOUS
SNOW DRIFT
DAVID
POLLINIZER

Fig. 2

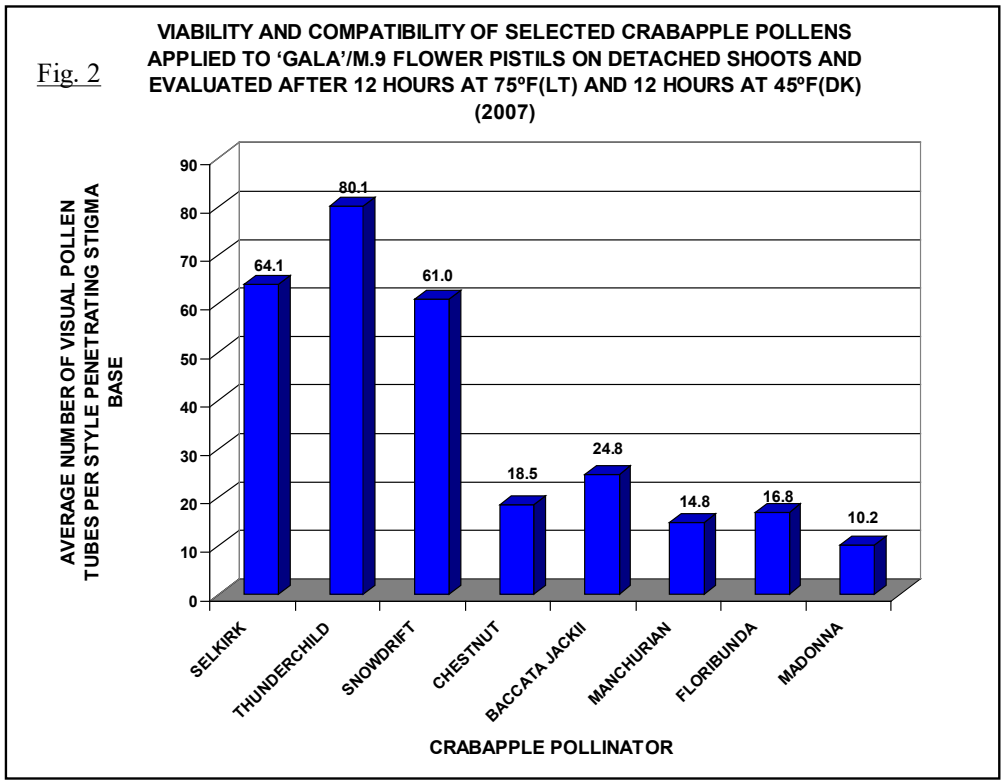

VIABILITY AND COMPATIBILITY OF SELECTED CRABAPPLE POLLENS APPLIED TO 'GALA'/M.9 FLOWER PISTILS ON DETACHED SHOOTS AND EVALUATED AFTER 12 HOURS AT 75ºF(LT) AND 12 HOURS AT 45ºF(DK) (2007)
AVERAGE NUMBER OF VISUAL POLLEN TUBES PER STYLE PENETRATING STIGMA BASE
90
80
70
60
50
40
30
20
10
0
64.1
80.1
61.0
18.5
24.8
14.8
16.8
10.2
SELKIRK
THUNDERCHILD
SNOWDRIFT
CHESTNUT
BACCATA JACKII
MANCHURIAN
FLORIBUNDA
MADONNA
CRABAPPLE POLLINATOR

Fig. 3

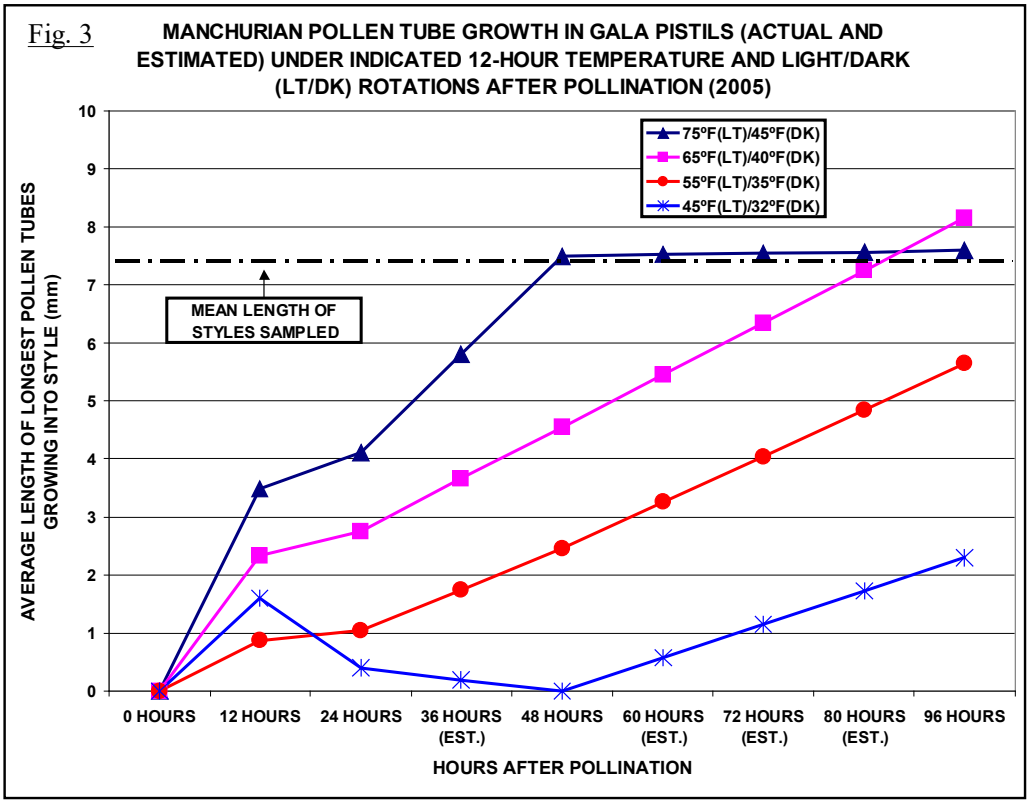

MANCHURIAN POLLEN TUBE GROWTH IN GALA PISTILS (ACTUAL AND ESTIMATED) UNDER INDICATED 12-HOUR TEMPERATURE AND LIGHT/DARK (LT/DK) ROTATIONS AFTER POLLINATION (2005)
75°F(LT)/45°F(DK)
65°F(LT)/40°F(DK)
55°F(LT)/35°F(DK)
45°F(LT)/32°F(DK)
MEAN LENGTH OF STYLES SAMPLED
AVERAGE LENGTH OF LONGEST POLLEN TUBES GROWING INTO STYLE (mm)
0
1
2
3
4
5
6
7
8
9
10
0 HOURS
12 HOURS
24 HOURS
36 HOURS (EST.)
48 HOURS
60 HOURS (EST.)
72 HOURS (EST.)
80 HOURS (EST.)
96 HOURS
HOURS AFTER POLLINATION

Fig. 4

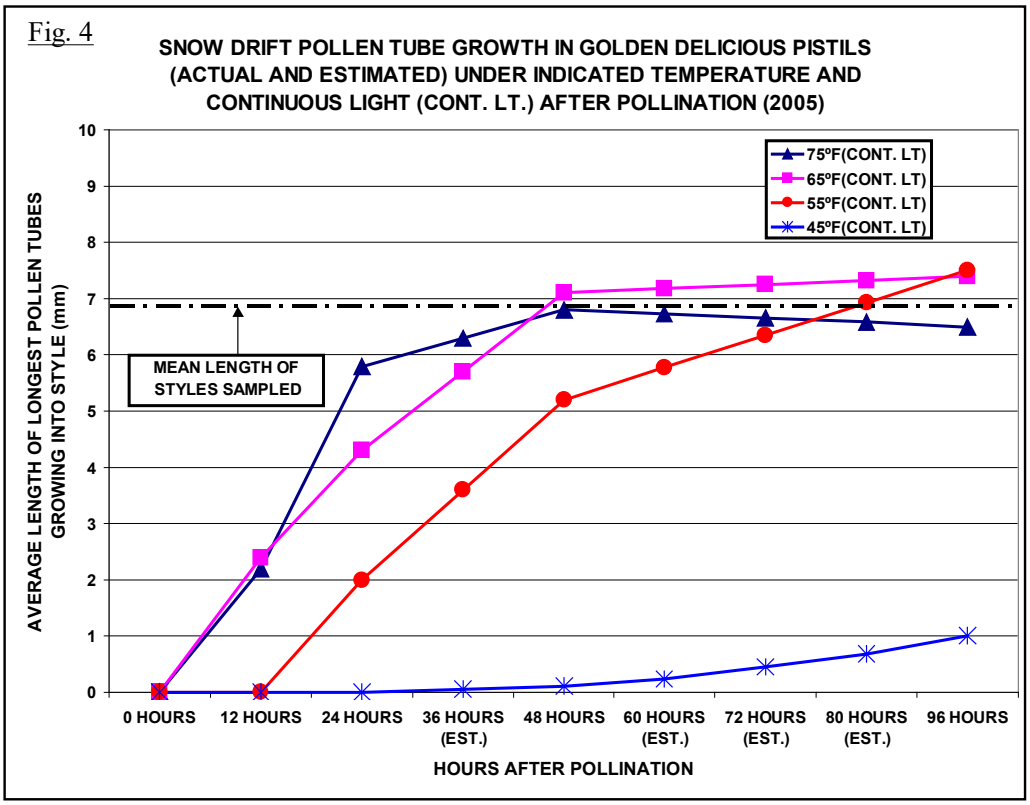
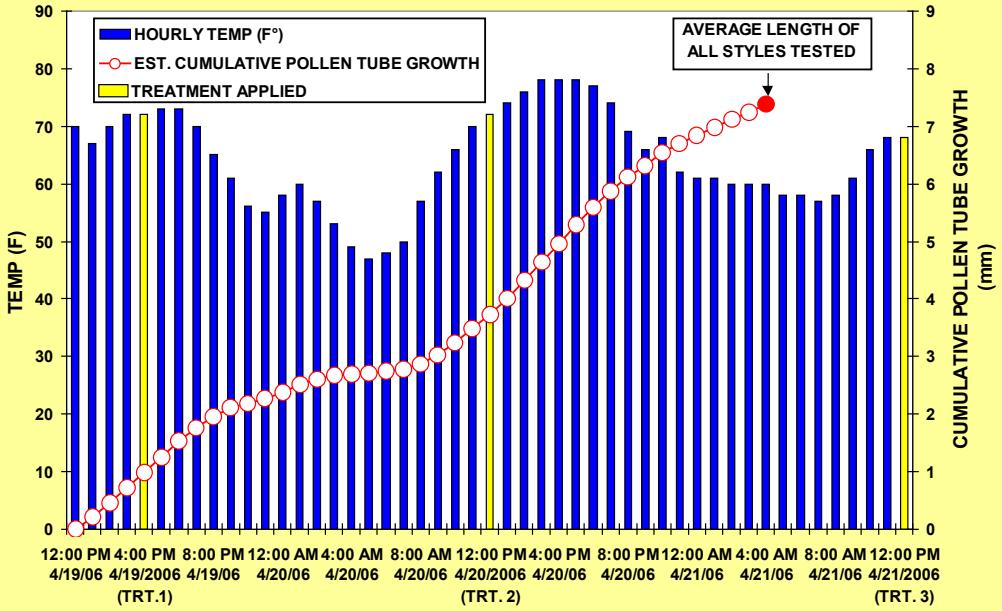

SNOW DRIFT POLLEN TUBE GROWTH IN GOLDEN DELICIOUS PISTILS (ACTUAL AND ESTIMATED) UNDER INDICATED TEMPERATURE AND CONTINUOUS LIGHT (CONT. LT.) AFTER POLLINATION (2005)
75°F(CONT. LT)
65°F(CONT. LT)
55°F(CONT. LT)
45°F(CONT. LT)
MEAN LENGTH OF STYLES SAMPLED
AVERAGE LENGTH OF LONGEST POLLEN TUBES GROWING INTO STYLE (mm)
0
1
2
3
4
5
6
7
8
9
10
0 HOURS
12 HOURS
24 HOURS
36 HOURS (EST.)
48 HOURS
60 HOURS (EST.)
72 HOURS (EST.)
80 HOURS (EST.)
96 HOURS
HOURS AFTER POLLINATION

Fig. 5

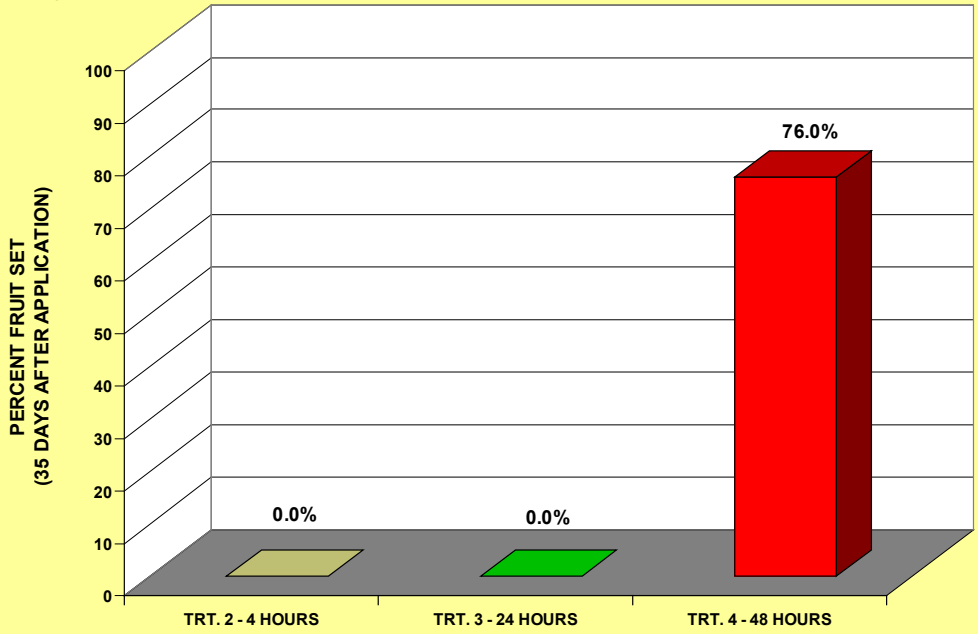
EFFECT OF TIMING OF APPLICATION OF LLS+CFO AT 4, 24, OR 48 HOURS AFTER POLLINATION ON POLLEN TUBE GROWTH IN HAND POLLINATED 'GOLDEN DELICIOUS'/M.27 FLOWER PISTILS IN FIELD (2006)



HOURLY TEMPERATURE AND ESTIMATED POLLEN TUBE GROWTH FROM POLLINATION TO TIME OF LAST TREATMENT APPLICATION

Fig. 6

Effect of timing of application of LLS+CFO on pollen tube growth and fruit set on hand pollinated 'Golden Delicious'/M.27 flower pistils in field at 4, 24, or 48 hours after pollination (2006)



LLS+CFO APPLIED AFTER POLLINATION

Fig. 7

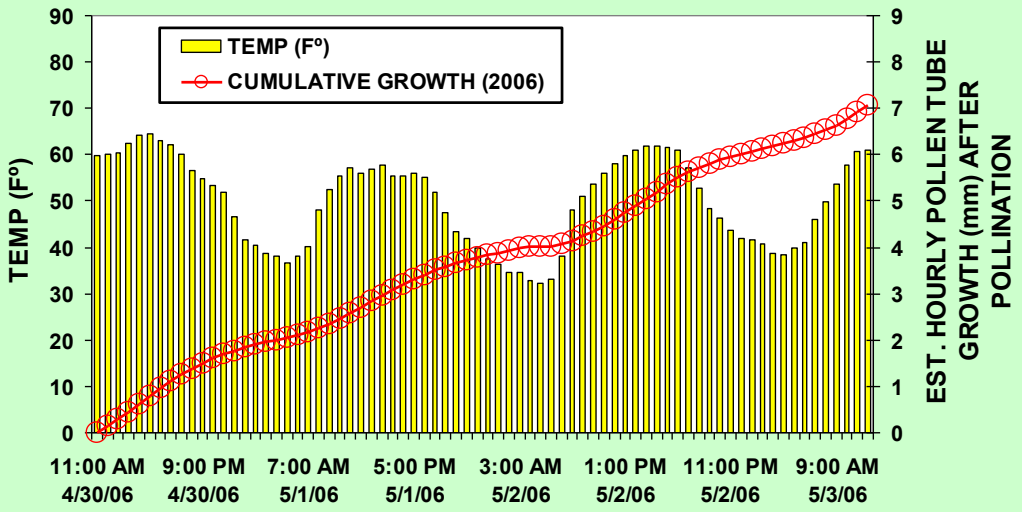

ESTIMATED POLLEN TUBE GROWTH FOR OMAK/POGUE FLATS, WA (2006)
TEMP (F°)
CUMULATIVE GROWTH (2006)
TEMP (F°)
EST. HOURLY POLLEN TUBE GROWTH (mm) AFTER POLLINATION
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
6
7
8
9
11:00 AM 4/30/06
9:00 PM 4/30/06
7:00 AM 5/1/06
5:00 PM 5/1/06
3:00 AM 5/2/06
1:00 PM 5/2/06
11:00 PM 5/2/06
9:00 AM 5/3/06

Fig. 8

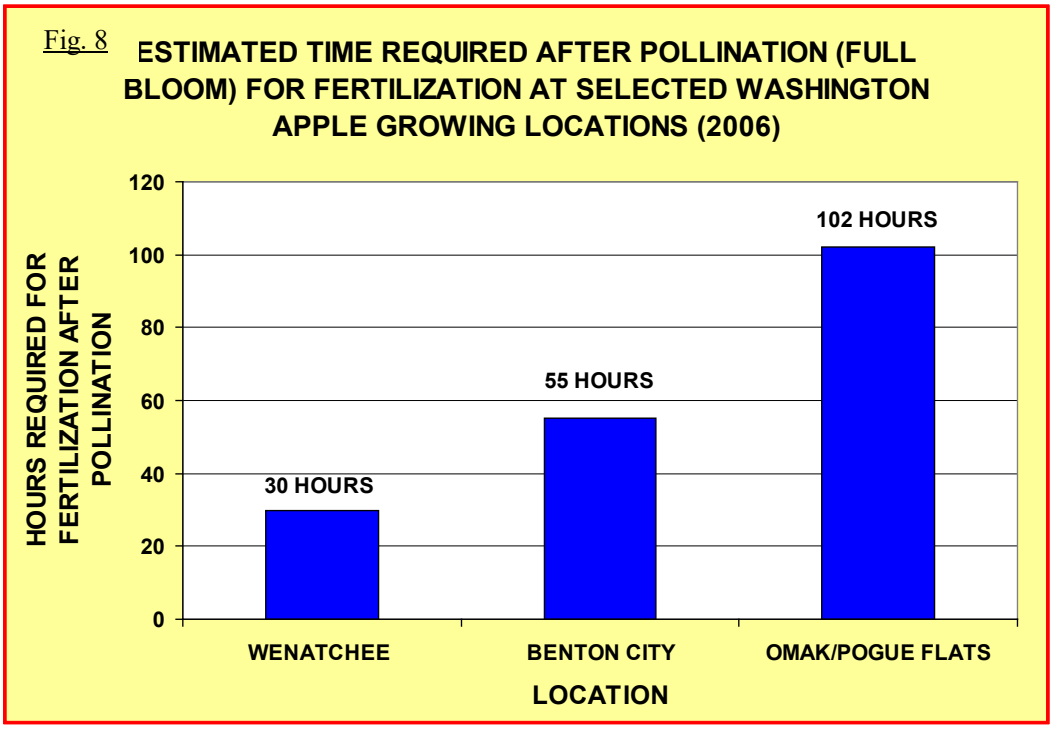

ESTIMATED TIME REQUIRED AFTER POLLINATION (FULL BLOOM) FOR FERTILIZATION AT SELECTED WASHINGTON APPLE GROWING LOCATIONS (2006)
HOURS REQUIRED FOR FERTILIZATION AFTER POLLINATION
0
20
40
60
80
100
120
30 HOURS
55 HOURS
102 HOURS
WENATCHEE
BENTON CITY
OMAK/POGUE FLATS
LOCATION

Fig. 9

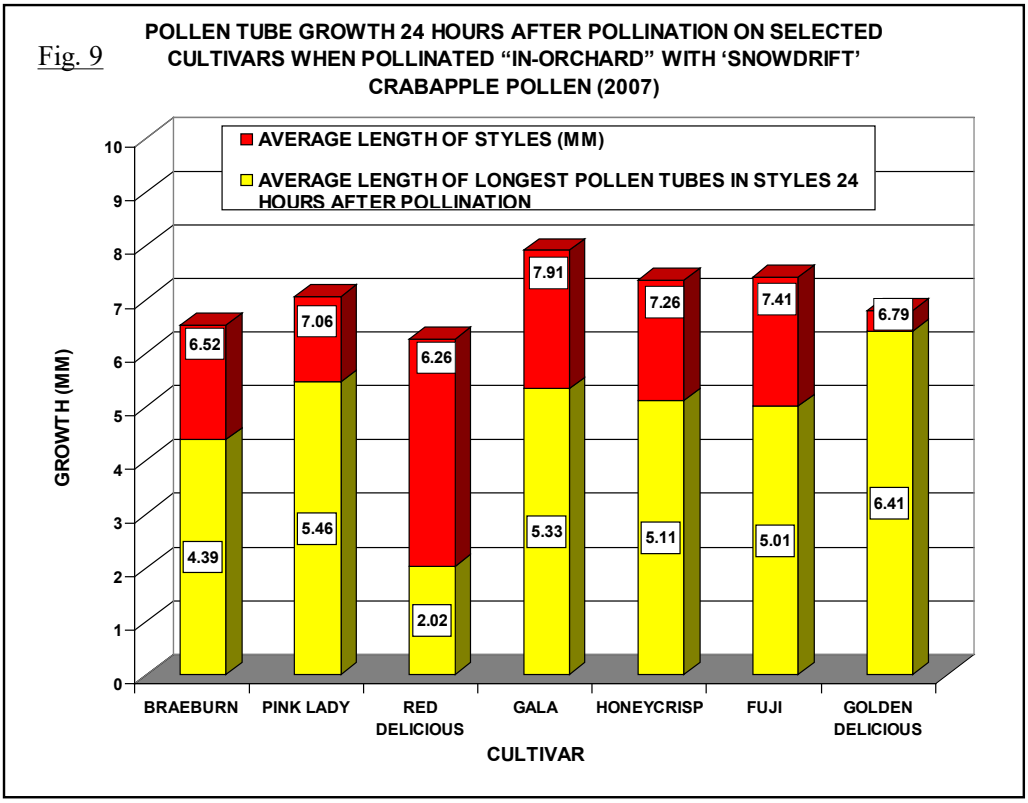
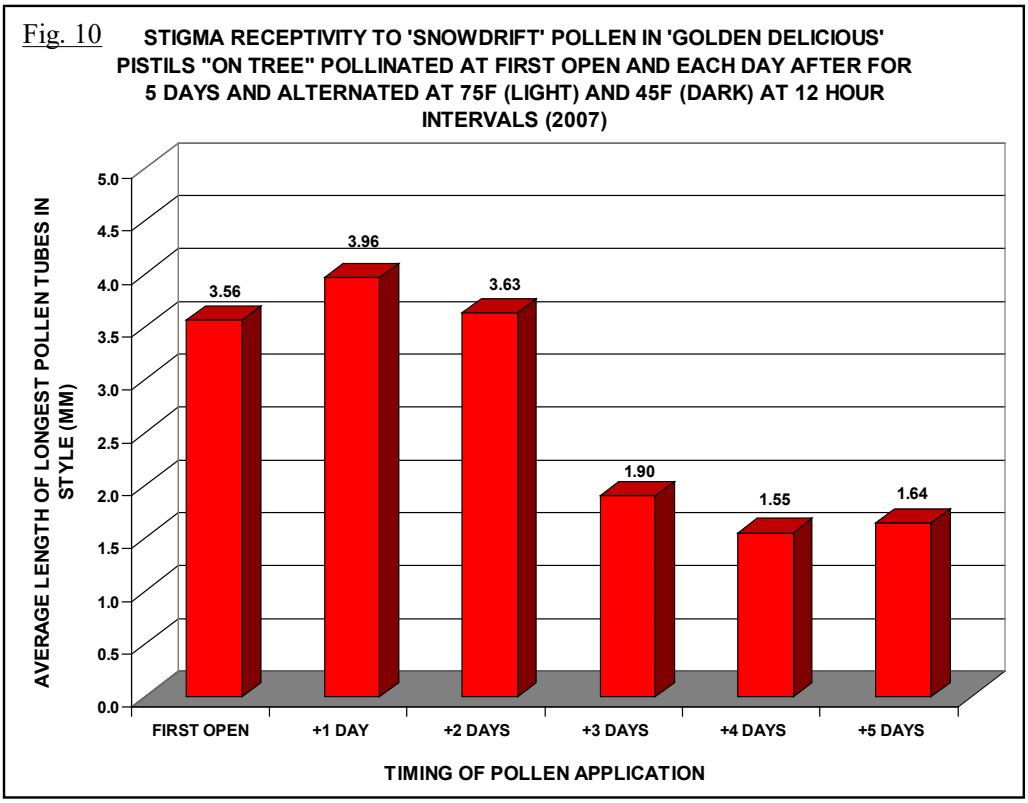

POLLEN TUBE GROWTH 24 HOURS AFTER POLLINATION ON SELECTED CULTIVARS WHEN POLLINATED "IN-ORCHARD" WITH 'SNOWDRIFT' CRABAPPLE POLLEN (2007)
AVERAGE LENGTH OF STYLES (MM)
AVERAGE LENGTH OF LONGEST POLLEN TUBES IN STYLES 24 HOURS AFTER POLLINATION
GROWTH (MM)
0
1
2
3
4
5
6
7
8
9
10
6.52
4.39
7.06
5.46
6.26
2.02
7.91
5.33
7.26
5.11
7.41
5.01
6.79
6.41
BRAEBURN
PINK LADY
RED DELICIOUS
GALA
HONEYCRISP
FUJI
GOLDEN DELICIOUS
CULTIVAR

Fig. 10


STIGMA RECEPTIVITY TO 'SNOWDRIFT' POLLEN IN 'GOLDEN DELICIOUS' PISTILS "ON TREE" POLLINATED AT FIRST OPEN AND EACH DAY AFTER FOR 5 DAYS AND ALTERNATED AT 75F (LIGHT) AND 45F (DARK) AT 12 HOUR INTERVALS (2007)
AVERAGE LENGTH OF LONGEST POLLEN TUBES IN STYLE (MM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
3.56
3.96
3.63
1.90
1.55
1.64
FIRST OPEN
+1 DAY
+2 DAYS
+3 DAYS
+4 DAYS
+5 DAYS
TIMING OF POLLEN APPLICATION

FINAL PROJECT REPORT
WTFRC Project Number: AH-05-507 (WSU Project #13C-3655-5299)

Project Title: Growth and crop load management in apple trees with bioregulators

PI: Don C. Elfving
Organization: WSU Tree Fruit Research and Extension Center
Telephone/email: 509-663-8181 x252; delfving@wsu.edu
Address: 1100 N. Western Avenue
City: Wenatchee
State/Province/Zip: WA 98801

Cooperators: Thomas D. Auvil, WTFRC; Eric A. Curry, USDA/ARS/TFRL; James R. McFerson, WTFRC; Dwayne Visser, WSU-TFREC

Other funding Sources

Agency Name: N/A

Total Project Funding: ***2005:*** 17,420 ***2006:*** 17,956 ***2007:*** 19,239

WTFRC Collaborative expenses:

Item	Year 3: 2007
Wages and benefits[1]	2,290
Travel[2]	200
Total	**2,490**

Footnotes:
[1]Calculate at $13/hour including benefits.
[2]Travel costs in 2007 based on fuel costs.

Budget History:

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	8,000	8,400	8,820
Benefits	2,720	2,856	2,999
Wages	2,000	2,000	2,000
Benefits	200	200	220
Equipment	0	0	0
Supplies	1,000	1,000	1,000
Travel	3,000	3,000	3,500
Miscellaneous	500	500	500
Total	**17,420**	**17,956**	**19,239**

Original objectives of the project:

1. Evaluate GA effects on return bloom in several alternating apple cultivars, e.g., 'Fuji,' 'Cameo,' and 'Braeburn.'
2. Examine single vs. multiple timings of various GA concentrations for efficacy in control of return bloom in apple to reduce amount of GA used, if possible.
3. Continue to test post-thinning ethephon as a method for stimulation of return bloom in cropping trees of several important apple cultivars, particularly those with alternate bearing problems.
4. Combine ethephon and GA programs in alternating cultivars as a possible strategy to help overcome alternate bearing.
5. Re-examine the integration of chemical thinning programs with GA/ethephon programs for beneficial effects on reduction of alternate cropping.
6. Test the use of cyclanilide and cytokinin bioregulators in high vigor sleeping-eye plantings for utility in programming lateral branch development into a specific canopy architecture as desired by the grower.

Additional objectives:

1. Evaluate various bioregulators for potential efficacy in stimulating bud activity and shoot growth on "blind wood."

Significant findings 2005:

1. The alternating flowering and yield effects observed this year in 'Fuji' trees treated with GA in 2003 were a direct result of altered flowering in 2004, **NOT** of altered fruit or crop load that year. **Hence, flowering intensity alone in 'Fuji' apples can induce an alternating bloom and production cycle.** It is likely that this fact explains the difficulty in controlling alternate bearing in 'Fuji' trees with thinning alone.
2. Even high concentrations of ethephon applied twice after the thinning period is over did not produce reliable improvement in flowering in cropping trees. Induction of good return bloom in cropping trees is very difficult.
3. New GA/alternate bearing trials established in 2005 have examined using GA, BA/GA or combinations of NAA/ethephon to more closely control the flowering cycle as a strategy for developing better control methods for alternate cropping in difficult cultivars such as 'Fuji.'
4. Cyclanilide works well for inducing branching in sleeping-eye trees, thus saving much labor cost, but it can reduce height growth to some degree.

Significant findings 2006:

1. Only the strongest bloom-suppressing GA treatment applied in 2003 (GA_7) continued to influence the flowering and yield of 'Fuji'/M.9 trees in 2006. Results from this trial and others have shown that an alternating cycle can be induced by a practice intended to reduce alternate bearing. New trials exploring the potential for yearly interventions to control flowering are now underway.
2. When flowering is reduced due to GA treatment the previous year, the potential exists for an increase in fruit set to take place. This phenomenon was observed in one trial in 2006. An increase in fruit set, if significant, might offset the benefit of reduced flowering on subsequent cropping.
3. New GA/alternate bearing trials established in 2005 are testing the continued use of GA, or the application of BA/GA or ethephon to more closely control the flowering cycle as a strategy for developing better control methods for alternate cropping in difficult cultivars such as 'Fuji.' GA_{4+7} at 100 mg/liter, Promalin at 100 mg/liter or ethephon at 600 mg/liter were applied in 2006 to manage flowering for 2007 with the objective of evening out the bloom and yield.
4. Cyclanilide works well for inducing branching in sleeping-eye trees, thus saving much labor cost, but it can reduce height growth to some degree. A large comparison trial between Promalin and cyclanilide for branch induction in sleeping-eye 'Fuji'/M.9 trees was undertaken in 2006.

Cyclanilide reduced leader-shoot elongation by about 30 cm, but may have induced thicker, better quality fruiting wood.

Significant findings 2007:

1. 2007 was the third year (second "off" year) of trials with GA and other bioregulators for control of alternate cropping in 'Fuji' and 'Braeburn trees. GA and Promalin applied in 2006 ("on" year) did not suppress return bloom in 2007 more than cropping alone. Only postbloom ethephon (Ethrel, 600 ppm, 6 weeks after full bloom) in 2006 improved the "off year" flowering and yield of 'Fuji' in 2007. Yield data in the 'Braeburn' trial were lost due to grower harvest.
2. Applications of cyclanilide continued to help produce branching at upper wires during the second (final) year of canopy development in sleeping-eye 'Aztec Fuji'/'Mark' apple trees in a high-density trellised planting. The grower made an observation that the cyclanilide-treated trees, though somewhat shorter than the Promalin-treated trees, had what looked to be more robust future fruiting wood. Follow-up studies will attempt to determine if there is a significant difference in early fruiting performance based on which branching agent is used during canopy development.
3. Cytokinin or cytokinin/gibberellin treatments in 2006 induced up to 5-fold increases in bud activity in latent buds on feathers of Jazz® ('Scifresh')/M.9 apple trees. A small percentage of those activated buds showed the presence of flower clusters in spring, 2007, but no effect of any cytokinin or cytokinin/GA combination on flower initiation itself was observed. A very few of these clusters set a fruit in 2007. Follow-up is planned to see if latent buds initiated into activity in 2006 will form spurs and flower more profusely in 2008, as would be normally expected.
4. Three cytokinins tested on 'Scifresh' latent buds in 2007 induced latent buds to break and grow. The cytokinins were thidiazuron, KT-30 chlorfenuron (CPPU, Kim-C1) and Prestige chlorfenuron (CPPU, Valent). Adding GA_{4+7} (ProVide) either alone or in combination with the cytokinin had no beneficial effect on bud development. Crushing the bark of young sleeping-eye 'Honeycrisp' and 'Fuji' apple trees at the trellis wire(s) where additional branches were needed did not increase branching. Treating crushed areas with cytokinins at up to 5,000 ppm also did not induce additional branch development. Higher concentrations may be required, but a limited number of available buds at each site may also account for the lack of new shoots.

Results and discussion

A. Control of flowering with GA in alternating apple cultivars (Objectives 1, 2, 4, & 5).

1. Previous trials showed the capacity of severely alternating cultivars to maintain this biennial cycle when treated with GA for inhibition of flower initiation. In fact, a single GA treatment can induce a continuing biennial cycle that can last for at least 4 years. In the various trials undertaken during this project, control of return bloom as a strategy for overcoming alternation has proven difficult. Supplementing an initial treatment with annual follow-up treatments that include: a) GA alone, b) GA + BA for some thinning or c) a late postbloom ethephon treatment to stimulate return bloom has also not proven to be consistently effective. In 2005, we reported evidence that the amount of bloom itself can induce bienniality in the absence of a significant difference in fruit load. This fact may be a part of the root cause of the stubbornly biennial behavior of some cultivars, such as 'Fuji' or 'Cameo'. If so, a more consistent flower-induction-controlling methodology is needed to attack this problem.

B. Induction of flowering with ethephon (Objective 3.)

1. In the alternate-bearing trials in this project, ethephon was applied annually in June to trees of 'Fuji'/M.26 and 'Braeburn'/M.9 that either received GA_{4+7} or were not treated during the first trial year ("off" year). While this strategy has not consistently led to improvement in return bloom, bienniality indices suggest that this approach may have merit. Perhaps higher doses or multiple applications can improve the response. The ethephon treatments applied in 2006 were

quite effective in 2007. What was it about the 2006 season that made this treatment stand out? Unfortunately we do not know.

C. Programming the induction of lateral branching in sleeping-eye apple trees with bioregulators (Objective 6).

1. In 2006, a test was established in newly planted 'Fuji'/M.9 apple trees to compare the effects of Promalin vs. cyclanilide in relation to stimulation of lateral branching at sequential trellis wires as the central leader developed vertically. Cyclanilide and Promalin sprays were applied to the shoot tips and nearby leaves whenever the shoot tip reached a wire. Cyclanilide was as effective as Promalin for inducing lateral shoots at wires, but resulted in somewhat shorter leader development (20-30 cm less). In year 2, a continuation of the program with lower concentrations of cyclanilide reduced the inhibitory effect of the bioregulator on leader extension growth while still producing good branch development. The lateral shoots developed from cyclanilide-treated trees appear a bit more "robust", leading to the supposition that they may produce better fruit when they begin to bear.

D. Stimulation of bud activity on "blind wood" in apple (New Objective 1).

1. Three cytokinins were tested for efficacy in stimulating growth activity from latent buds in five trials with 'Scifresh' (Jazz), 'Cameo' and 'Granny Smith' apple trees. Thidiazuron (TDZ) painted onto the basal halves of feathers at green-tip in second-leaf and newly planted Jazz trees produced a strong budbreak response. When applied at 2,500 mg/liter a.i., chlorfenuron (CPPU) was also effective, but 6-BA (Maxcel) produced only a marginal effect on budbreak, even at 5,000 mg a.i./liter. Although budbreak was induced, very few of the activated buds formed any sort of shoot. Combining nicking cuts with TDZ application to lateral shoots of 'Cameo' trees at green-tip produced the strongest budbreak response, but nicking only improved the response by about 10%. Crushing shoot bark in conjunction with other applications did not improve the response. High concentrations of TDZ, CPPU and 6-BA were applied to two-year-old wood and the base of one-year-old leader shoots on three-year-old 'Honeycrisp'/M.9 trees. The older wood produced almost no response, while the leader shoots did show some evidence of increased budbreak, but the overall response was poor. Higher concentrations might have helped, but the low vigor of the trees may have been the principal factor limiting the growth response. Good vigor is an important factor facilitating the bud development response to exogenous applications of cytokinins. Interestingly, the addition of GA in these trials did not have the bud-growth-stimulating effect seen when similar treatments are applied to sweet cherry.

Acknowledgments:
The assistance and support of the following persons and organizations are gratefully acknowledged: Dave Allan, Travis Allan, Bruce Allen, Tom Auvil, Bill Borton, Humberto Camacho, Felipe Castillo, Phil Dormaier, Dan Fulbright, Mark Gores, Jeff Henry, Steve Hull, Dr. Chris Ishida, Rick Kamphaus, Heidi Künzel, Dr. Jim McFerson, Chris Olsen, Byron Phillips, Steve Scheib, Major Tory Schmidt, Dr. Earl Seeley, David Silvernail, Kris Thomas, Dwayne Visser, Allan Bros. Orchards, Apple-Eye Orchards, BASF Corp., Bayer Environmental Science, Blue & White Orchard, Borton & Sons Orchards, Columbia Reach Pack, Inc., Dormaier Orchards, Firewood Orchards, Gores Orchards, Highland Partnership, Mountain View Orchards, Valent BioSciences, Washington Tree Fruit Research Commission, WSU Agricultural Research Center.

Publications 2007:

Elfving, D.C. and D.B. Visser. 2007. Improving the efficacy of cytokinin applications for stimulation of lateral branch development in young sweet cherry trees in the orchard. HortScience 42:251-256.

Elfving, D.C., S.R. Drake, A.N. Reed and D.B. Visser. 2007. Preharvest applications of sprayable 1-methylcyclopropene in the orchard for management of apple harvest and postharvest condition. HortScience 42:1192-1199.

Schmidt, T.R. and D.C. Elfving. 2007. Crop load management of apple via induced plant stress. J. Amer. Pom. Soc. 61:167-169 (2nd place U.P. Hedrick award 2007).

Elfving, D.C. and D.B. Visser. 2007. The use of bioregulators in the production of deciduous fruit trees. Acta Hort. 727:57-66.

Elfving, D.C. and D.B. Visser. 2007. Optimizing vegetative and reproductive growth. Proc. Wash. State Hort. Assn. 102:55-56.

Elfving, D.C. 2007. Bioregulator sprays. p. 74-86. *In*: T.J. Smith (coord.). 2007 Crop Protection Guide for Tree Fruits in Washington. EB 0419.

Elfving, D.C. and D.B. Visser. 2007. Bioregulator effects on growth, flowering and cropping in apple trees. Poster, Wash. State Hort. Assn. Annual Meeting, Wenatchee, WA.

Elfving, D.C. and D.B. Visser. 2007. Branch induction in pear trees with bioregulators. Poster, Wash. State Hort. Assn. Annual Meeting, Wenatchee, WA.

Elfving, D.C. and D.B. Visser. 2007. Bioregulators for managing growth, cropping and fruit quality in sweet cherry. Poster, Wash. State Hort. Assn. Annual Meeting, Wenatchee, WA.

Manuscripts accepted for publication:

Lenahan, O., M. Whiting, and D. Elfving. Gibberellic acid is a potential sweet cherry crop load management tool. Acta Horticulturae.

Schmidt, T.R., D.C. Elfving et al. GA and fruit maturity. HortTechnology.

FINAL REPORT
WTFRC Project Number: AP-07-708

Project Title: Sprayable 1-MCP for managing apple harvest and postharvest quality

PI: Don C. Elfving
Organization: WSU Tree Fruit Research and Extension Center
Telephone: 509-663-8181 x252
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Cooperators: Eugene M. Kupferman, Horticulturist, WSU-TFREC; Dwayne Visser, Agric. Technol. III, WSU-TFREC

Total project funding request: **Year 1: $12,000**

Other funding Sources: NONE

Budget:

Organization Name: WSU-Tree Fruit Research and Extension Center	**Contract Administrators:** Mary Lou Bricker; Kevin J. Larson
Telephone: 509-335-7667; 509-663-8181 x221	**Email address:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	2007
Salaries	5,220
Benefits	1,780
Wages	3,590
Benefits	410
Equipment	0
Supplies	200
Travel	800
Miscellaneous	0
Total	**12,000**

Objectives:

1. Evaluate effects of ethephon applied 4 weeks or 4 and 3 weeks before normal harvest on fruit quality attributes at harvest 2 or 1 week(s) before normal harvest and at normal harvest.
2. Examine effects of postharvest SmartFresh treatment with or without early ethephon on storability and fruit quality attributes for each harvest date after short-term RA and medium-term CA storage.
3. Assess whether combinations of ethephon applied 4 or 3 weeks before normal harvest and SmartFresh postharvest can enable earlier than normal harvest of fruit with comparable quality and storage characteristics to untreated fruit harvested at the normal timing.

Significant findings:

1. Two treatments of ethephon at 150 mg a.i./liter at a weekly interval had less effect on stimulating drop than a single treatment with 300 mg a.i./liter.
2. Both ethephon treatments induced significant drop; a stop-drop treatment of NAA should be used if this program is repeated (combining ReTain and ethephon treatment on 'Cripps Pink' apple stimulates, rather than retards, fruit drop).
3. Over a two-week interval (three harvests), ethephon stimulated fruit C_2H_4 production in proportion to treatment concentration, not number of applications.
4. Ethephon significantly increased C_2H_4 production for the first two harvests and enhanced starch hydrolysis on the second harvest date.

Methods:

One trial was established in a cropping 'Cripps Pink' apple orchard in Wapato. Multiple-tree plots were chosen to allow sufficient fruit for sequential harvests over 3 weeks. A randomized complete block experimental design was used, with pre-harvest treatments in a one-way treatment arrangement, subdivided into a 3X2 factorial when harvested fruit samples were separated into replicate non-treated or SmartFresh-treated fruit. Harvest evaluations of fruit characteristics were made, to be followed by subsequent evaluations at 60 days of RA storage, as well as 90 and 120 days of CA storage. Experimental harvest in this trial began 2 weeks before normal commercial harvest, to simulate the avoidance of an early freeze event that otherwise might severely damage a large percentage of the crop.

Results and discussion:

Preharvest ethephon did not result in softer fruit flesh at harvest, a common observation that does not indicate how fruit will behave in storage. Starch hydrolysis and C_2H_4 production were the two fruit characteristics most affected at harvest by preharvest ethephon treatment. The lack of accumulating differences in fruit characteristics at the third harvest suggests that the substantial crop loss to drop may have adjusted the population of remaining fruits such that less mature fruit were the only ones left on ethephon-treated trees at commercial harvest. If so, this observation is not of serious concern, since any program in which ethephon would be used preharvest would also automatically schedule harvest earlier than the normal commercial harvest time in order to retain a larger proportion of the crop in an early fall freeze event. Follow-up evaluations are planned for short-term RA (60 days) and medium-term CA (90 and 120 days).

Acknowledgments:

The assistance and support of the following persons and organizations are gratefully acknowledged: Lynnell Brandt, Nancy Buchanan, Clyde Buechler, Dr. Steve Drake, Jeff Henry, Dr. Gene Kupferman, Dr. Nate Reed, Chris Sater, Dwayne Visser, Marcia Walters, Mike Young, AgroFresh, Inc., Bayer Crop Science, E.W. Brandt & Sons, Inc., Washington Tree Fruit Research Commission, WSU Agricultural Research Center.

Publications 2007:

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Schmidt, T.R. and D.C. Elfving. 2007. Crop load management of apple via induced plant stress. J. Amer. Pom. Soc. 61:167-169 (2nd place U.P. Hedrick award).

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Elfving, D.C. and D.B. Visser. 2007. Bioregulator effects on growth, flowering and cropping in apple trees. Poster, Wash. State Hort. Assn. Annual Meeting, Wenatchee, WA.

Elfving, D.C. and D.B. Visser. 2007. Branch induction in pear trees with bioregulators. Poster, Wash. State Hort. Assn. Annual Meeting, Wenatchee, WA.

D.B. Visser and D.C. Elfving. 2007. Bioregulators for managing growth, cropping and fruit quality in sweet cherry. Poster, Wash. State Hort. Assn. Annual Meeting, Wenatchee, WA.

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Schmidt, T.R. and D.C. Elfving. Crop load management of apple via induced plant stress. J. Amer. Pom. Soc. (2nd place U.P. Hedrick award 2007).

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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Jim McFerson and Tom Auvil, WA tree Fruit Research Commission;
John Fellman, Dept of Hort & LA, WSU, Pullman,
Gordon Brown, Scientific Horticulture P/L, Tasmania, Australia

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.

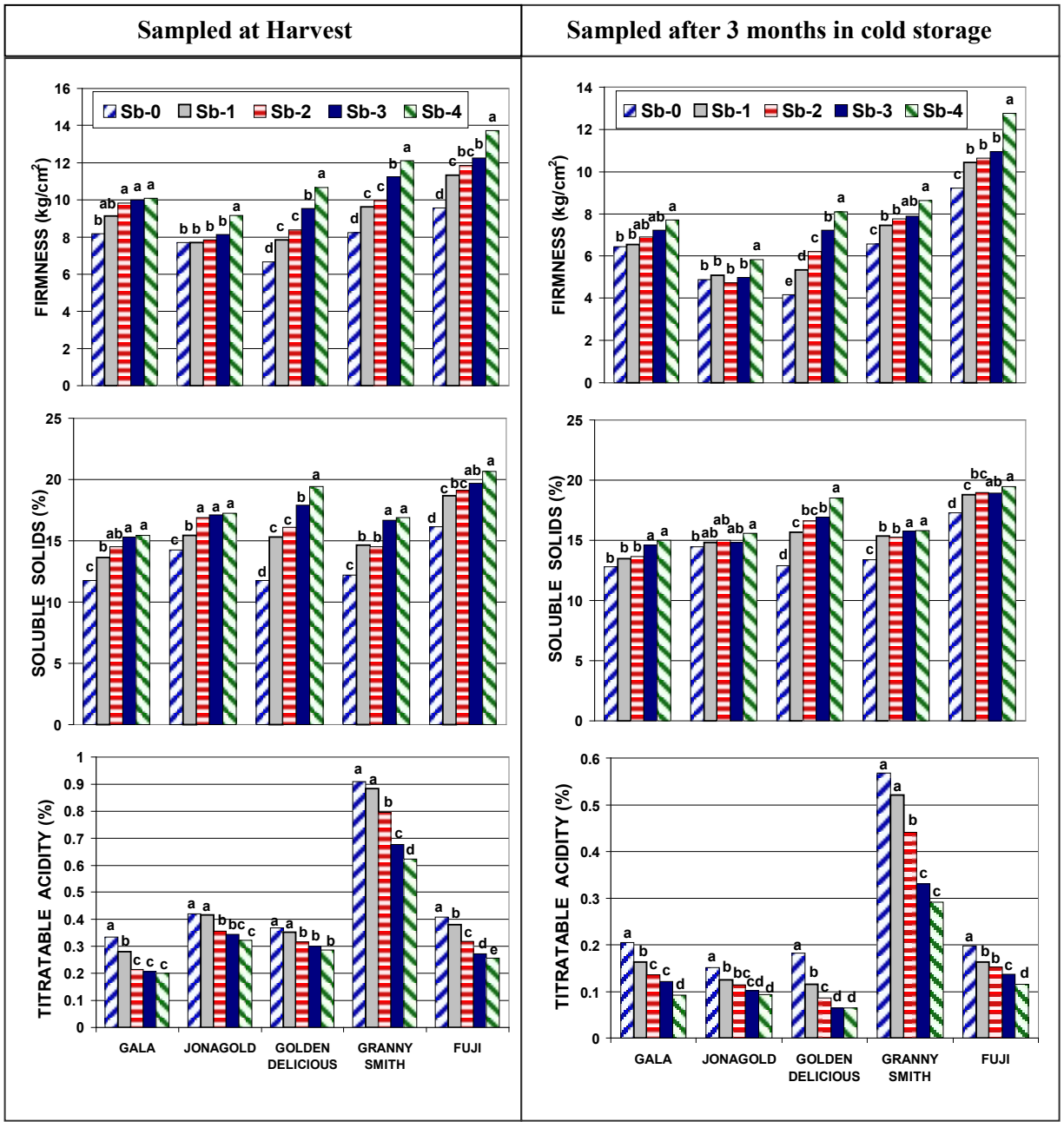

Fig. 1. Firmness, SSC, and TA in five cultivars of apples at harvest and at 3 months after harvest. Five classes of sunburn ranging from no sunburn (Sb-0) to severe sunburn browning (Sb-4) were compared for Gala, Jonagold, Golden Delicious, Granny Smith, and Fuji.

2. Fuji Flecking—Effects on Fruit Quality

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

3. Golden Delicious Russet—Effects on Fruit Quality

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

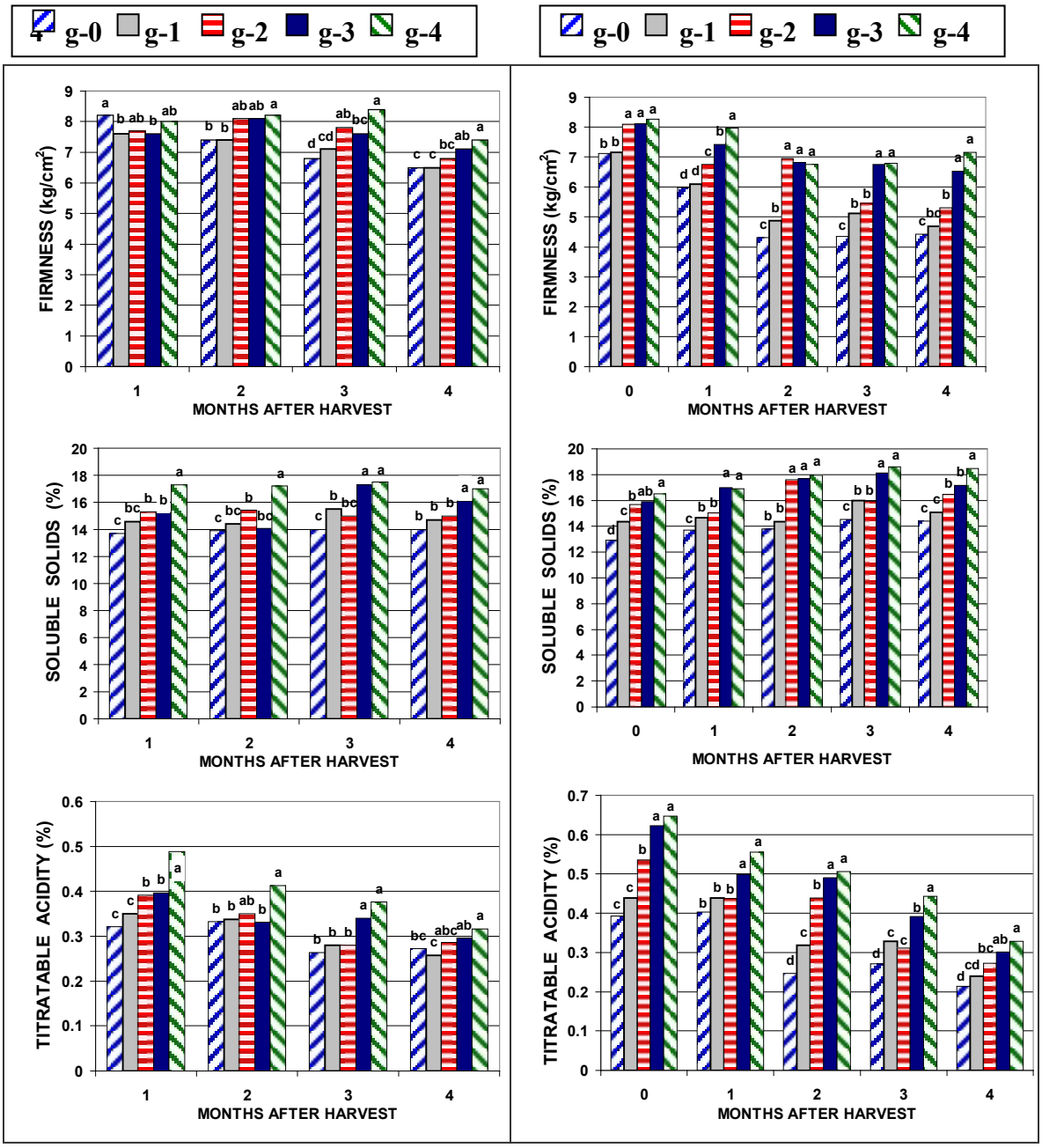

Fig. 2. Firmness, SSC, and TA in Fuji apples after 1, 2, 3, and 4 months of storage. Five classes of flecking ranging from no flecking (g-0) to severe flecking (g-4) were compared.

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).

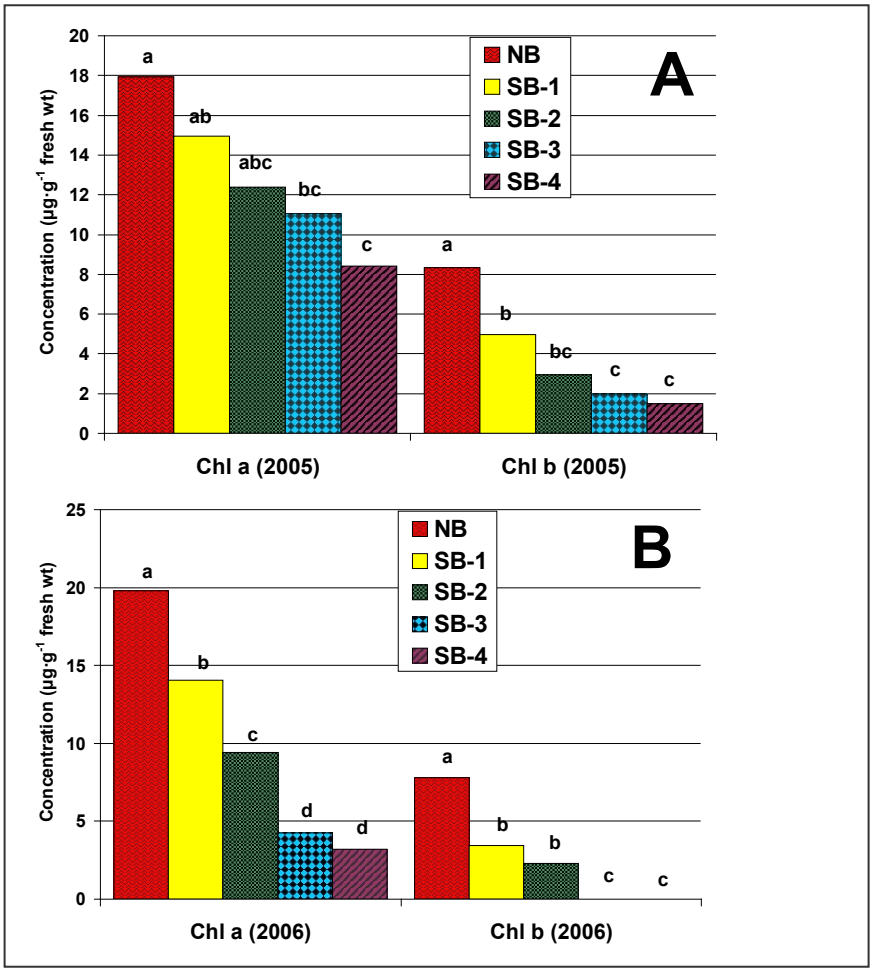

Fig. 4. Changes in chlorophyll a and b concentrations in Fuji apples with different degrees of sunburn damage. Fig. A is for 2005 and Fig. B is for 2006.

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.

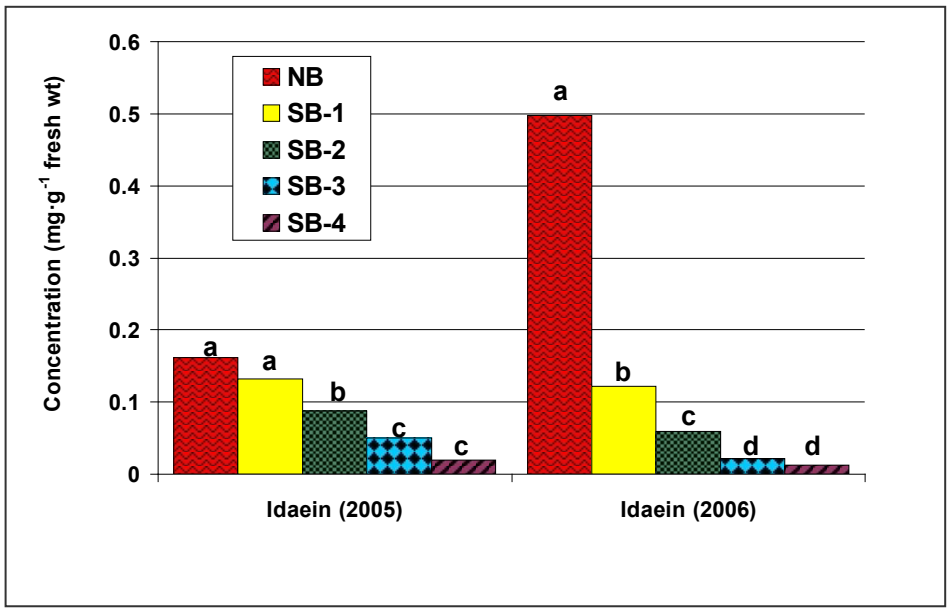

Fig. 5. Changes in Anthocyanin (idaein) concentrations in Fuji apples with different degrees of sunburn damage in 2005 and in 2006.

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.

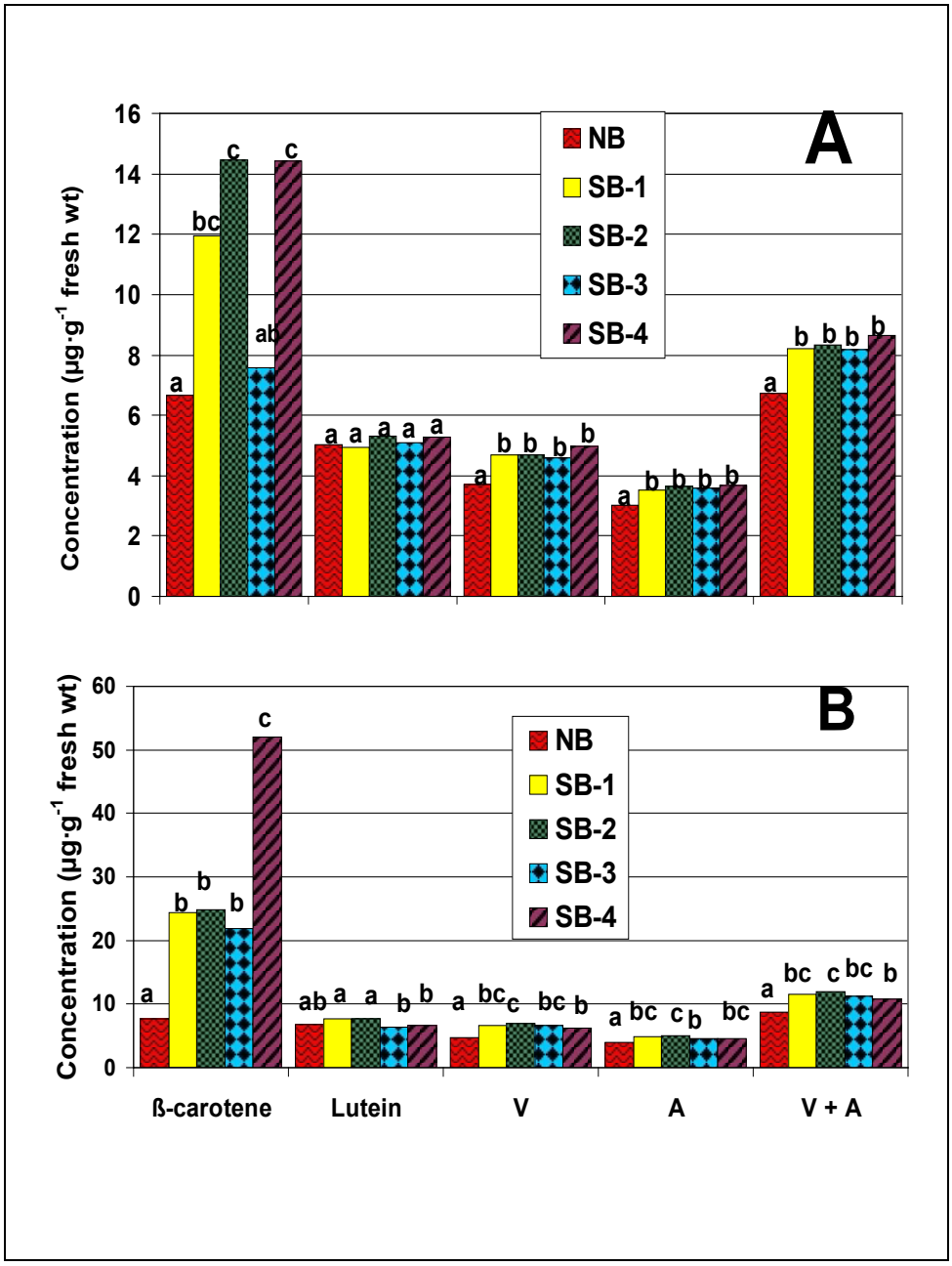

Fig. 6. Carotenoid concentrations for 2005 (A) and 2006 (B) peel samples. V = Violaxanthin; A = Antheraxanthin

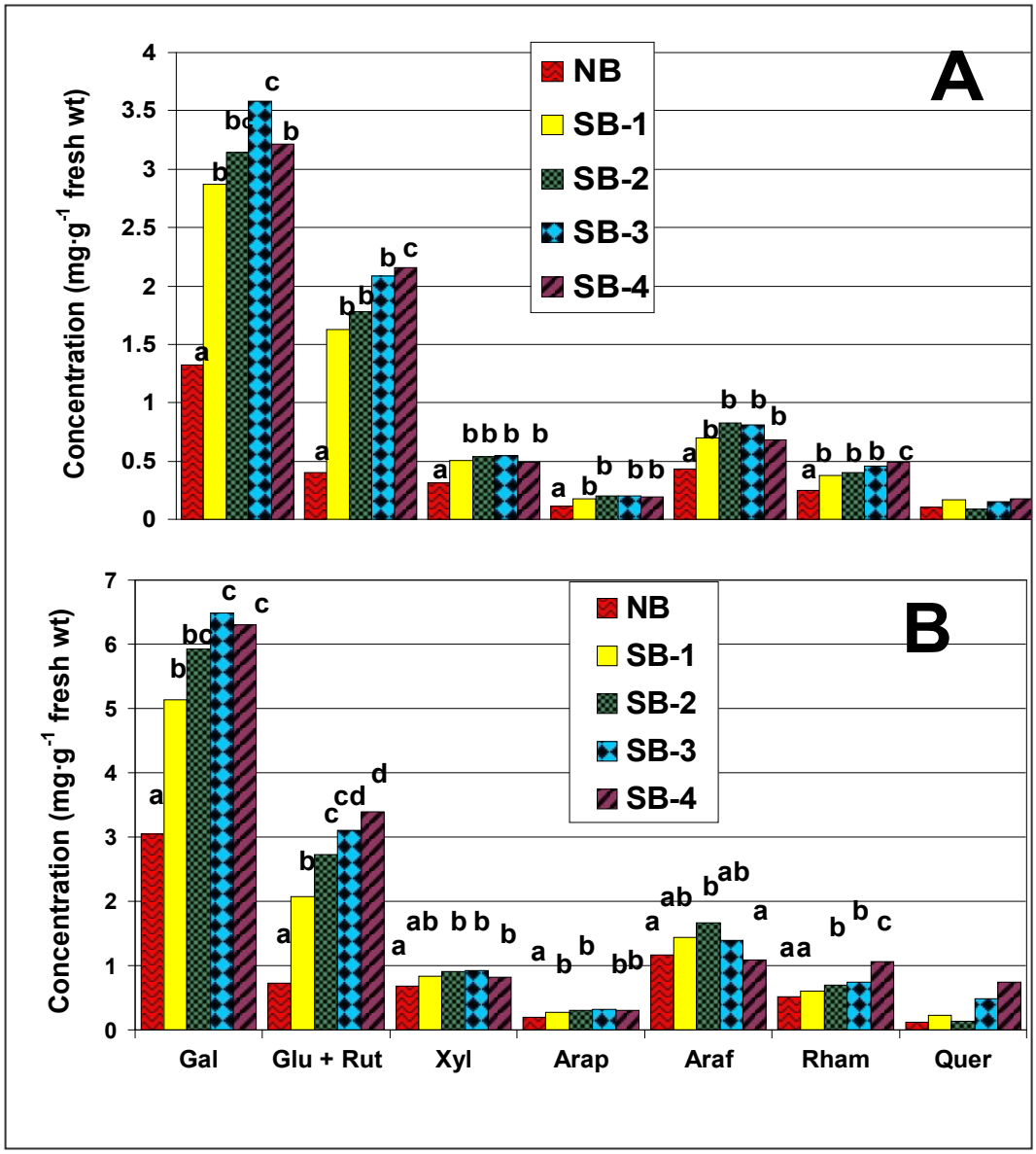

Fig. 7. Quercetin glycoside concentrations for 2005 (A) and 2006 (B) peel samples. Gal = quercetin galactoside; Glu + Rut = quercetin glucoside and quercetin rutinoside; Xyl = quercetin xyloside; Arap= quercetin arabinopyranoside; Araf = quercetin arabinofuranoside; Rham = quercetin rhamnoside; Quer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

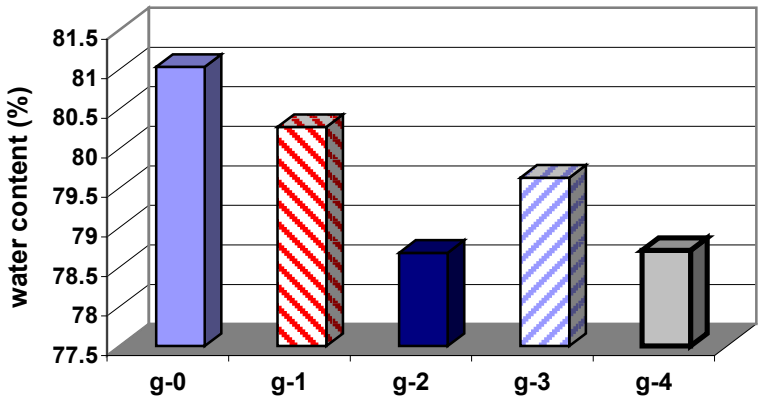

Fig. 8. Water content of Fuji apples with different grades of flecking.

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.

FINAL PROJECT REPORT
WTFRC Project Number: AP-06-604

Project Title: High temperature stress on apple fruit peel: physiology and detection

PI:	Lailiang Cheng	**Co-PI:**	Larry Schrader
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Cooperators:

Other funding Sources

Agency Name:
Amount awarded:
Notes:

Total Project Funding: $82,967

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	22,000	23,188	
Benefits	8,249	9,070	
Wages			
Benefits			
Equipment	0	0	
Supplies	10,230	10,230	
Travel	0	0	
Miscellaneous	0	0	
Total	40,479	**42,488**	

Objectives

The overall objective is to better understand the underlying physiology of high temperature stress to apple fruit peel with an ultimate goal of detecting and reducing high peel temperature-induced fruit disorders both preharvest and postharvest. The specific objectives are:

1) To determine how high temperature affects the balance of photooxidation and photoprotection of apple fruit peel, leading to sunburn browning;
2) To determine if chlorophyll fluorescence reflects the damage of high temperature on fruit peel and varietal differences during the growing season;

Significant Findings

1) Maximum photosystem II (PSII) quantum efficiency (Fv/Fm) of the sun-exposed peel of well-exposed fruit in the southwest canopy decreased during the day in response to high peel temperatures, and very little recovery was made during overnight dark relaxation, indicating that the high peel temperature has damaged the PSII centers of the peel.

2) After a couple days of high temperature exposure, more fruit in the west side of the canopy had very low Fv/Fm value than those in the east side. This difference corresponds to the different profiles of peel temperatures and sunburn occurrence between the two sides of the canopy. This along with the diurnal Fv/Fm data indicates that Fv/Fm is a very sensitive indicator of high temperature stress in apple peel.

3) Compared with the non-sunburned peel, the sunburned peel had lower chlorophyll content, lower Fv/Fm, lower net oxygen evolution rate, and lower activities of key photosynthetic enzymes, but higher activities of antioxidant enzymes and higher content of antioxidant metabolites and higher xanthophyll cycle activity on a chlorophyll basis, and higher hydrogen peroxide and malondialdehyde content. This indicates that high peel temperature most likely has increased the photooxidation potential, rather than decreased the photoprotective capacity of fruit peel.

4) Controlled temperature treatments of fruit peel samples in the dark showed that high peel temperature led to decreases in Fv/Fm and net O_2 evolution, and appearance of "K" step in chlorophyll a fluorescence transient. This indicates that high temperature has damaged the oxygen evolution complex of the PSII, leading to oxidative stress.

5) Simultaneous high temperature and high light treatment decreased Fv/Fm and O_2 evolution of Gala peel more than high temperature or high light alone. The clear "K" step in chlorophyll fluorescence, which appeared in the high temperature treatment, disappeared under simultaneous high temperature and high light treatment. This indicates that high temperature mainly affects the oxygen evolution complex of the PSII (the donor side) whereas high light mainly affects the acceptor side of the PSII.

6) Apple cultivars differ in their responses to high temperature and high light stress. Of the cultivars tested, Red Delicious is most tolerant of high temperature and high light stress whereas Cameo is the least tolerant. Our data show that chlorophyll fluorescence is an effective tool for testing varietal difference in tolerance to high temperature and high light stress.

Methods

<u>1. Determine diurnal changes of Fv/Fm in relation to peel temperature</u> This experiment was carried out on mature Gala/M.9 trees (spacing:15 X 6.5 feet) at WSU TFREC on July 21. Fifty well-exposed fruit on the southwest part of the canopy were selected the day before and the temperature of the sun-

exposed side of each fruit was monitored with a thermocouple connected to a data logger. In addition, the temperature of the shaded side of 3 fruit was also monitored along with ambient temperature. Every 4 hours starting from pre-dawn (5:00), ten fruit were dark-adapted for 30 min and then measured for Fv/Fm. The pre-dawn values of Fv/Fm were also measured the next day.

2. Determine the distribution of fruit peel Fv/Fm and sunburn occurrence on the east side and west side of the canopy after exposure to high temperatures. Ten well-exposed fruit from each side (east and west) of the canopy were selected and their peel temperatures were monitored as above from July 23 to 25. All the fruit from the east side and west side of the canopy were harvested separately and Fv/Fm of the sun-exposed peel was measured at pre-dawn on July 25. The percentage of fruit with sunburn was counted on separate trees with similar canopy size and structure in the morning on July 26.

3. Compare the sunburned and non-sunburned fruit in terms of photosynthetic capacity, chlorophyll fluorescence, and antioxidant system. The sun-exposed peel of non-sunburned and sunburned fruit (80 fruit each) was taken from the east and west side of the canopy from 9:15 to 10:00AM and from 4:00 to 4:45PM on July 25, respectively. The samples were immediately frozen in liquid nitrogen and stored until analysis.

4. Determine chlorophyll fluorescence and oxygen evolution of the sun-exposed peel of 'Fuji' fruit in response to controlled high temperature treatments. At approximately 100 days after full bloom (mid-August), well-exposed fruit on the west side of the canopy of Fuji/M.9 trees were taken right after sunset and the sun-exposed side of each fruit was marked. All the fruit were dark-adapted overnight at 22C and fruit peel samples (0.5 mm thick, 1 cm^2) were taken from the sun-exposed side. The peel samples were placed between two layers of wet paper towel and the assembly was put onto the bottom of a small aluminum foil vessel with the top covered with aluminum foil. Then, the vessel was directly floated on water in a water bath, the temperature of which was controlled by a refrigerated water bath and the temperature equilibrium between the fruit peel and water was reached within 1 to 2 min. The peel samples were exposed to 25, 35, 40, 42, 44, 46 or 48°C in the dark for 30 min. Chlorophyll a fluorescence transient and photosynthetic O_2 evolution were measured after the peel samples had been kept in the dark at room temperature for 30 min after each temperature treatment.

5. Determine the effect of simultaneous high temperature and high light stress on the sun-exposed peel of Gala fruit. Well-exposed fruit were harvested from the west side of Gala/M.9 trees on August 16, 2007, wrapped in wet paper towel and put into plastic bags immediately. After overnight dark adaptation in the lab, the peel discs with 3 mm thickness were cut from the sun-exposed side and were put onto 4 layers of wet cheesecloth in a stainless steel water jacket, the temperature of which was controlled by a refrigerated water bath. Light was provided by a tungsten lamp. Peel disc were treated with high temperature (45 ºC) in the dark, high light (1600 μmol m^{-2} s^{-1}) at room temperature, or cross stress of high light and high temperature (45 ºC, 1600 μmol m^{-2} s^{-1}) for 0, 15, 30 and 45 min, respectively. After dark-adaptation for one hour, chlorophyll a fluorescence transients and O_2 evolution were measured.

6. Determine varietal difference in response to high temperature and high light stress. In late August, well-exposed fruit of Cameo, Fuji, Gala, Golden Delicious and Red Delicious were harvested from the west side of the canopy, wrapped in wet paper towel and put into plastic bags immediately. After overnight dark adaptation in the lab, the peel discs with 3 mm thickness were cut from the sun-exposed side and were put onto 4 layers of wet cheesecloth on a stainless steel water jacket, the temperature of which was controlled by a refrigerated water bath. The light intensity was controlled by a tungsten lamp. Discs were treated with different temperatures (30, 35, 40, 42, 44, 46 and 48 ºC) in the dark or in the light (1200 μmol m^{-2} s^{-1}) for 30 min. After dark adaptation for 1 h, chlorophyll a

fluorescence transients were measured. For all the experiments, chlorophyll fluorescence was measured with a Handy PEA and oxygen evolution was measured with ChloroLab 2 (Hansatech, UK).

Results and Discussion

1. Diurnal changes of PSII quantum yield (Fv/Fm) of the sun-exposed peel and the shaded peel in relation to peel temperature on a hot day in central WA

On July 21 of 2006, the temperature of the sun-exposed peel of well-exposed fruit in the southwest part of the canopy increased almost linearly from 5:00 to 15:20, reaching 50C at 15:20, and then stayed above 45.9C till 17:30. In contrast, the shaded peel only reached a maximum of 40.7C between 17:00 and 17:30 when the highest ambient temperature was 35.5C.

Fv/Fm of the sun-exposed peel at pre-dawn was around 0.53, and remained essentially unchanged till 9:00. However, the Fv/Fm decreased linearly from 9:00 to 17:00, and then increased slowly from 17:00 to 5:00 the next day. The rapid decrease in Fv/Fm from 9:00 to 17:00 corresponds well with the period of high fruit peel temperature. Even at pre-dawn the next day Fv/Fm has not fully recovered to the previous pre-dawn level, indicating severe oxidative damage has occurred in the peel. In contrast, Fv/Fm of the shaded peel was higher than in the sun-exposed peel and remained unchanged throughout the entire day. The large error bar in the Fv/Fm data for the sun-exposed peel is due to the fact that there were large variations in Fv/Fm value among individual fruits.

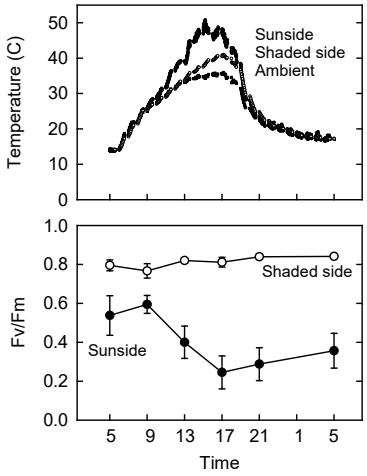


Fig 1

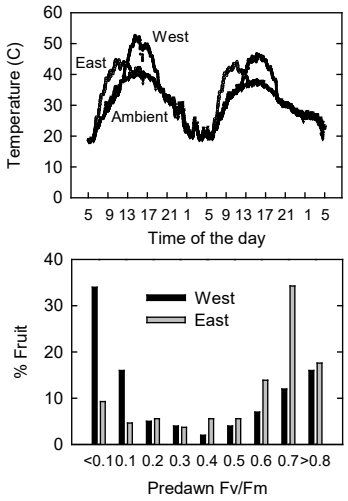


Fig 2

Fig 1. Diurnal changes of peel temperature and Fv/Fm in the sun-exposed peel and the shaded peel of well-exposed fruit in the southwest part of the canopy on July 21, 2006.

Fig 2. Diurnal changes of the temperature of the sun-exposed peel in the east and west sides of the canopy on July 23 and 24 and fruit distribution in terms of Fv/Fm measured at pre-dawn on July 25, 2006.

2. Distribution of fruit peel Fv/Fm and sunburn occurrence in the east side and west side of the canopy after exposure to high temperatures.

We compared the distribution of fruit Fv/Fm between east and west sides of the canopy after a couple days of high temperature exposure (July 23 and 24). As shown in Fig 2, the % fruit with an

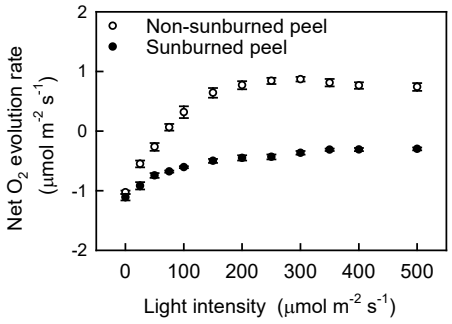
Fv/Fm value less than 0.1 was much higher in the west side (34%) than in the east side (9%) of the tree canopy whereas the % fruit with an Fv/Fm value between 0.7 and 0.8 was much lower in the west side (13%) than in the east side (34%). These numbers correspond well with the difference in temperature profiles between the two sides. Interestingly, both east and west sides had 16 to 18% of the fruit with an Fv/Fm value higher than 0.8, which indicates that both sides have equal number of shaded fruit. Counting the number of sunburned fruit on each side showed that the west side had 21.9% with sunburn whereas the east side had only 6.1%.

3. Comparison of sunburned and non-sunburned fruit peel

Photosynthetic oxygen evolution: As light level increased, net O_2 evolution rates for both non-sunburned and sunburned peels increased almost linearly first, then reached a saturation point, beyond which O_2 evolution showed little response to increasing light level (Fig 3). At each given light level, photosynthetic O_2 evolution rate was significantly lower in the sunburned peel than in the non-sunburned peel (Fig. 3). The quantum yield for O_2 evolution (the initial slope of each curve) was much lower in the sunburned peel than in the non-sunburned peel, whereas the light saturation point was higher in the sunburned peel than in the non-sunburned peel.

Hydrogen peroxide and malondialdehyde: The sunburned peel had higher concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA, an indicator of oxidative lipid metabolism) compared with the non-sunburned peel (Fig. 4), which clearly indicates that oxidative damage has occurred.

Activities of key photosynthetic enzymes: Compared with the non-sunburned peel, the sunburned peel had lower activities of key photosynthetic enzymes, including ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), NADP-glyceraldehyde-3-phosphate dehydrogenase, phosphoribulokinase, and stromal fructose-1,6-bisphosphatase (Data not shown). However, the activities of these enzymes decreased to a lesser extent than the net O_2 evolution rate.



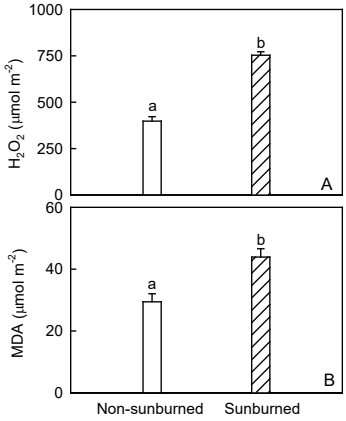


Fig 3 Fig 4

Fig 3. Light response of net oxygen evolution of sunburned and non-sunburned peels.
Fig 4. Hydrogen peroxide and malondialdehyde (MDA) content in sunburned and non-sunburned peels.

Reflectance and pigments: The sunburned peel had higher reflectance averaged over 400 – 700 nm. Reflectance spectra showed that the sunburned peel reflected more light in the range between 420 and 700 nm than the non-sunburned peel (Data not shown).

Chlorophyll, xanthophyll cycle pool size and lutein contents expressed on a peel area basis, and β-carotene and neoxanthin contents expressed on a peel area or Chl basis were lower in the sunburned peel than in the non-sunburned peel, whereas the contents of xanthophyll cycle pool size, zeaxanthin and antheraxanthin, and lutein expressed on a chlorophyll basis were higher in the sunburned peel than in the non-sunburned peel. This indicates that more chlorophylls were degraded relative to xanthophylls and other carotenoids. Almost all the xanthophyll cycle pool was converted to zeaxanthin and antheraxanthin in both sunburned and non-sunburned peels.

Antioxidant enzymes and metabolites: Activities of ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase were higher in the sunburned peel than in the non-burned peel, whereas there was no significant difference in superoxide dismutase or catalase activity between the two peel types (Data not shown).

The content of total ascorbate (reduced + oxidized), total glutathione (reduced + oxidized) and reduced glutathione was higher in the sunburned peel than in non-sunburned peel, but the ratio of both reduced ascorbate to total ascorbate and reduced glutathione to total glutathione were lower in the sunburned peel than in the non-sunburned one. No significant difference was observed in reduced ascorbate content between the sunburned and the non-sunburned peels (Data not shown).

Chlorophyll a fluorescence transients: When overnight dark-adapted fruit was exposed to a saturating pulse of light, chlorophyll a fluorescence showed a characteristic rise from minimal fluorescence (Fo) to maximum fluorescence (Fm) in the non-sunburned peel (Fig 5). However, the fluorescence signal of the sunburned peel was much lower and reached Fm at a much earlier stage followed by little change in fluorescence intensities. Chlorophyll fluorescence turns out to be the most sensitive of all the responses we have measured on the sunburned peel.

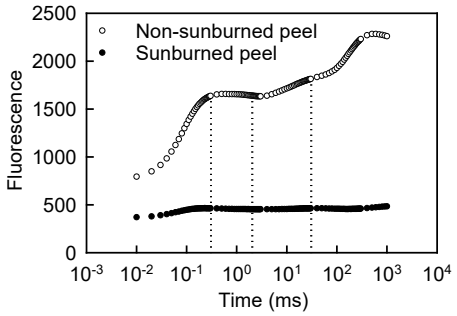


Fig 5. Chlorophyll fluorescence induction curves of sunburned and non-sunburned fruit.

4. Chlorophyll a fluorescence transients, Fv/Fm and photosynthetic oxygen evolution of the sun-exposed peel in response to temperature treatments

When the peel temperature increased from 25 to 35C, neither Fo nor Fm showed any significant change (Fig 6A). However, as peel temperature increased further, Fm decreased whereas Fo increased. In addition, the shape of the chlorophyll fluorescence induction curve changed. When the peel temperature reached 44 - 48°C, a very clear peak (called "K" step) at 300 μs appeared, followed by a pronounced dip. After exposure to 46 and 48°C, maximal fluorescence was already reached at "K" step followed by a rapid decrease to a level close to or below Fo (Fig. 6A). Fv/Fm changed very little from 25 to 40°C, and then dropped rapidly with further increase in temperature (Fig. 6B).

Net photosynthetic O_2 evolution rates remained unchanged as temperature increased from 25°C to 40°C, then dropped rapidly with any further increase in temperature (Fig. 6C). After

exposure to 46 and 48C, the net O_2 evolution rate became negative. However, heat stress showed no effects on dark respiration.

Decreases in Fv/Fm and net O_2 evolution, coupled with appearance of "K" step in chlorophyll a fluorescence transient indicate that high temperature has damaged the oxygen evolution complex of the PSII, leading to oxidative stress. However, the lack of a clear K step in the sunburned peel (Fig 6) suggests that there is interaction between high peel temperature and high light.

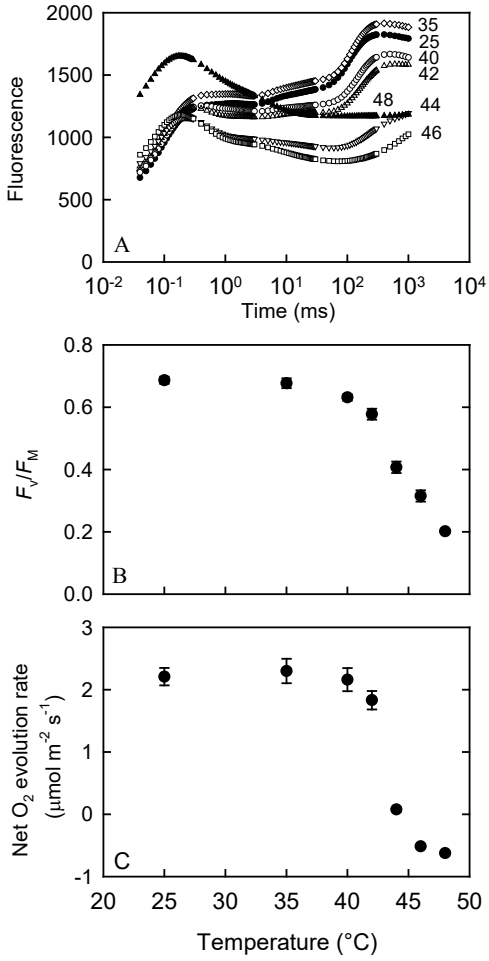


Fig 6. Chlorophyll fluorescence transient (A), maximum PSII efficiency, Fv/Fm (B), and net oxygen evolution of the sun-exposed peel of Fuji fruit in response to temperature treatments.

5. Responses of Fv/Fm, chlorophyll a fluorescence transients and photosynthetic oxygen evolution of the sun-exposed peel in response to high temperature and high light treatments

Simultaneous high temperature and high light treatment decreased peel Fv/Fm more than high temperature or high light treatment alone (Fig 7). Peel oxygen evolution rate was significantly lower in the simultaneous high temperature and high light treatment than in the high temperature or high light treatment alone (Fig 8A). Dark respiration was not significantly affected (Data not shown). This clearly indicates that high temperature coupled with high light causes more damage to fruit peel than high temperature or high light alone.

Chlorophyll fluorescence transients at the end of the 45 min treatment showed a clear "K" step in the high temperature treatment (in the dark) alone, but the "K"step disappeared in the

simultaneous high temperature and high light treatment. This suggests that high temperature mainly affects the oxygen evolution complex of the PSII (the donor side) whereas high light mainly affects the acceptor side of the PSII.

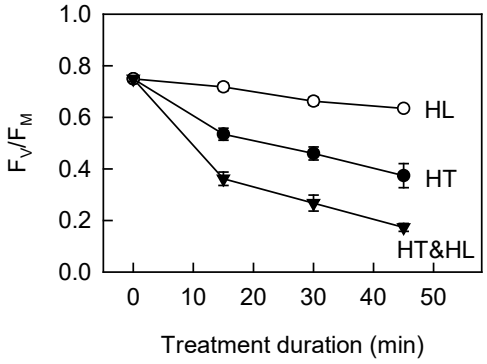


Fig 7. Maximum quantum yield of PSII (Fv/Fm) of the sun-exposed side of Gala apple peel in response to high temperature (45 ºC, HT), high light (1600 μmol m^{-2} s^{-1}, HL), and high temperature with high light (HT&HL) treatments.

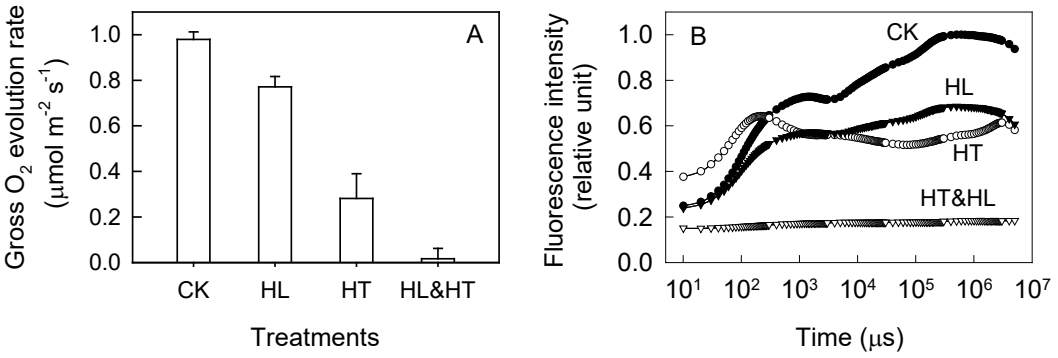


Fig 8. Oxygen evolution rate (A) and chlorophyll fluorescence transients (B) of 'Gala" peel in response to high light (HL), high temperature (HT) and high light with high temperature (HL&HT) treatments for 45 min.

6. Varietal difference in response to high temperature and high light stress

When treated in the dark, the Fv/Fm value of the sun-exposed peel of Cameo, Fuji, Gala, Golden Delicious and Red Delicious remained unchanged as the treatment temperature increased from 30 to 40 °C (Fig 9A). With further increases in treatment temperature, varietal difference showed up. Fv/Fm of Cameo peel started to decrease at 42°C, whereas that of Red delicious didn't decrease until temperature increased to 46°C. At any given temperature from 42 to 48 °C, Red Delicious had the highest Fv/Fm whereas Cameo had the lowest Fv/Fm.

When treated under high light, Fv/Fm of the sun-exposed peel of all cultivars tested decreased as the treatment temperature increased (Fig 9B). At each given temperature, Red Delicious had the highest Fv/Fm whereas Cameo had the lowest Fv/Fm.

Our data indicate that apple cultivars differ in terms of tolerance to high temperature and high light stress. However, the tolerance mechanism remains to be elucidated.

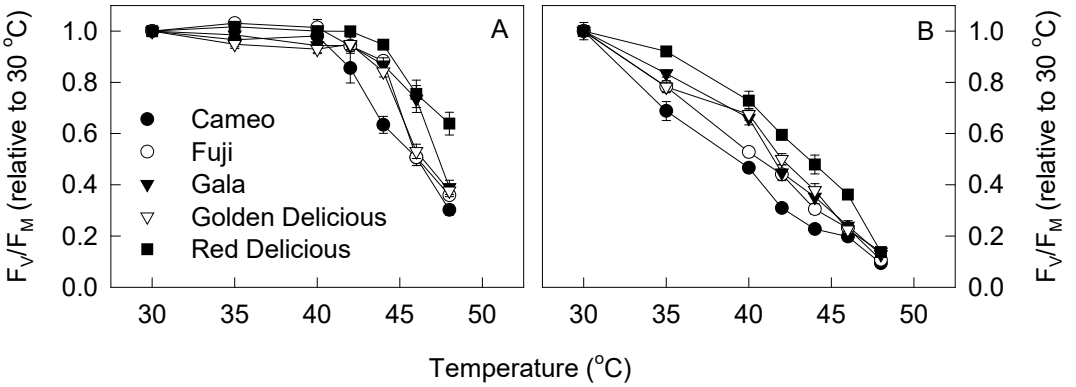


Fig 9. Maximum quantum yield of PSII (Fv/Fm) of the sun-exposed peel of five apple cultivars in response to temperature treatment for 30 min in the dark (A) or under 1200 μmol m^{-2} s^{-1} light (B).

FINAL PROJECT REPORT
WTFRC Project Number: PH-05-504

Project Title: Defining ethylene regulation of apple fruit quality traits

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Other funding Sources

Agency Name: N/A
Amount awarded:
Notes:

Total Project Funding: $128,295

Budget History:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries	$19,326	$20,292	$21,307
Benefits	$10,109	$10,651	$11,146
Wages			
Benefits			
Equipment			
Supplies	$ 9,500	$12,000	$11,000
Travel	$ 1,000	$ 1,000	$ 1,000
Miscellaneous			
Total	**$39,935**	**$43,907**	**$44,453**

Objectives:
Our overall goal was to define the role of ethylene in the functional regulation of apple fruit quality. This was accomplished by using transgenic apple fruit modified in their capacity to synthesize endogenous ethylene or wild type fruit modified in their ethylene response via the application of 1-methylcyclopropene (1-MCP). Tissues were obtained from ripening wild type, transgenic and treated fruit (ethylene gas of 1-MCP) displaying significant differences in phenotypic traits responsive to ethylene. These differences in phenotypes were matched with changes in gene expression patterns which were used to identify ethylene regulated genes in apple fruit tissues. We created specialized resources and mining tools to utilize the information available in GeneBank to annotate the genes that we identified. Validation of the ethylene regulated genes was accomplished by quantitative real time PCR (RT-PCR) along with the analysis of metabolites from the same tissues to define the biochemical pathways by identifying the key metabolites, their precursors and the enzymes involved.

Objective 1. Identify specific transcripts that are differentially regulated in transgenic apple fruit silenced for ethylene synthesis or perception, and correlate them with flavor and texture development - The primary aim of this objective is to compile and annotate the most highly regulated transcripts expressed during postharvest apple fruit development. The transcriptome of developing apple fruit has been sampled over time, with particular emphasis on transcripts expressed in cortical and peel tissues. Our approach has been on two fronts one is to develop or deploy bioinformatic tools to do a digital analysis of expressed genes available in GenBank (NCBI) and to develop and deploy microarray technologies to investigate ethylene dependent pattern of expression in apple fruit. Microarray analysis revealed the expression pattern of ethylene regulated genes some of these were validated using real time PCR (RT-PCR). We have also developed the resource to visualize apple expression data as pathways to better understand the relationships between the expressed patterns of genes in tissues obtained from different treatments and their regulation by ethylene.

Objective 2. Functional validation of pathways *via* analysis of key metabolites and enzymes regulated by ethylene – We have focused on 2 transgenic lines that make very low ethylene these are 68G expressing antisense ACC oxidase (ACO) and 103Y a line expressing a sense version of the apple ACC synthase gene (ACS). We have refined our analysis by focusing on peel and cortical tissues obtained from samples harvested in 2005 and 2006. We have carried out phenotypic, biochemical, enzymatic and metabolic analysis focusing on the postharvest behavior of gene activity. We would like to discover the subset of genes that are regulated by 'system 2' ethylene regulation, i.e., those genes/traits that are specifically regulated by autocatalytic ethylene biosynthesis. The phenotypic, metabolic and biochemical data has been integrated using gene set enrichment analysis to visualize the functional caregories of genes regulated by ethylene.

Significant findings/accomplishments

1. Development of unique study design that involves 2 transgenic, 1 treated and 1 wild type fruit to create 9 phenotypically distinct treatments.
2. Validation of the study design demonstrated the ethylene is positively correlated with color, starch and weight and negatively correlated with firmness and acidity. Ethylene is not correlated with soluble solids.
3. Successful deployment of microarray resources and analysis tools to dissect the transcriptome of apple fruit.
4. Differential gene expression patterns obtained by microarrays identified 3029 genes significantly regulated in apple fruit with 658 in cortical, 2169 in peel tissue and 381 in both tissues respectively that are likely regulated by ethylene.
5. Expression of genes related with ethylene biosynthesis (ACO, ACS and ERF3), aroma volatiles-related biosynthesis (AAT, LOX, LOX1) and sorbitol biosynthesis (NAD-SDH5,

NAD-SDH6, S6PDH, SDH4 and SDH5) down regulated in the GS (control) fruit treated with 1-MCP.
6. Sugar-acid balance is differentially regulated in cortical but not in peel.
7. Metabolite profiles showed that most of the statistically significant metabolites identified in cortical and peel are precursors of aroma-volatile compounds.
8. Ethylene is a modulator of most of the aroma related volatiles, especially the alcohol acyltransferase (AAT) enzyme activity.

Methods:

Plant material: The experiments utilized transgenic apple fruits suppressed in ethylene biosynthesis obtained from 2 different lines and one coltrol line grown in an experimental orchard.

Fruit collection and handling: Apples (Golden Delicious cv. 'Greensleeves', GS) were harvested from the research orchard when GS fruit was in a pre-climacteric stage (internal ethylene concentration lower than 0.3 $\mu L\ L^{-1}$) prior to the initiation of autocalylytic ethylene biosynthesis (Fig 1). Apples were transported to the Postharvest Pomology Research Laboratory at UC Davis and sorted to select those that were free from defects. Matched samples of 1 to 5 apples per replicate were prepared with 3 to 5 biological replicates per treatment.

Treatments: Fruits from selected 'Greensleeves' apples lines including transgenic 68G (ACO-antisense), 103Y (ACS-sense). Half of the fruit from each line was kept at 20ºC in an ethylene-free atmosphere and the other half was stored at 20ºC under the flow of air containing 80 $\mu L\ L^{-1}$ ethylene during storage. Fruit were sampled at 1 day after 14 days of storage at 20ºC. The controls GS were also harvested at the same time as the transgenic lines and these were treated with 0 (control) or 1□LL^{-1} 1-MCP in a 20L sealed glass jar for 20 h at 20ºC before storage at 20ºC for 14 days. Relative humidity was maintained close to 90-95%. The untransformed fruit (GS) and treated fruit were sampled at 1 day after 14 days of storage at 20ºC in air (ethylene-free atmosphere). After storage fruit tissues were dissected to obtain peel and cortical which were frozen in liquid N_2 and kept at -80 ºC until analysis. For all biochemical analysis, three replicates of five fruit each was used.

Ethylene and respiration rate measurements: Within each experiment ethylene production and respiration rates were determined at 1 and 14 days after storage for individual fruits using a static system. Exit air samples was collected from each jar and analyzed for CO_2 concentration (by an infrared gas analyzer) and ethylene concentration (by a flame ionization gas chromatograph) (Defillipi et al., 2004).

Maturity and quality parameters: An initial sample from each harvest was evaluated for skin color (by a Minolta Chromameter), starch pattern (by IKI staining), soluble solids content (by a refractometer), titratable acidity (by an automatic titration system), cortical firmness (by a Guss fruit texture analyzer and an Aweta Acoustic Firmness Sensor). All of these were used to document the mature apple phenotype and provide a "quality" reference for samples tested for comparison with ethylene-silenced fruit.

Microarray analysis of the transcriptome: A custom 12K oligonucleotide microarray was designed by CombiMatrix using the UniGene Build#14 (05 Apr 2006) their bioinformatics pipeline and design criteria and utilizing only the apple unigene entries that came from the 34 fruit libraries from GeneBank. Combimetrix synthesized the desired oligonucleotides on the chip surface and then sent us the chips. We isolated RNA from the two tissues (peel and cortex) from the 9 treatments (Fig 1), labeled RNA using the standard one color biotin labeling kit. The hybridization, imaging, stripping and re-hybridization was performed at the Microarray Core Facility at UC Davis as per the

CombiMatrix protocols available on their website (http://www.combimatrix.com/docs/PTL006_00_12K_Hyb_Imaging.pdf). The chips were scanned at this facility using a GenePix scanner and the spots were manually aligned to the grid supplied by ComBimatrix to obtain a tiff image. The tiff image was preprocessed with global median scaling normalization, background (av of lowest 5% of signal of control probes) subtraction to obtain gene expression data. R package LMGene was used to perform one-way ANOVA to obtain p-value for each gene. R package multtest was used to BH adjust all p-values for multiple hypotheses. R package limma was used to obtain pair wise comparisons to identify tissue and treatment specific genes.

Determination of sugars, acids and related enzymes activities: Fruit cortical and peel tissues obtained from the various transgenic lines were analyzed for soluble sugars (sucrose, fructose, glucose, sorbitol), and acids (malic and citric acids) using a high resolution GC/MS equipment. Enzyme that regulate sugar-acid balance was assayed using methods described by Dey and Harborne (1990).

Additional Metabolite analysis: Metabolite analysis of samples obtained from all treatments was carried out at the Metabolomic facility of the Davis campus (http://fiehnlab.ucdavis.edu/) under the direction of Prof. Oliver Fiehn and the manager of the facility Dr. Valdimir Tolstikov. Cortical and peel tissues carefully separated and frozen in liquid N_2 and kept at -80 ºC, until sample preparation was carried out at the Metabolomic facility. Using their high resolution GC/MS equipment a relative abundance was obtained for 400 compounds that can be resolved using their protocol for each of our samples.

Results and Discussion

1. Development of unique study design that involves 2 transgenic, 1 treated and 1 wild type fruit to create 9 phenotypically distinct treatments.

Our overall goal was to define the role of ethylene in the functional regulation of apple fruit. The availability of transgenic apple fruit modified in their capacity to synthesize endogenous ethylene and the use of 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, was used in our study design as they have distinct phenotypes demonstrated earlier by us (Dandekar et al., 2004). The various treatments used in our study are outlined below in Fig 1. Fruit harvested at a preclimacteric stage before the onset of system 2 ethylene formation and kept at 20ºC for 14 days. Transgenic lines 68G and 103Y were incubated with and without ethylene whereas the controls were incubated with or without 1-MCP. This gave us the 9 treatments that we investigated in our study to define the ethylene responsive genes. The Fig 1 is adapted from our earlier publication that shows the autocatylytic ethylene biosynthesis in the wild type GS controls at day 14 but not in the transgenics.

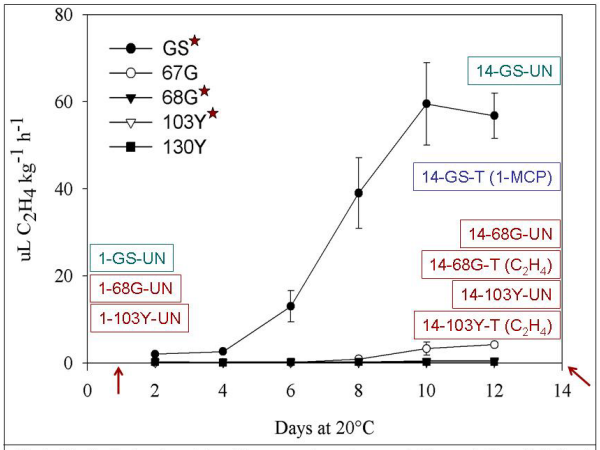

Fig 1: Study design involving 2 transgenic and one wild type yielding 9 distinct treatments modulated by or responsive to ethylene levels

2. Validation of the study design demonstrated the ethylene is positively correlated with color, starch and weight and negatively correlated with firmness and acidity. Ethylene is not correlated with soluble solids.

Application of 1-MCP to GS (control) apple fruit completely suppressed ethylene production. Application of exogenous ethylene to the 68G and 103Y transgenic lines did not produce increased ethylene biosynthesis.Weight, external color, firmness, starch index, and total acidity were regulated by ethylene in both 2005 and 2006, but total soluble solids were ethylene-independent (Fig 2). The apple fruit from the 2006 crop was affected by unseasonal and sustained heat at maturity and quality indicators showed ethylene dependence, but not color. Firmness, a primary measure of maturity and quality, is regulated by ethylene biosynthesis. Firmness was most affected in the 1-MCP treated fruit; the effect of ethylene suppression on firmness in transgenic lines was less obvious. Firmness after 1 day storage at 20 ºC was measured using a Guss fruit texture analyzer (destructive measurement method) and after 14 days firmness was measured first by an Aweta Acoustic Firmness sensor (AFS, non-destructive method) and then with the same fruits by the Guss Fruit Texture Analyzer. In 2005 the firmness value obtained using the the Guss fruit analyzer didn't showed good correlation with the firmness index obtained with the Aweta instrument. However in the second year of use the correlation was very high, a more extensive study including different varieties is needed to validate the Aweta non destructive method. This phenotypic analysis validated the phenotypic distinctions in the 9 treatments of our study design, the tissues (peel and cortex) form which were used to dissect the gene regulation.

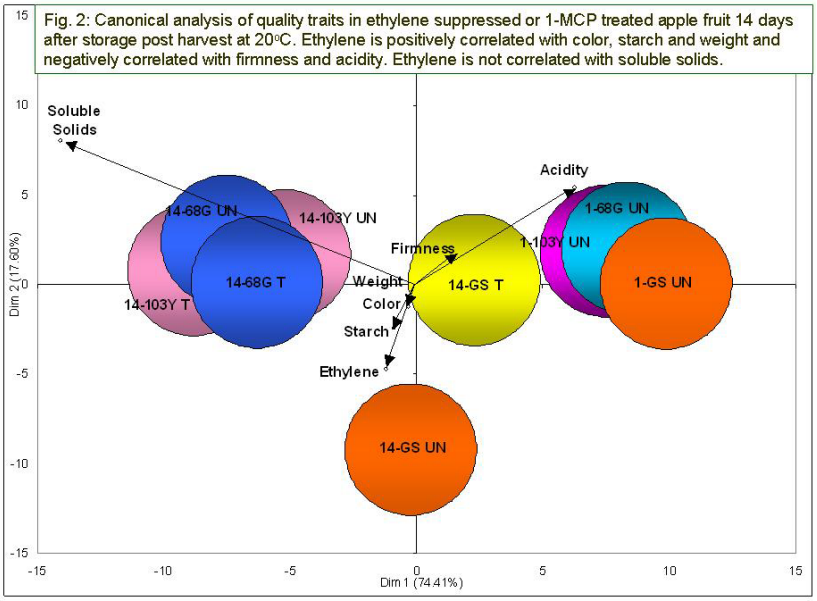

Fig. 2: Canonical analysis of quality traits in ethylene suppressed or 1-MCP treated apple fruit 14 days after storage post harvest at 20ºC. Ethylene is positively correlated with color, starch and weight and negatively correlated with firmness and acidity. Ethylene is not correlated with soluble solids.

3. Successful deployment of microarray resources and analysis tools to dissect the transcriptome of apple fruit.

We successfully investigated the expressed genes in apple through the assembly of a cDNA anlaysis pipeline at the College of Agriculture and Environmental Science Core Genomics Facility (CA&ES CGF) at Davis. This pipeline is a series of programs that help examine all information stored in the GenBank public database, and it allows us to access raw sequence information for apple ESTs, which are terminal DNA sequences representing either the 5' of 3' end of an apple mRNA. These sequences were downloaded and stored in an Oracle database at Davis, then sorted to remove extraneous sequences that represent *E. coli*, chloroplast, or mitochondria DNA sequences. Only high quality sequence information was retained for further analysis. At the time we examined 160,620 of the current 256,249 entries in the public database for Apple (GenBank, NCBI). Other available resources are the GDR which is the Rosaceae community wide resource and is more current and upto date in the analysis of all available apple EST sequences. Our resource and analysis is still web-accessible through the Core Genomics facility (CGF) website (http://cgf.ucdavis.edu/), available by clicking on the apple icon. Of the analyzed 160,620 ESTs, 45,414 (28.3% discovery rate) of the genes correspond to a unigene set, with a majority (25,232; 15.7%) being singletons (represented once in our database) and 20,182 (12.6%) being 'contigs' (represented more than once in our online database). This provided an estimated 45,414 unigenes. Since most of the apple ESTs represent 5' ends of mRNAs without any 3' anchor ESTs the unigene set we could derive was quite redundant. To avoid this

problem we used the current 'unigene' (NCBI) assembly at the time which was build #14 (05 Apr 2006; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene). This particular build had about 17,180 unique genes that came from 106 different cDNA libraries. We divided these into two groups one vegetative and one fruit derived. We have utilized the fruit derived sequences to design oligonucleotides for microarray analysis.

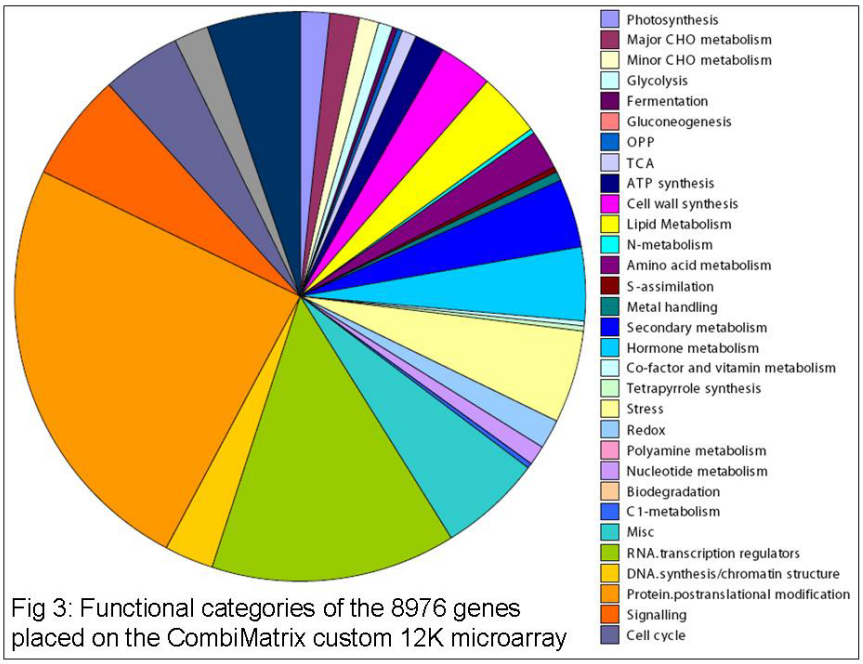

Fig 3: Functional categories of the 8976 genes placed on the CombiMatrix custom 12K microarray

A Combimatrix 12K custom microarray was designed by us using their design criteria and 8813 sequences from the UniGene Build#14 that came from the 34 fruit libraries and 163 genes of interest to us that we added for a total of 8,976 apple genes. The total number of probes synthesized were 12,395 of which 474 (4%) were control probes and 11,921 (96%) were apple probes. Oligo nucleotides were synthesized on the surface of the chip. Shown in Fig 3 are the functional categories of the genes that are represented on our chip. We used the MapMan tool developed by Mark Stitt et al. at the German Resource Center for Genome Research to develop the functional categories (http://gabi.rzpd.de/projects/MapMan/). MapMan project collaborators have developed an ontology which classifies Arabidopsis genes into 35 broad categories, and nearly 2000 sub-categories that correspond to all known functions in Arabidopsis.

4. Differential gene expression patterns obtained by microarrays identified 3029 genes significantly regulated in apple fruit with 658 in cortical, 2169 in peel tissue and 381 in both tissues respectively that are likely regulated by ethylene.

The microarray analysis involved 36 microarray hybridizations that included 6 treatments (14-GS-UN, 14-GS-T, 14-68G-UN, 14-68G-T, 14-103Y-UN and 14-103Y-T) 2 tissues (cortex and peel) and 3 replications. A one-way ANOVA model was first applied to each gene, respectively. Then the standard errors were modified using empirical Bayes methods. The resulting p-values were BH adjusted (Benjamini and Hochberg 2001) for multiple hypotheses testing. Genes with adjusted p-values less than 0.05 were considered differentially expressed. Therefore, we report positive hits with high confidence. R package limma (Smyth, 2004) was used to perform the pair wise comparisons shown in Table 1.

Table 1. Pairwise comparisons of differentially expressed genes in peel and cortical tissues in different treatments obtained from fruit after 14 days at 20ºC		
Comparisons	**PEEL**	**CORTICAL**
14-GS-T – 14-GS-UN	239	472
14-68G-UN – 14-GS-UN	389	626
14-68G-T – 14-GS-UN	1641	322
14-103Y-UN – 14-GS-UN	50	319
14-103Y-T – 14-GS-UN	680	507
14-68G-UN – 14-GS-T	388	20
14-68G-T – 14-GS-T	581	6
14-103Y-UN – 14-GS-T	70	3
14-103Y-T– 14-GS-T	1010	6
14-68G-T – 14-68G-UN	5	16
14-103Y-UN –14-68G-UN	4	67
14-103Y-T– 14-68G-UN	7	46
14-103Y-UN – 14-68G-T	13	14
14-103Y-T – 14-68G-T	32	14
14-103Y-T – 14-103Y-UN	2	28

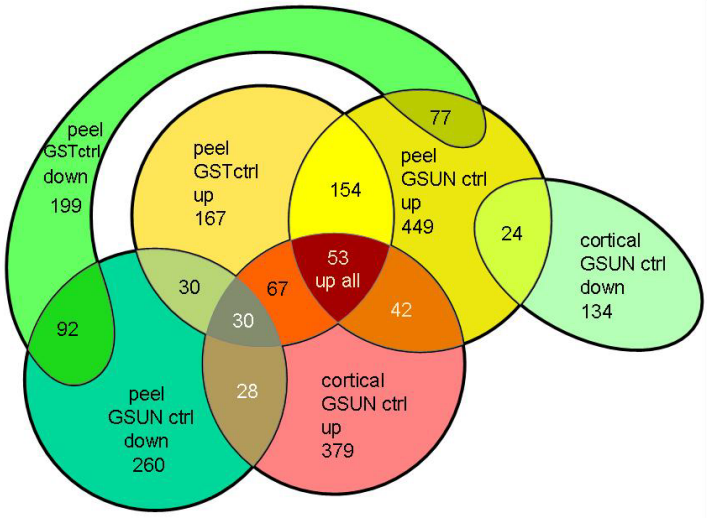

Fig 4: Expression patterns of 2339 genes up or down regulated in peel or cortex tissues among the treatments

The pair wise comparisons conducted for each of the 6 treatments (14-GS-UN, 14-GS-T, 14-68G-UN, 14-68G-T, 14-103Y-UN and 14-103Y-T) contain both up and down regulated genes. One can see that most number of different genes occurs when one compares the transgenics to either the control (14-GS-UN) or the control treated with 1-MCP (14-GS-T). Clearly the peel is much more transcriptionally active as compared to the cortex. This is a view of the complexity of gene expression patterns based on tissue type and treatment. The complex ven diagram presented in Fig 4 provides a view of expression patterns of 2339 genes in these various comparisons focusing on comparisons of the transgenics with either the control or the control treated with 1-MCP. The numbers indicate the number of different genes that are either up regulated or down regulated and the numbers within the intersections indicate genes that are ether up or down regulated in more than one tissue type or in more than one treatment. Fig 4 shows the complex expression pattern of ethylene regulated genes in peel and cortex tissues among the treatments. Since the comparisons involve tissues from our transgenics we can say that those particular genes are regulated by ethylene and in the case of the 1-MCP treatments are regulated by the ethylene response.

5. Expression of genes related with ethylene biosynthesis (ACO, ACS and ERF3), aroma volatiles-related biosynthesis (AAT, LOX, LOX1) and sorbitol biosynthesis (NAD-SDH5, NAD-SDH6, S6PDH, SDH4 and SDH5) down regulated in the GS (control) fruit treated with 1-MCP.

The expression of 47 target genes related mainly with ethylene biosynthesis and response, aroma biosynthesis, softening/texture, carbohydrate metabolism, amino acid metabolism and flavanoid biosynthesis were tested with cortical tissue using Real time (RT) PCR using a dual-labeled florogenic probe TaqMan Probe. The purpose of this analysis is the validation of data obtained from the microarray analysis. Expression of 12 out of 47 genes were shown to be significant at the

threshold p-value<0.01, gene expression was represented as fold changes compared to the wild type GS control at harvest. Ethylene biosynthesis related genes, 1-aminocyclopropane-1-carboxylate oxidase (ACO), 1-aminocyclopropane-1- carboxylate synthase (ACS) and ethylene-response factor ERF3 showed a major reduction of ACO, ACS and ERF3 gene expression in GS treated with 1-MCP and stored for 14 days at 20ºC. 68G and 103Y samples treated with ethylene during storage showed an increase in ACS and AC0 gene expression, respectively (Table 2).

Table 2. ACO, ACS ERF3 and AAT genes expression[a]

	ACO		ACS		ERF3		AAT2	
Sample	**1 day**	**14 day**	**1 day**	**14 day**	**1 day**	**14 day**	**1 day**	**14 day**
GS	1.3544a	13.444b	1.3558a	58.21a	1.0264a	2.7990ab	1.0893a	3.1030ab
GS + 1MCP		0.1080b		0.120b		1.138b		0.0470b
68G	0.0142b	0.0290b	0.1010b	39.30a	1.1387a	2.6140ab	0.7307a	3.7720a
68G + C_2H_4		0.0790b		52.10a		1.9940ab		1.2450ab
103Y	0.3087b	7.6070b	0.2462b	1.630b	1.4774a	4.6310ab	0.6677a	0.3050b
103Y + C_2H_4		28.882a		1.120b		6.012a		2.5800ab

[a]Values represent fold changes compared to the mean of GS-1day samples. Different letters a or b indicate differences among sample at significance level p=0.05 within 1 day or 14 day. ACO = 1-aminocyclopropane-1-carboxylate oxidase, ACS = 1-aminocyclopropane-1- carboxylate synthase, EFR3 = ethylene-response factor and AAT = alcohol acyltransferase

Alcohol acyltranferase (AAT2), lipoxygenase (LOX), LOX1 gene expression, which are related with the biosynthesis of aroma-volatiles compounds showed a decrease in expression for GS control treated with 1-MCP and stored at 20ºC for 14 days. Whereas 14-103Y-T sample treated with ethylene showed a increase in AAT gene expression after harvest at 14 days. 68G and 103Y sample treated with ethylene showed an increase in LOX gene expression after the 14 day storage period at 20ºC. LOX2.3 gene expression showed and increase in GS control treated with 1-MCP, and in sample 103Y treated with ethylene after the the 14 day storage period at 20ºC (Tables 2 and 3).

Table 3. Lipoxygenases genes expression[a]

	LOX		LOX1		LOX2.3	
Sample	**1 day**	**14 day**	**1 day**	**14 day**	**1 day**	**14 day**
GS	1.2068a	14.2710ab	1.2963a	30.31a	1.5584a	2.0110b
GS + 1MCP		0.0670b		0.360b		7.7630b
68G	0.2103b	10.2460ab	0.7086a	22.930ab	1.4068a	1.6050b
68G + C_2H_4		21.264a		17.790ab		NA
103Y	0.0509b	9.9350ab	0.2719a	6.8700ab	1.0979a	7.6480b
103Y + C_2H_4		16.7750ab		1.360ab		20.894a

[a]Values represent fold changes compared to the mean of GSUN-1day samples. Different letters a or b indicate differences among sample at significance level p=0.05 within 1 day or 14 day. LOX = lipoxygenase.

Other significant genes related with sugar/acid balance are the genes associated with sorbitol metabolism. NAD-dependent sorbitol dehydrogenase 5 (NAD-SDH5), NAD-SDH6, sorbitol-6-phosphate dehydrogenase (S6PDH), sorbitol dehydrogenase 4 (SDH4) and SDH4 showed a decrease in gene expression for the GS control treated with 1MCP after 14 day storage period at 20ºC when compared with GS control stored for 1 day (Table 4).

Table 4	NAD-SDH5		S6PDH		SDH4		SDH5	
Sample	**1 day**	**14 day**	**1 day**	**14 day**	**1 day**	**14 day**	**1 day**	**14 day**
GS	1.1587b	0.0706a	1.1149a	0.1881a	1.010b	0.28730ab	1.0080b	0.814b
GS + 1MCP		0.0309a		0.2221a		0.5901a		0.928b
68G	0.3948b	0.0032a	0.9062ab	0.5178a	1.118b	0.0333b	0.8791b	0.411b
68G + C_2H_4		0.0805a		0.2815a		0.3212ab		3.804a
103Y	2.1382a	NA	0.2881b	0.2158a	4.961a	0.0063b	1.6840a	0.510b
103Y + C_2H_4		0.0371a		0.2820a		0.0868ab		1.425b

[a]Values represent fold changes compared to the mean of GSUN-1day samples. Different letters a or b indicate differences among sample at significance level p=0.05 within 1 day or 14 day. NAD-SDH = NAD-dependent sorbitol dehydrogenase, S6PDH = sorbitol-6-phosphate dehydrogenase, SDH = sorbitol dehydrogenase.

6. Sugar-acid balance is differentially regulated in cortical but not in peel.

Flavor is one of the important non visual quality parameters that influences consumer acceptance. Flavor composition has been defined as a complex attribute of quality in which the mix of sugars, acids and volatiles play a primary role. The sugars sucrose, glucose and fructose are responsible for sweetness, with some contribution of sorbitol in apple fruit (Baldwin 2002). The behavior of individual sugars, malic and citric acid was analyzed in the various treatments in response to ethylene regulation in cortical and peel tissues using a high resolution GC/MS. The response of the sugars and acids in apple cortical and peel was different. Fig 5 is a canonical analysis of target sugar and acid compounds in both apple tissues. In the cortex ethylene is not correlated with citric acid, fructose, glucose and sucrose, but negatively correlated with malic acid and sorbitol. Fructose, glucose and sucrose are closely correlated with each other in a positive direction (Fig 5). In the peel, ethylene is slightly correlated with malic acid, fructose, glucose and sorbitol and sucrose are negatively correlated. Fructose, glucose and sucrose are closely related with each other in a positive direction and malic acid is strongly positively correlated with fructose (Fig 5).

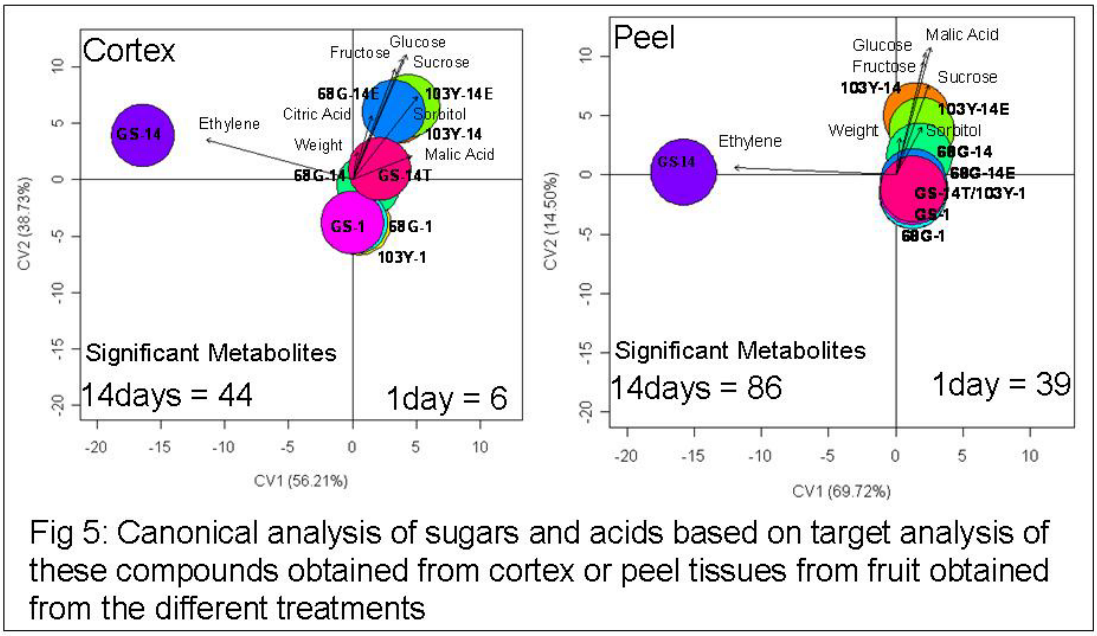

Fig 5: Canonical analysis of sugars and acids based on target analysis of these compounds obtained from cortex or peel tissues from fruit obtained from the different treatments

7. Metabolite profiles showed that most of the statistically significant metabolites identified in cortical and peel are precursors of aroma-volatile compounds.

The average number of metabolites detected in cortical tissue after 1 day and 14 days of storage at 20ºC were 155 and 136, respectively. The average number of metabolites detected in peel tissue after 1 day and 14 days of storage at 20ºC were 132 and 125, respectively. In both cases the metabolites observed included sucrose, glucose, fructose, sorbitol, and malic acid. After the statistical analysis we found that for cortical tissue 6 and 44 metabolites for 1 day and 14 days, respectively, are statistically different at the level of significance of $p<0.05$ (Fig 5). For peel tissue 39 and 86 metabolites for 1 and 14 days, respectively, are statistically different at the level of significance of $p<0.05$ (Fig 5). In cortical and peel tissue many of the significant metabolites identified are precursors of aroma volatile-related compounds, among these we found alcohols (arabitol and erythritol), fatty acids (palmitic acid, oleic acid and linoleic acid) and amino acids (valine, alanine, serine, glutamic acid and isoleucine). These metabolites can be used to evaluate differences among genotypes and treatments. Metabolite profiles can also be used to further define pathways that are operative in different tissues among treatments and can be used to validate the pathway data obtained from the microarray analysis.

8. Ethylene is a modulator of most of the aroma related volatiles, especially the alcohol acyltransferase (AAT) enzyme activity.

Fruit aroma is a complex trait, particularly in terms of the number of different biosynthetic pathways involved, accumulation of the final metabolites and their regulation. The aroma volatiles-related enzymes involved are alcohol acyltransferase (AAT), alcohol dehydrogenase (ADH) and lipoxygenase (LOX). And the main precursors of aroma volatiles are amino acids and fatty acids as mention above.

177 volatiles compounds were detected in cortical tissue by GC/MS using the method described by Defilippi et al (2004). 25 volatiles compounds that were present in 100% of the samples were statistically analyzed. The statistic results showed that the volatile profile of cortical tissue was dominated for aldehydes, alcohols and ketones at harvest. Alcohols and aldehydes showed an increase after 14 days of storage at 20ºC only for the control sample. Under ethylene suppression conditions, GS treated with 1-MCP, 68G and 103Y lines showed a major reduction of all groups of aroma volatiles. No recovery of volatiles compounds were observed when transgenic lines were exposed to ethylene. The reduction in aroma volatiles in 'Greensleves' cortical tissue treated with 1-MCP, an inhibitor of ethylene action, support the findings of early studies that ethylene is a modulator of volatiles responsible of aroma production (Defilippi et al 2004, 2006.; Lurie 2002). .

AAT the main enzyme in ester biosynthesis, showed and increase of enzyme activity of 40-60% between harvest and the end of storage in non transformed line. In transgenic lines lower levels of enzyme activity at harvest relative to the non-transformed lines were measured, and no significant changes in activity were observed until the end of the storage period. These results suggest that ethylene plays an important role in modulating AAT enzyme activity in GS apples. This observation is also supported by the use of 1-MCP, in which we observed an inhibition of enzyme activity relative to the non treated fruit. The AAT activity levels in peel was higher than in cortical for nontransformed and transformed lines at harvest and after 14 days.

ADH the enzyme responsible for the interconversion between aldehydes and alcohols, initially increase and then gradually declined in peel tissue or remain steady in all lines during the holding period. Enzyme activity did not show any significant changes between the measurements done at harvest an after 14 days, under any conditions.

Lipoxygenase pathway plays an important role in the generation of aroma volatile-related during ripening. LOX enzyme activity levels showed a minor increase during the holding period, especially peel tissue obtained from GS line, application of ethylene only caused a minor increase in LOX activity in fruit from the 68G line.

Significance to the industry and potential economic benefits

Understanding the metabolic network and biosynthetic pathways active in apple fruit could facilitate extending postharvest life of flavor, aroma, and texture to match appearance. This, in turn, would promote consumption of fresh apple fruits and reduce losses during postharvest storage, stimulating demand while reducing costs to handlers and consumers. Identifying genes that determine and regulate fruit quality phenotypes can provide a new set of tools to improve management, breeding, and selection of existing and new cultivars.

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Executive Summary

Our overall goal of this project was to define the role of ethylene in the functional regulation of apple fruit quality. This was accomplished by using apple fruit obtained from 2 transgenic lines modified in their capacity to synthesize endogenous ethylene or wild type fruit modified in their ethylene response via the application of 1-methylcyclopropene (1-MCP). The study design used these unique resources to create 9 phenotypically distinct treatments. Validation of the study design demonstrated the ethylene is positively correlated with color, starch and weight and negatively correlated with firmness and acidity. These differences in phenotypes were matched with changes in gene expression patterns obtained by the successful deployment of microarray resources and analysis tools to dissect the transcriptome of apple fruit which were used to identify ethylene regulated genes in apple fruit tissues. Differential gene expression patterns obtained by microarrays identified 3029 genes significantly regulated in apple fruit with 658 in cortical, 2169 in peel tissue and 381 in both tissues respectively that are likely regulated by ethylene. These genes were functionally categorized to define their metabolic role in apple fruit. Expression of genes related with ethylene biosynthesis, aroma volatiles-related biosynthesis and sorbitol biosynthesis were down regulated in the control fruit treated with 1-MCP. Genes involved in sugar-acid balance were differentially regulated in cortical but not in peel. Metabolite profiles of the tissues validated the gene expression analysis and showed that most of the statistically significant metabolites identified in cortical and peel are precursors of aroma-volatile compounds. Thus ethylene is an important modulator of most of the aroma related volatiles, and a key gene that regulates this activity encodes the enzyme alcohol acyltransferase (AAT).

FINAL PROJECT REPORT **YEAR**: 3 of 3
WTFRC Project Number: Internal project

Project Title: Chemical thinning of apple

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Budget 1:
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: (509) 665-8271 **Email address:** kathy@treefruitresearch.com

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries			
Benefits			
Wages	30,000	30,000	30,000
Benefits (16%)	4,800	4,800	4,800
Equipment			
Supplies	3,000[1]	3,000[1]	3,000[1]
Travel	500	500	500
Miscellaneous			
Total	**38,300**	**38,300**	**38,300**

Footnotes: [1] Chemicals, fruit purchase
NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

OBJECTIVES:

- Evaluate pre-bloom, bloom, and post-bloom chemical thinning agents with particular focus on complete programs to achieve three goals:
    1. Minimize costs of green fruitlet thinning
    2. Maximize fruit quality
    3. Encourage annual bearing
- Investigate influence of important variables (drying conditions, spray technology, carrier volume) on chemical thinner efficacy and fruit finish
- Expand collaborative efforts with other research programs

SIGNIFICANT FINDINGS:

Effective chemical thinning programs reduce hand-thinning, improve fruit size and quality, and increase return bloom; bloom thinners generally achieve these goals more consistently than postbloom programs (Tables 3, 7)

Fruit size and return bloom are often improved by chemical thinners, even when fruit set is not significantly reduced (Tables 3, 7)

Oil (dormant, summer, vegetable, fish) + lime sulfur programs are the most efficacious options for bloom thinning; results with Crocker's Fish Oil are most consistent (Table 3)

Thinning programs using high rates of lime sulfur or oil + lime sulfur are gaining industry acceptance and generally superior to ATS programs (Table 3)

Novel bloom thinning programs including Raynox, vinegar + oil, urea, Pacific Natural fish emulsion + lime sulfur, NAA, ethephon, and Tergitol have been largely ineffective in WTFRC trials (data not shown)

Thinning efficacy and fruit finish were not clearly affected by variations in spray technology (AccuTech vs. Proptec vs. airblast), carrier volume (100 vs. 200 gal/acre), or drying conditions (dawn vs. noon vs. dusk sprays) of chemical thinning programs in WTFRC trials (Tables 4, 5); trials will be repeated in 2008

All formulations of BA (MaxCel, Exilis Plus, Riteway, Genesis) perform equally well for postbloom thinning and fruit sizing (data not shown)

BA + carbaryl thinning programs give results equal or superior to NAA + carbaryl or ethephon + carbaryl programs; BA often shows a positive effect on fruit size (Tables 6-9)

Additive effects of bloom thinning + postbloom thinning increase chances for successful crop load management (Tables 8, 9)

Factorial field trials indicate that chemical thinning (bloom and postbloom) and PGR (BA or BA+GA) programs are not affected by the presence/absence of Extenday throughout the growing season, including at time of application; trials should be repeated to corroborate initial results (data not shown)

Screening of new thinning chemistries (organic acids, essential plant oils) supplied by Rom program (U of Arkansas) has yielded few positive results, but salicylic acid and clove oil warrant further investigation (data not shown)

Summer applications of NAA have not increased return bloom in WTFRC trials; summer applications of ethephon have sporadically yielded positive results (data not shown)

Earlier work (Schmidt, Elfving) suggests use of gibberellins in "off" years may help mitigate biennial bearing; field trials will be evaluated in spring of 2008

Collaborative efforts across disciplines, institutions, and regions (Greene, McArtney, Hirst, Schupp, Yoder, Rom, Fallahi, Sugar, van Nocker, Whiting, Elfving, Schrader, Beers, Xiao, Lewis, Toye) have increased relevance and impact of all crop load management research

BACKGROUND:
The internal research program of the WTFRC conducted 19 apple chemical thinning trials in commercial orchards around the state of Washington in 2005, 18 more in 2006, and another 15 in this past season. Results from these trials add to our already sizable body of chemical thinning data, drawing from approximately 200 field trials since 1998 on eleven cultivars and ten rootstocks representing all important growing districts in the state. The downward trend in trial number reflects a shift in focus in our program away from screening of various chemical thinners toward more intricate trials focused on questions about comprehensive crop load management, plant physiology, and variability in treatment response. Three 2007 trials were applied by grower-cooperators, with the balance being applied by WTFRC staff using the Proptec research sprayer; historically, roughly half of our trials have been applied by grower-cooperators with their own equipment.

We have identified three measurable targets which are directly tied to a grower's economic bottom line:

1. Reduction of green fruitlet hand-thinning
2. Improved fruit size and quality
3. Increased return bloom/annual bearing

The degrees to which our chemical thinning programs achieve each of these goals are reflected in our data labeled fruitlets/100 floral clusters, harvest fruit size, and percent return bloom, respectively.

Our protocols generally call for two applications of each bloom thinning program, at 20% and 80% full bloom. Likewise, most postbloom thinning programs are applied twice, typically at 5mm and 10mm fruitlet size. Programs investigated over the course of this project are reflected in Table 1; in programs which show a range of possible rates, higher concentrations are typically reserved for cultivars known to be difficult to thin, such as Fuji and Golden Delicious. In most cases, additional chemical thinning programs were left to the discretion of individual grower-cooperators as long as all experimental plots received the same treatments.

Table 1. Typical chemical thinning programs evaluated. WTFRC 2005-2007.

BLOOM THINNERS
3.4 – 4 gal Ammonium thiosulfate (ATS)/A
5 gal NC99/A
6-8% Lime sulfur (LS)
2% Crocker's Fish Oil (CFO) + 2-4% LS
2% Pacific Natural Fish Emulsion + 2.5% LS
1% Wilbur-Ellis Supreme Oil (WES) + 3% LS
5% Canola, Corn, or Soybean Oil Emulsion
2% Canola, Corn, or Soybean Oil Emulsion + 2% LS
10% Vegetable Oil Emulsion (VOE)
17% VOE + 17% Vinegar
15% GS Long thinner (unnamed)
40 lbs Urea/A
2 pts Tergitol/A
20% Raynox
3 pts Ethrel (ethephon)/A
3 oz NAA/A
POSTBLOOM THINNERS
1.5-3 qts Sevin (carbaryl)/A
3 qts MaxCel, Exilis, Genesis BA (BA)/A
1-3 pts Ethrel (ethephon)/A
3 oz NAA/A
2 oz Amid Thin (NAD)/A

BLOOM THINNING:

While chemical bloom thinning was the primary focus of the WTFRC internal program crop load management research program several years ago, its prominence in our research portfolio has begun to decline, due in part to our success in establishing effective lime sulfur-based programs which are gaining widespread use in commercial operations. Our work with bloom thinners is now increasingly geared toward increasing their predictability, supporting research to explore their molecular and physiological effects, and understanding their role in comprehensive crop load management programs. We continue to screen new chemistries which may offer alternatives to currently available options (Table 1); unfortunately, none of the materials currently under evaluation have demonstrated enough thinning efficacy, practicality, and commercial viability to merit beta testing on a larger scale.

In 2007, we did have encouraging results from both clove oil and salicylic acid in cooperative trials with Curt Rom and Jason McAfee (U of Arkansas), who are working to identify novel chemistries (primarily plant oils and organic acids) which may act as pollenicides to achieving fruit thinning. We plan to continue this collaboration in 2008 to confirm these results. Additionally, we have achieved modest success in thinning with vegetable oil emulsions tank mixed with lime sulfur (Table 2). While these programs have not been as effective as other options, they are potentially important to both conventional and organic growers if fish- and petroleum-based oils become more scarce and expensive.

Table 2. Crop load effects of bloom thinning programs. WTFRC 2006.

Trial	Thinning program	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	2007 return bloom
			%	%	g	%
Gala / Bud.9	Lime sulfur	75 ab	56 ns	22 ns	182 ns	160 ab
- Chelan	CFO + LS	69 b	60	20	185	166 a
	Canola oil emulsion	85 ab	53	20	179	75 c
	Canola oil emulsion + LS	90 ab	51	22	179	121 abc
	Corn oil emulsion	72 ab	58	20	187	91 bc
	Corn oil emulsion + LS	80 ab	56	20	178	109 abc
	Soybean oil emulsion	85 ab	54	19	183	109 abc
	Soybean oil emulsion + LS	80 ab	55	21	176	134 abc
	NC99	68 b	62	18	189	113 abc
	Control	97 a	51	19	176	114 abc

No thinning program we have evaluated yet outperforms oil + lime sulfur combinations. Table 3 summarizes results from all apple bloom thinning trials conducted by the WTFRC since 1999, reflecting a very conservative standard by which to assess our most frequently studied programs.

Table 3. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials WTFRC 1999-2007.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
Ammonium thiosulfate	15 / 55 (27%)	10 / 58 (17%)	3 / 46 (7%)
NC99 (Mg^{++}/Ca^{++} Cl^{-} brine)	15 / 30 (50%)	7 / 32 (22%)	2 / 26 (8%)
Lime sulfur	25 / 54 (46%)	12 / 48 (25%)	9 / 45 (20%)
Crocker's Fish Oil + lime sulfur	57 / 96 (59%)	25 / 88 (28%)	19 / 78 (24%)
JMS Stylet Oil + lime sulfur	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
Wilbur-Ellis Supreme Oil + lime sulfur	14 / 27 (52%)	4 / 26 (15%)	4 / 26 (15%)
Vegetable Oil Emulsion	13 / 29 (45%)	4 / 28 (14%)	2 / 29 (7%)

[1]Does not include data from 2007 trials.

[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

VARIABLES AFFECTING THINNING EFFICACY AND FRUIT FINISH:

While our results have clearly demonstrated the efficacy of several chemical thinning programs, we seek to improve their consistency and predictability. We have conducted a series of trials to investigate the effects of drying conditions, spray technology, and application carrier volume on efficacy of proven chemical bloom and postbloom thinning programs and their impact on fruit finish.

Table 4. Chemical thinner drying condition effects on crop load and fruit finish. WTFRC 2007.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
			%	%	g		%
Golden	Bloom+PB – 6 AM	51 ab	57 ns	35 ns	188 ns	101	29 a
Delicious/	Bloom+PB – Noon	64 ab	49	40	185	103	20 ab
M.26	Bloom+PB – 6 PM	56 ab	57	34	206	93	27 a
- Manson	PB only – 6 AM	51 ab	59	32	183	104	12 b
	PB only – Noon	71 ab	50	33	192	99	24 ab
	PB only – 6 PM	47 b	63	28	194	98	17 ab
	Control	80 a	49	30	187	102	21 ab
Fuji/M.26	Bloom+PB – 6 AM	89 ns	49 ns	24 ns	199 ab	96	50 ns
- Manson	Bloom+PB – Noon	93	42	31	197 ab	97	32
	Bloom+PB – 6 PM	86	45	32	197 ab	97	46
	PB only – 6 AM	114	32	34	184 b	104	45
	PB only – Noon	112	32	34	194 ab	98	41
	PB only – 6 PM	83	45	34	201 a	95	47
	Control	95	41	33	190 ab	100	56

Table 4 represents two trials in which identical bloom (CFO + LS) and postbloom (carbaryl + BA) chemical thinning programs were applied at different times of the same days. Morning applications were typically during cool (53-58ºF) and damp, but warming conditions; midday conditions featured temperatures continuing to rise from 60-65ºF; evening sprays occurred in relatively dry conditions cooling from 62-67ºF. As in 2006, treatments on Golden Delicious reduced fruit set, but trends in data were not clear enough to be statistically significant. No thinning was observed by any treatment in Fuji, and no treatment in either trial showed any effect on fruit finish. Inconsistent and/or contradictory data has made interpretation of results from these trials difficult in both 2006 and 2007; we plan to try these programs one last time in 2008.

Table 5. Chemical thinner spray technology and carrier volume effects on crop load and fruit finish. WTFRC 2007.

Trial	Sprayer	Carrier volume	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		gal/acre		%	%	g		%
Fuji/M.26 - Quincy	AccuTech	100	64 ns	59 ns	24 ns	298 ab	64	52 ns
	AccuTech	200	59	58	28	298 ab	64	47
	Proptec	100	79	49	60	306 a	62	45
	Proptec	200	60	60	24	306 a	62	54
	Turbo-mist	100	63	58	27	305 a	63	34
	Turbo-mist	200	65	54	30	284 b	67	40
	Control	na	73	53	28	296 ab	64	49

At another site, we applied identical chemical thinning programs at consistent timings using different sprayers and carrier volumes (Table 5). Despite using aggressive thinning programs, we observed no effects on fruit set or fruit finish, let alone discerning any trends relative to spray technology or dilute vs. concentrate spraying.

POSTBLOOM THINNING:

The primary focus of our postbloom thinning work is to identify effective programs which do not rely on carbaryl, which is facing considerable regulatory pressure. While 2007 trials showed few treatment effects (Table 6), we are encouraged by past successes with BA + NAA programs (Table 7). Table 7 also confirms our past assertions that carbaryl + BA programs are often superior to standard carbaryl + NAA programs. Perhaps most striking about Table 7 is the overall dearth of significant effects from any postbloom chemical thinning program; when compared to the general success rates of bloom chemical thinners (Table 3), it becomes all the more clear that early, aggressive thinning is critical to effective crop load management.

Table 6. Crop load effects of postbloom thinning programs. WTFRC 2007.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
			%	%	g		%
Gala / M.26	BA	73 ns	51 ns	30 ns	220 ns	87	32 ab
- Orondo	BA + ethephon	65	55	30	215	89	14 bc
	BA + NAA	61	53	36	217	88	20 abc
	NAA	71	52	30	225	85	32 ab
	NAA + ethephon	62	56	30	219	87	34 a
	Carbaryl	56	60	27	220	87	20 abc
	Carbaryl + ethephon	68	51	34	221	86	12 c
	Carbaryl + BA	67	54	31	235	81	37 a
	Carbaryl + NAA	60	56	32	212	90	35 a
	Control	67	55	29	210	91	10 c
Braeburn/	BA	50 ns	62 ns	27 ns	185 abc	103	5 ns
M.9	BA + ethephon	40	66	28	178 bc	107	7
- Grandview	BA + NAA	50	63	27	180 bc	106	5
	NAA	42	66	28	187 abc	102	7
	NAA + ethephon	38	66	30	169 c	113	4
	Carbaryl	47	61	33	192 abc	99	10
	Carbaryl + ethephon	33	73	21	196 ab	97	11
	Carbaryl + BA	43	66	27	207 a	92	12
	Carbaryl + NAA	46	62	31	188 abc	101	9
	Control	55	56	34	174 bc	110	12

Table 7. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials WTFRC 1999-2007.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
6-benzyladenine (BA)	2 / 18 (11%)	0 / 19 (0%)	0 / 16 (0%)
Carbaryl + BA	23 / 65 (35%)	7 / 65 (11%)	7 / 54 (13%)
Carbaryl + naphthaleneacetic acid (NAA)	9 / 46 (20%)	6 / 46 (13%)	3 / 40 (8%)
Carbaryl + NAA + ethephon	0 / 5	0 / 5	2 / 5
Carbaryl + BA + NAA	0 / 8	0 / 8	3 / 8
BA + NAA	2 / 6	0 / 6	0 / 2

[1]Does not include data from 2007 trials.

[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

COMBINED BLOOM AND POSTBLOOM PROGRAMS:
Our results continue to demonstrate that comprehensive chemical thinning programs are typically necessary to reduce crop load to appropriate levels. Table 8 details that modest effects were obtained by either approach individually, but only when bloom and postbloom programs were combined was fruit set significantly reduced. Likewise in grower-applied trials on 'Pacific Rose' and 'Fuji,' the additive effects of CFO + LS and carbaryl + BA delivered positive results (Table 9). Our 2007 'Honeycrisp' trial further reinforced that postbloom thinning with BA + NAA can be as effective as carbaryl-based programs.

Results from trials sprayed with the Proptec paralleled those from grower-applied trials for several years after our program purchased it in 1999; in recent seasons, however, we have not seen as much consistency in results from Proptec trials as those applied by grower-cooperators (Table 9). We plan to replace our aging sprayer in 2008 and hope that the new machine will both reflect innovative spray technology and produce results which more tightly reflect those of typical commercial sprayers.

Table 8. Crop load effects of bloom + postbloom thinning programs (Proptec). WTFRC 2006.

Trial	Bloom thinner	Postbloom thinner	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	2007 return bloom
				%	%	g	%
Golden Delicious/	ATS		47 a	65 bc	25 a	198 bcd	43 b
M.7	ATS	Sevin + NAA	41 ab	68 bc	24 a	220 ab	94 ab
Othello	ATS	Sevin + MaxCel	15 c	88 a	11 b	209 abcd	98 ab
	CFO + LS		35 ab	71 bc	22 ab	187 cd	44 b
	CFO + LS	Sevin + NAA	35 ab	70 bc	26 a	210 abc	110 ab
	CFO + LS	Sevin + MaxCel	25 bc	78 ab	18 ab	219 ab	121 a
		Sevin + NAA	40 ab	68 bc	25 a	227 a	66 ab
		Sevin + MaxCel	31 abc	75 abc	20 ab	211 abc	105 ab
		MaxCel	33 ab	72 bc	23 a	199 bcd	76 ab
	Control		46 a	63 c	29 a	182 d	46 b

Table 9. Crop load effects of chemical thinning programs (grower applied). WTFRC 2007.

Trial	Bloom thinner	Postbloom thinner	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
				%	%	g		%
Pacific Rose/	CFO + LS		68 b	63 a	18 ns	282 ab	68	18 ns
M.26	CFO + LS	Carbaryl + BA	60 b	64 a	20	321 a	59	28
- Brewster		Carbaryl + BA	72 b	60 a	20	279 ab	68	28
	Control		92 a	52 b	19	267 b	71	22
Fuji/M.9	CFO + LS		119 b	43 b	20 b	222 ns	86	80
- Royal City	CFO + LS	Carbaryl + BA	90 c	53 a	20 b	239	80	93
		Carbaryl + BA	99 c	49 ab	22 b	233	82	92
	Control		143 a	29 c	27 a	231	83	100
Honeycrisp/	CFO + LS	BA + NAA	64 b	57 a	27 ns	260 ns	73	72 ns
M.9	CFO + LS	Carbaryl + BA	63 b	58 a	26	255	75	86
- Wiley City	CFO + LS	Carbaryl + NAA	66 b	56 ab	26	236	81	66
	CFO + LS	None (control)	78 a	51 b	28	250	76	74

RETURN BLOOM PROGRAMS:
We have been disappointed by our inability to increase return bloom with summer NAA programs like those used successfully by researchers in North Carolina and many Washington growers. Despite following advice for treatments from those researchers and growers, as well as the manufacturer, we have rarely observed significant effects on flowering from any NAA program in 4 years of trials. Return bloom was increased sporadically in those trials by summer ethephon applications, but even those results have not been consistent enough to inspire any degree of confidence. We will strive to conduct future return bloom trials using other spray technologies to determine whether our poor results may be in part related to use of our Proptec sprayer.

In 2007, we initiated a new series of trials to investigate application of GA_3 (Falgro) to *reduce* return bloom in blocks set up to be heavily cropped in 2008. The material was applied at 10mm fruitlet size in three lightly cropped Fuji blocks around the state; the effect of these treatments will not be clear until spring of 2008. The manufacturer of Falgro (Fine) has expressed a willingness to register their product for this use if results are promising and we are hopeful this approach can help growers mitigate biennial bearing in their apple blocks.

NEW PROJECT PROPOSAL **PROPOSED DURATION**: 3 YEARS

Project Title: Crop load management of apple

PI: Tory Schmidt
Organization: WTFRC
Telephone/email: (509) 665-8271 x4; tory@treefruitresearch.com
Address: 1719 Springwater Ave.
City: Wenatchee
State/Province/Zip WA 98801

Cooperators: Jim McFerson, Felipe Castillo, Ines Hanrahan, Tom Auvil – WTFRC

Total Project Request: **Year 1:** Internal funding

Other funding Sources

Agency Name: Private companies including Valent, Fine, Nufarm, GS Long, Extenday, Amvac
Amount requested or awarded: variable (typically $10,000 - $20,000 per year)
Notes: external funding requests are typically based upon costs incurred to evaluate new products or conduct research of potential benefit to specific companies

WTFRC Collaborative expenses:

Item	2008	2009	2010
Stemilt RCA room rental	6400	6400	6400
Crew wages & benefits	32,000	30,000	28,000
Shipping			
Supplies	2500	2500	2500
Travel	1500	1500	1500
Miscellaneous			
Total	**42,400**	**40,400**	**38,400**

Footnotes:
Project is funded through the WTFRC internal program budget; figures are shared for informational purposes only; total expenses will be offset by external funding

RCA room used Aug-Nov each year to hold fruit samples for quality analysis; supply money to cover limited chemical purchase and grower compensation in case of damaged fruit or crop-destruct treatments; travel dollars are for in-state travel to trial sites

OVERVIEW

Effective crop load management has always been important for profitable apple production, but is increasingly so in today's tightening labor market. Our program has been able to outline effective chemical thinning programs to help growers more efficiently manage their crops and we seek to build on those successes by investigation of developing mechanical and molecular technologies which can be brought to bear on horticultural applications. We expect that our objectives and approaches will continue to evolve as new technologies and opportunities for collaboration develop, but we will begin this next phase of research with the following objectives.

OBJECTIVES

1. Ongoing screening of new chemical thinners and PGR chemistries in replicated field trials

2. Investigation of carbaryl-free postbloom chemical thinning programs

3. Field testing of new innovations for mechanical and automation-assisted manual thinning

4. Development of practical PGR programs to manipulate flowering and mitigate biennial bearing

5. Elucidation of cultural and environmental factors which cause variability in plant response to chemical thinning and PGR programs

6. Assay long-term reproductive and vegetative growth patterns of individual trees to profile plant-to-plant variability and develop historical databases of specific trees (plant growth phenotypes) to be utilized in physiological and genomic studies

7. Expand collaborative studies with other research programs, especially in crop physiology and genetics/genomics/breeding

Note: This new project will be presented at the internal research review in Ellensburg, February 6.

CONTINUING PROJECT REPORT
Note: continuing funding will be considered at the internal research review February 6 in Ellensburg**.**

Project Title: Programs to suppress sunburn, russet and lenticel breakdown of apples

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Cooperators: Jim McFerson, Tom Auvil, Felipe Castillo, Tory Schmidt, WTFRC, Wenatchee, WA, Larry Schrader, WSU-TFREC, Wenatchee, WA

Budget 1:
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: 1 509 665 8271 **Email address:** Kathy@treefruitresearch.com

Item	Year 1: 2006	Year 2: 2007	Year 3: 2008
Salaries			
Benefits			
Wages		10,000	
Benefits		1,600	
Equipment			
Supplies			
Travel			
Miscellaneous			
Total		11,600	

Footnotes: All chemicals and harvest supplies were provided by industry vendors.

NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

Acknowledgement: We would like to thank Pace Intl., BASF, Fine Americas, Valent, Monterrey Ag, Wilbur Ellis, Nufarm, Globachem, and D & M Chemicals for graciously donating chemicals. Special thanks to our cooperating growers: Stan Olson, Clyde Buchler, Rick Kamphaus, McDougall & Sons, Jason Matson.

OBJECTIVES:

1. Investigate chemical programs to improve fruit finish of 'Fuji' and 'Golden Delicious' apples.
2. Compare sunburn protectant efficacy in apple and evaluate ease of cleanup in the warehouse.
3. Facilitate field testing of promising approaches to mitigate lenticel breakdown in apples.

SIGNIFICANT FINDINGS:

Russet: **Standard GA programs typically enhanced the percentage of premium fruit.**
Some GA alternatives reduced russet in 2007.
No treatment significantly reduced Fuji flecking.
2007 return bloom was not affected by 2006 russet treatments.

Sunburn: **All materials tested increased the percentage of sunburn-free fruit.**
Most materials cleaned easily off fruit flanks. Residues of particle films remained visible in the stem bowl and calyx after drying.

LB: **No consistent treatment effect was noted after late-season application of hydrophobic materials (summer supreme oil, soybean oil, Raynox) in 2006.**

METHODS

Russet suppression: In 2007, we conducted 4 fruit finish trials (3 x Golden Delicious, 1 x Fuji).
(A) Trials evaluating standard GA programs vs. alternatives were sprayed with a PropTec sprayer at 200 gal/acre using a randomized complete block design with 4 replications and 5 trees/treatment/rep. We tested the following materials alone or in combination: ProVide (GA_{4+7}), Novagib (GA_4), Falgro 20 SP (GA_3), FAL 900 (GA_7), TypRus 1 + 2 (all at 20ppm), Raynox at 2.5 gal/acre, Platina at 0.11 gal/acre, BlueStim at 4lbs/acre and 0.5 pt/acre surfactant, Surround WP (25lbs/acre), SylTac 1pt/acre,. Materials were applied at five weekly timings starting at petal fall, reflecting standard industry practice.
(B) Trials (one each) evaluating Platina timings and effects of application timing relative to drying conditions of standard GA programs were sprayed with a hand-held sprayer to run-off. A completely randomized design was applied using 6 single tree reps/trt. Platina was applied in four timing combinations (PF, PF + 7, PF + 14, 5 x weekly, starting at PF) at 0.11 gal/acre.

Sunburn suppression: Two trials were established (Granny Smith/M.106, Golden Delicious/M.26 Manson; both near Manson) testing a variety of commercially available sunburn protectants (Cocoon, Eclipse, Fruit Shield, Invelop, Raynox Plus, Sun Guard, Surround WP). All materials were applied four times using each product's respective label rate and starting on July, 1st. At harvest, individual fruit was graded for sunburn according to the Schrader/McFerson system (0 = clean, 6 = necrosis). The ease of cleanup was evaluated by running fruit over the USDA-ARS packingline in Wenatchee. No wax was applied. Fruit was allowed to dry for 24 hours before evaluation.

Lenticel breakdown: In 2006, we conducted 4 trials (Selah, Manson, Royal City, Desert Aire). All trials were sprayed with a PropTec sprayer at 80 gal/acre using a randomized complete block design with 4 replications and 10-20 trees/treatment/rep. We tested the following materials: Summer Supreme Oil (1 or 2%), soybean oil (1 or 2%), and Raynox at 2.5 gal/acre. Spray programs included the following timings: single applications 1, 2, or 3 weeks before anticipated harvest. Samples were stored under RA conditions at the Stemilt RCA rooms and evaluated for LB incidence after 3-4 and 5-6 months with the waxing test. Standard maturity parameters were taken at harvest and after storage.

In 2007, we conducted 2 trials sprayed with a PropTec (100 gal/acre, Desert Aire & Royal City) testing the following materials: Summer Supreme Oil (2%), soybean oil (2%), and SylTac (2pt/acre). Timings were: 4, 2, 1 weeks before anticipated harvest alone or in combination. Samples were stored under CA conditions and will be evaluated for LB incidence after 5 and 8 months of storage (data not available until March 2008). Secondly, we applied BlueStim (at 4lbs/acre and 0.5 pt/acre surfactant) to Gala's and Fuji's (Orondo) using a hand-held sprayer and utilizing a completely randomized design with 6 single tree replications/treatment. In addition, Gala apple samples were taken from several Extenday trials and the Wapato rootstock trial. All samples are currently in storage (5 month CA; data not available until March 2008).

RESULTS AND DISCUSSION

Russet suppression: Fruit russet is typically induced early in the growing season and is likely aggravated by a combination of weather conditions, spray chemicals, and/or topical biotic pests. Few practical options are available to orchardists to suppress russet. Standard gibberellic acid programs include up to five weekly applications starting around petal fall and amount to considerable spray material costs ($100-300/acre). In 2007, we tested additional GA products and commercial materials with novel chemistries (Tables 1, 2; Graph 1). We examined the effect of drying conditions on russet development following standard GA programs in a separate test (Table 3).

Table 1. Commercial products utilized in WTFRC fruit finish trials in 2007.

Active Ingredient	Commercial product(s)
Gibberellic acid mixture	ProVide, NovaGib, Falgro, FAL 900, TypRus
Plant wax	Raynox
Clay particles	Surround
Vegetable oil+silicone surfactant	SylTac
Glycine Betaine (osmoregulator)	Bluestim
L-Tryptophan (auxin synthesis)	Platina

Graph 1. Difference in percentage of premium fruit after application of standard GA and alternative spray programs for russet suppression in 2007.

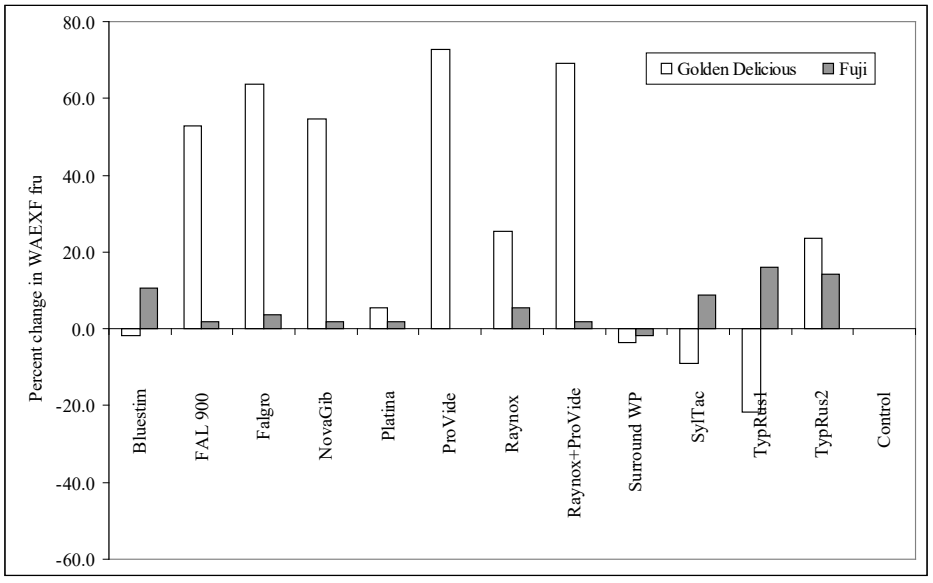

Table 2. Fruit finish effects of various spray materials on Golden Delicious and Fuji apples in 2007.

	COMMERCIAL GRADE				
	WAEXF	WAF	US #1	CULLS	FLECKING
TREATMENT	%	%	%	%	%
Wapato Golden Delicious / M.111					
Bluestim	54 cde	44 abc	3 ns	0 ns	
FAL 900	84 abcd	16 bcd	0	0	
Falgro	90 ab	8 cd	3	0	
NovaGib	85 abc	15 bcd	0	0	
Platina	58 cde	43 abcd	0	0	
ProVide	95 a	5 d	0	0	
Raynox	69 abcde	23 abcd	3	0	
Raynox+ProVide	93 ab	6 d	1	0	
Surround WP	53 cde	48 ab	0	0	
SylTac	50 de	50 ab	0	0	
TypRus1	43 e	55 a	3	0	
TypRus2	68 bcde	31 abcd	1	0	
Control	55 cde	46 ab	1	0	
Lakeview Orchard Fuji / Royal City					
Bluestim	62 ns	10 ns	1 ns	0 ns	27 ns
FAL 900	57	10	1	0	32
Falgro	58	11	0	0	31
NovaGib	57	8	1	0	33
Platina	57	14	0	0	29
ProVide	56	15	0	0	29
Raynox	59	12	0	0	29
Raynox+ProVide	57	12	1	0	31
Surround WP	55	13	1	0	31
SylTac	61	8	0	0	31
TypRus1	65	8	0	0	27
TypRus2	64	7	1	0	28
Control	56	11	1	0	33

Golden Delicious: All GA products evaluated, except TypRus 1, increased the number of premium fruit (WAEXF) in 2007. Among the alternative products, Platina (5.5%) and Raynox (25.5%) lead to more premium grade fruit. Although Raynox increased the number of clean fruit when applied by itself, no additive effect was obtained by combining ProVide and Raynox. No significant treatment effects relative to drying conditions (time of day) of standard GA programs were noted. Since we had low russet pressure in 2007 (see Table 3, WAEXF for control 81-90% in a susceptible orchard), we will repeat these trials in 2008.

Fuji: The results for Fuji are less clear. No spray program significantly increased the amount of WAEXF fruit. Within the GA product series, only TypRus 1+2 increased the percentage of premium fruit (14.3-16.1%). Among alternative products, Bluestim and SylTac had a positive

influence on the percentage of WAEXF fruit (10.7/ 8.9%), perhaps indicating that russet in Fuji has slightly different characteristics than typical 'Golden' russet (Graph 1).
Applying Raynox and ProVide together did not yield significant fruit finish improvement, as compared to the effect either of material when applied alone. Fuji flecking was not influenced by any spray program tested in 2007.

Return bloom effects: Gibberellins are known to be inhibitors of floral initiation in apple, especially materials containing GA_7, such as ProVide. We did not observe any effects on return bloom in spring of 2007 for trials sprayed with GA products in 2006.

Conclusion: Standard GA programs are still the most reliable strategy for improving apple fruit finish. Some GA alternatives (BlueStim, Raynox, Platina, SylTac) have shown some effectiveness for improving Fuji fruit finish.

Table 3. Fruit finish effects of application timing of Platina on Golden Delicious. Effects of application timing relative to drying conditions (time of day) of standard gibberellic acid programs on Golden Delicious. WTFRC 2007.

	COMMERCIAL GRADE			
	WAEXF (%)	WAF (%)	US #1 (%)	CULLS (%)
Wapato Handgun 'Golden Delicious'/M.111				
Platina PF	91 ns	9 ns	0 ns	0 ns
Platina PF+7	90	13	0	0
Platina PF+14	90	10	0	0
Platina 5X (PF-28)	88	12	0	0
Control	90	13	0	0
Wapato Handgun Golden Delicious 'Time of Day' /M.111				
ProVide morning	92 a	10 b	0 ns	0 ns
ProVide evening	92 a	12 b	0	0
NovaGib morning	70 b	28 a	2	0
NovaGib evening	90 ab	10 b	0	0
Control	81 ab	18 ab	2	0

Sunburn suppression: Sunburn is the primary physiological cause of cullage, sometimes damaging up to 50% of the fruit in a given orchard. Previously, WTFRC trials have shown calcium-based products (Eclipse, FruitShield) to perform as well as industry standards (Raynox, Surround WP). We revisited the question of sunburn protection product efficacy this last field season, testing six commercially available materials with varying chemical composition (Table 4).

Table 4. Sunburn protectants used in 2007 WTFRC comparative trials.

Type	Product(s)
Plant wax	Raynox Plus
Kaolin clay	Surround WP, Cocoon
Talc	Invelop
Calcium carbonate	SunGuard, Eclipse, FruitShield

All materials tested increased the percentage of sunburn-free fruit (Table 5). A common concern with sunburn protectants is the ease of cleanup in the warehouse. Ideally, fruit emerges free of spray residue after a standard washing and rinsing. We simulated this process by running fruit over the USDA-ARS packingline in

Wenatchee. Visible residues were recorded before placing fruit on the line and after 24 hours of drying time. All materials cleaned easily off fruit flanks. Residues remained in the stem bowls (about 50% of fruit) and the calyx end (about 25%) regardless of material used.

Table 5. Sunburn severity readings at harvest in Golden Delicious and Granny Smith apples. WTFRC 2007.

		SUNBURN INCIDENCE[a]				
TREATMENT	Clean (%)	Y1 (%)	Y2 (%)	Y3 (%)	Tan (%)	Black (%)
Beebe Golden Delicious / M.26 Manson						
Cocoon	47 bc[b]	22 ab	22 a	10 ns	0 ns	0 ns
Eclipse	56 ab	21 ab	17 ab	6	0	0
FruitShield	54 abc	22 ab	15 b	9	0	0
Invelop	58 a	17 b	17 ab	7	0	0
Raynox Plus	56 ab	19 b	16 ab	9	0	0
SunGuard	55 abc	20 b	17 ab	8	0	0
Surround WP	58 a	18 b	16 b	8	0	0
Control	42 c	26 a	20 ab	8	1	0
Beebe Granny Smith / M.106 Manson						
Cocoon	76 a	13 abcd	7 ns	5 ns	0 ns	0 ns
Eclipse	77 a	13 abc	6	4	0	0
FruitShield	77 a	10 d	8	4	1	0
Invelop	77 a	12 bcd	7	4	0	0
Raynox Plus	77 a	13 abcd	7	3	1	0
SunGuard	74 a	14 ab	8	4	1	0
Surround WP	77 a	11 cd	8	3	1	0
Control	69 b	15 a	9	5	1	1

[a] based on 'Schrader-McFerson' scale; [b] data transformed with arcsin, $p \leq 0.1$

Lenticel breakdown: The complete data set for the 2006 field trials will be discussed, since it was not yet available at last year's research review. The 2007 data set will be available in March 2008. In 2006 we set up 4 trials to determine if the application of hydrophobic materials within 3 weeks of harvest would alleviate LB development after storage.

One problem encountered when scheduling appropriate applications was the uncertainty of harvest. When working with grower cooperators, harvest decisions are adjusted on an ongoing basis, making it difficult to harvest fruit at the ideal experimental timing. Thus, we ended up with different preharvest spray intervals for all our trials (Table 6).

All fruit was harvested at commercial maturity suitable for long term CA storage. We found no differences for common maturity parameters at harvest between control and treated fruit (data not shown). LB symptom expression was strongest after 7 days at room temperature. Fruit from all orchards expressed symptoms after 3-4 months of RA storage (low: 9%, high: 33%). We observed slight orchard-to-orchard variation, with fruit from Manson having the highest LB incidence, possibly due to advanced maturity; other factors may have included Gala strain, rootstock, harvest date, orchard elevation, and tree age. No significant treatment effect was seen regarding oil type or concentration, spray frequency, or Raynox application. Manson apples treated with Soy 21 showed

significantly fewer LB symptoms than fruit treated Soy 3x, indicating highly variable LB susceptibility within the same lot (both treatments received only one preharvest spray).

Table 6: Effects of preharvest application of hydrophobic materials on LB development of Gala apples after 3 and 5 months of RA storage during the 2006-07 storage season. WTFRC 2007.

		3 months RA		5 months RA	
TREATMENT	Actual # days before harvest	LB - 2d[2] (%)	LB - 7d (%)	LB - 2d (%)	LB - 7d (%)
Royal Gala / M.9 Manson					
Soy 21[1]	2	16 b	20 b	6 ns	13 ns
Soy 3x	2	33 a	34 a	4	16
Supreme 21[1]	2	26 ab	21 b	4	13
Supreme 3x	2	29 ab	33 a	8	13
Control		23 ab	25 ab	15	18
Brookfield Gala / M.9 Selah					
Raynox	15	19 ns	21 ns	3 ns	14 ab
Soybean 2%	15	18	20	8	17 a
Soybean 1%	15	16	19	4	13 ab
Supreme 2%	15	18	22	4	15 ab
Supreme 1%	15	20	23	5	5 b
Control		16	19	5	16 a
Galaxy Gala / M.9 Royal Slope					
Raynox	20	19 ns	19 ns	3 ns	5 b
Soybean 2%	20	15	18	4	6 b
Soybean 1%	20	9	13	8	21 a
Supreme 2%	20	13	16	6	16 ab
Supreme 1%	20	16	19	10	14 ab
Control		14	17	5	10 ab
Imperial Gala / M.26 Desert Aire					
Soy 21[1]	17	11 ns	15 ns	5 ns	11 ab
Soy 14[1]	10	15	16	2	15 a
Soy 7[1]	3	15	19	4	4 b
Soy 3x	all above	13	17	5	11 ab
Supreme 21[1]	17	13	13	1	5 ab
Supreme 14[1]	10	21	23	4	11 ab
Supreme 7[1]	3	13	16	4	9 ab
Supreme 3x	all above	13	16	3	13 ab
Control		18	20	5	8 ab

[1] refers to planned number of days prior to harvest

[2] days at 72F

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Apple Rootstock and Scion Evaluation
Note: continuing funding will be considered at the internal research review February 6 in Ellensburg**.**

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Collaborators: Dr. Bruce Barritt, WSU-TFREC, Wenatchee,
Dr. Gennaro Fazio, USDA-ARS, Geneva, New York

Cooperators: Dave Allan, Bob Brammer, Ray Fuller, John Verbrugge, Del Feigal, Ron Wilcox, Dale Goldy, Tim Welsh and Gus Heinecke

Total project funding request: Year 1: $28,300 **Year 2**: $30,280 **Year 3**: $35,000

WTFRC Collaborative expenses:

Item	2006	2007	2008
Stemilt RCA room rental			
Crew labor	25,500	23,000	26,000
Shipping			
Supplies	300	5,680[2]	7,300[2]
Travel[1]		1,600	1,700
Miscellaneous	2,500		
Total	**28,300**	**30,280**	**35,000**

Footnotes: [1] mileage is based on 120 miles round trip at 45 cents/mile, 8 trial sites up to 6 trips per site per season for data or plot maintenance.
[2]Tree expense for scion evaluation

OBJECTIVES:

1. Evaluate apple rootstocks, particularly disease resistant rootstocks, in commercial settings in Washington State with known replant conditions.
2. Integrate the processes of evaluation and industry adaptation.
3. Extend procedures for rootstock evaluation into scion breeding program.
4. Establish protocol for scion evaluation program.

Significant findings:

- G.935 is a productive rootstock. The high productivity has calmed the non-bearing vigor of G.935 to keep it in the tree size range of the M.9 class rootstocks.
- G.11 is a very productive M.9 class rootstock. G.11 is fireblight resistant, but not replant tolerant nor woolly aphid resistant. It appears to be smaller in tree size than M.9-337
- Bud 9 is the smallest tree in the trials, planting Bud 9 at higher density can overcome this trait. Bud 9 can stunt out, particularly with tough to grow scions such as Honeycrisp or sandy soils.
- Rootstock influence on fruit size is trending favorably with G.41,G.935 and G.11. Statistically significant differences are not consistent from trial to trial, though the trend is consistent that these stocks have equal fruit size or better than M.9 class rootstocks.
- Honeycrisp performs well on dwarf rootstocks if vigor is maintained. Canopy development can stall easily with all rootstocks with Honeycrisp as scion. Bitterpit and poor fruit color are problems on the semi-dwarf rootstocks
- Supporter 1,2 and 3 can be substituted for M.9. None of them are disease resistant. Supporter 2 suffered the most tree loss, about 1/3 of trees failed. Supporter 3 is comparable to the large M.9 rootstocks in vigor and production.

Discussion:

- The Geneva rootstocks are outperforming industry standards of M.9 class rootstocks in terms of yield and fruit size. Suseptible varieties such as Pink Lady and Jazz should be planted on fireblight resistant rootstocks.
    - Bud 9 is resistant to fireblight in field conditions with scion infections. Bud 9 can stunt out from replant disease or overcropping.
- G.41 is tending to be smaller than M.9-337, but appears replant tolerant and produces larger sized fruit similarly to M.9. This stock is known to be winter hardy, woolly aphid immune and fireblight resistant. It will be a couple of years before small quantities of G.41 will appear. Order requests will help progress G.41's availability.
- G.935 is a larger tree than M.9 – 337, but is likely to be smaller than M.9-Nic 29. Fruit size is similar to M.9 class rootstocks. G.935 is not woolly aphid resistant, but is replant tolerant and fireblight resistant. Orders for G.935 will bring this rootstock to market. It grows vigorously as a non bearing tree, but tree vigor calms better than M.9-Nic 29. Commercial availability is a couple of years away.
- Commercially, G.11 is the Geneva stock most available.
- G.202 is performing well in New Zealand. Trials look promising in Washington but are not old enough to provide an opinion.
- M.9-Nic 29 and Pajam 2 will likely be too vigorous for many plantings above 1100 trees per acre, especially with varieties such as Gala or Fuji.

Summary of plant evaluation trials:

Apple
Rootstock
Replant site - plant in place

Year Planted	Location	Cooperator	Cultivar	# of Rootstocks
2006	Vantage	Feigel	Fuji bench grafts	28 dwarf

Apple
Rootstock

Year Planted	Location	Cooperator	Cultivar	# of Rootstocks
2003	Frenchman	Verbrugge	Honeycrisp	18 dwarf / 6 semi
2003	Chelan	Fuller	Honeycrisp	18 dwarf / 6 semi

Apple Rootstock - Replant
Fumigated / Unfumigated

Year Planted	Location	Cooperator	Cultivar	# of Rootstocks
2004	Naches	Allan	Honeycrisp	16 dwarf
2004	Wapato	Wilcox	Gala	12 dwarf
2004	Chelan	Fuller	Gala	12 dwarf
2006	Wapato	Wilcox	Gala	22 dwarf /12semi
2006	Brewster	Brammer	Fuji	16 dwarf / 8 semi

Apple scion

Year planted	Location	Cooperator	Company	# varieties/Total # trees
2007	Brewster	Bob Brammer	Crane & Crane	4 / 230
2007	Quincy	Dale Goldy	Stemilt	4 / 230
2007	Mattawa	Tim Welsh	Columbia	4 / 230
2007	Prosser	Dave Allan	Yak.Val.Orch.	4 / 230

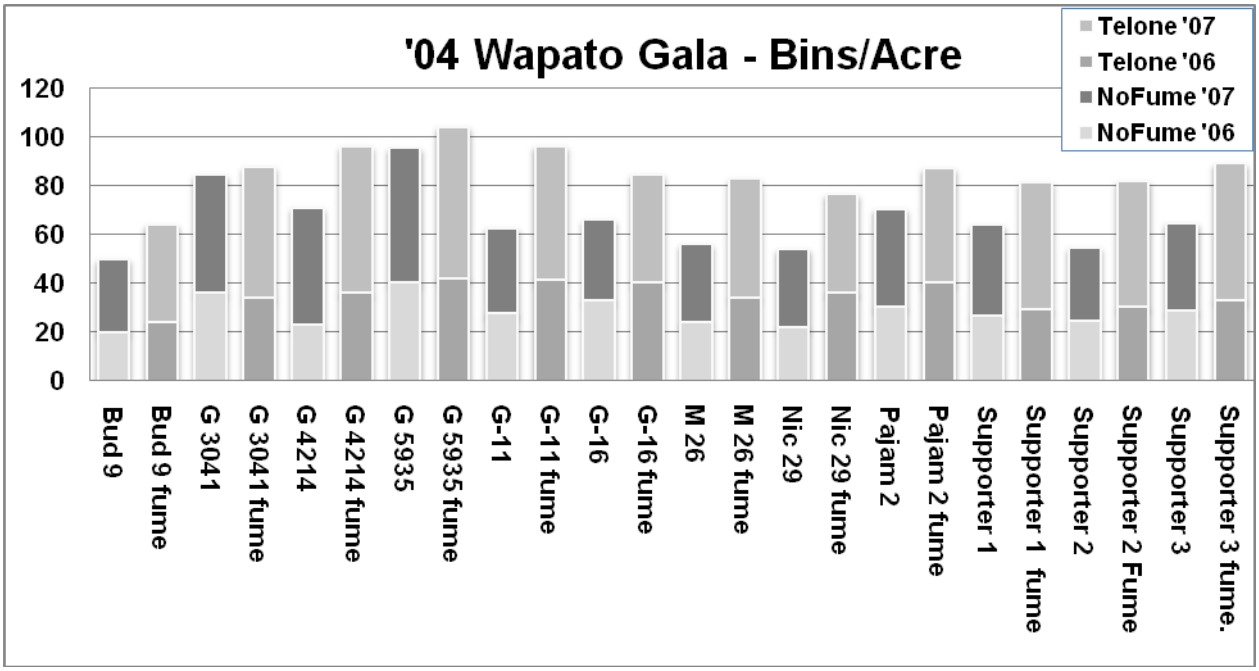
2007 Accomplishments:

- Apple scion evaluation trials have been established. Written work plans are being established.
- G.41 production has quality assurance monitoring for genetic true to type traits. One tissue culture line and two layer beds were removed from production. One layer bed was identified as being G.11 not G.41.

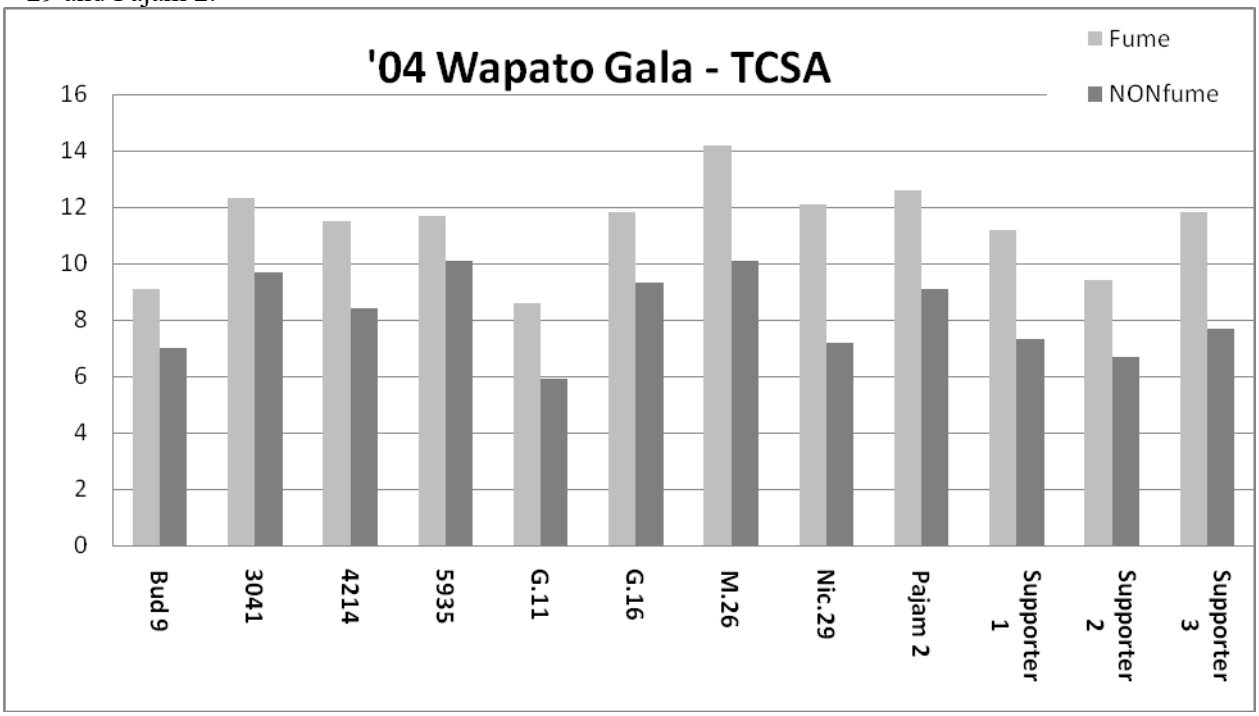
Observations:

- Fumigation in all replant trials has enhanced tree growth and yield of all rootstocks, even those that show promise of being replant tolerant.
- Drip irrigation is an important tool in rapidly establishing a full and consistent canopy. Trials with sprinkler, or micro sprinkler are not as consistent as trials with drip irrigation.
- Rootstock vigor or tree size in Washington State can be different than trial results in Eastern US. Examples include M.26 tree canopy volume in the east is nearly double what is observed in the irrigated west. Ottawa 3, on the other hand is opposite, it is much larger than M.26 in the irrigated west than in the East.
- All the replant trials, indicated by fumigated and nonfumigated data show strong response to fumigation. There is not yet a reliable replacement for fumigation in organic production. Planting larger rootstocks will not mitigate the yield impacts of replant disorders. The difference in first crop volumes on vigorous rootstocks or nonfumigated plots are striking. Early yields are vital to the economic success of new plantings.

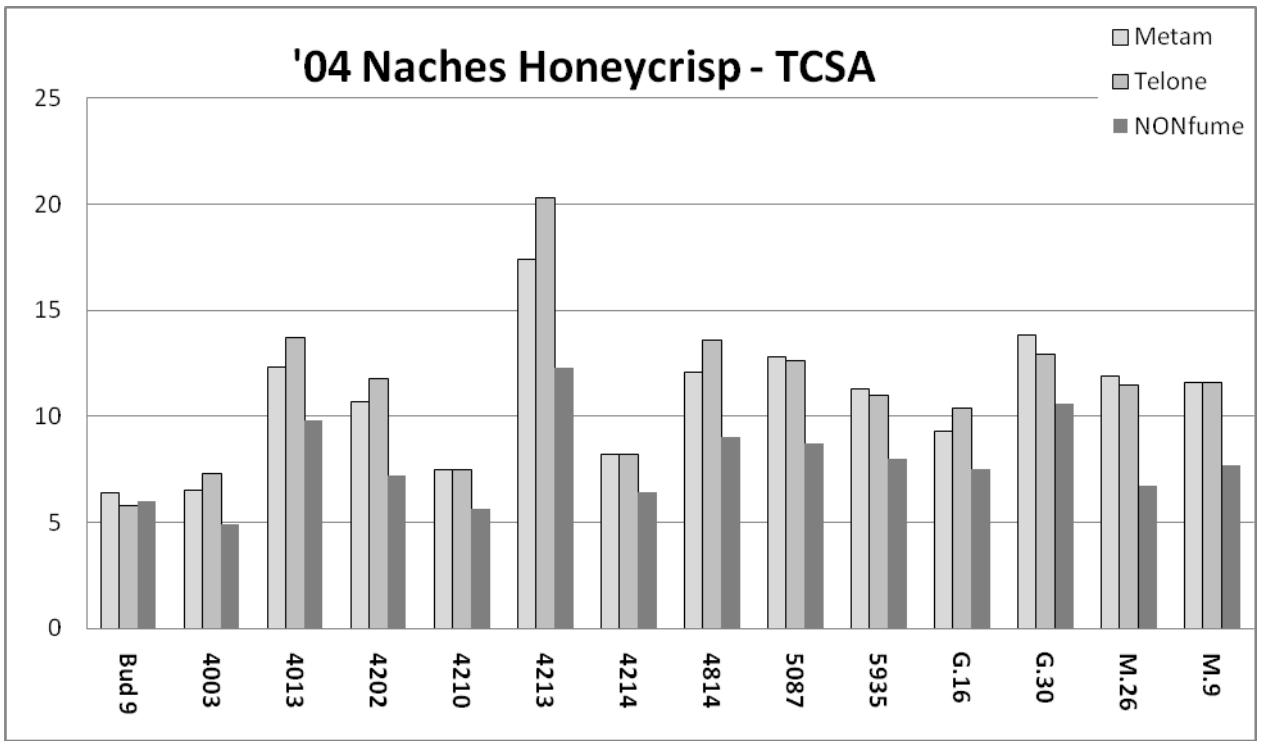
Data:



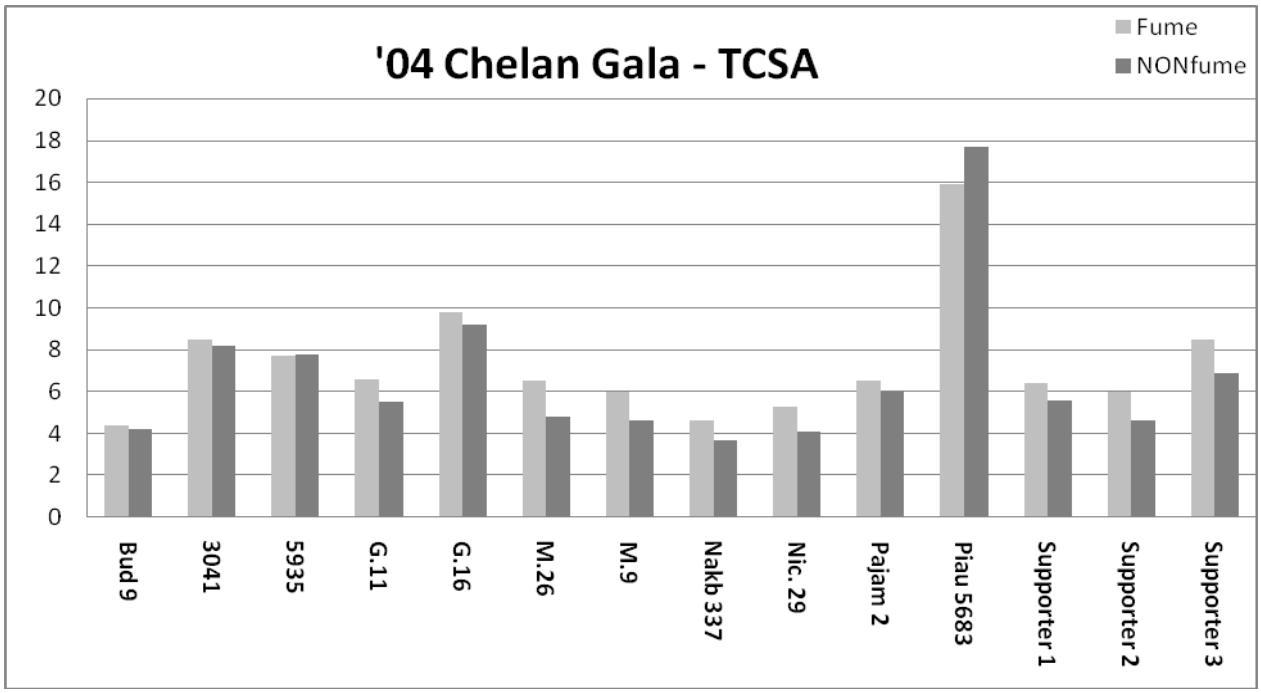
The ’04 Wapato Gala trial has filled its space and is a mature canopy. G.11, 3041 (G.41) and 5935 (G.935) had better yields and fruit size trended larger than the M.9 class standards of Nic. 29 and Pajam 2.



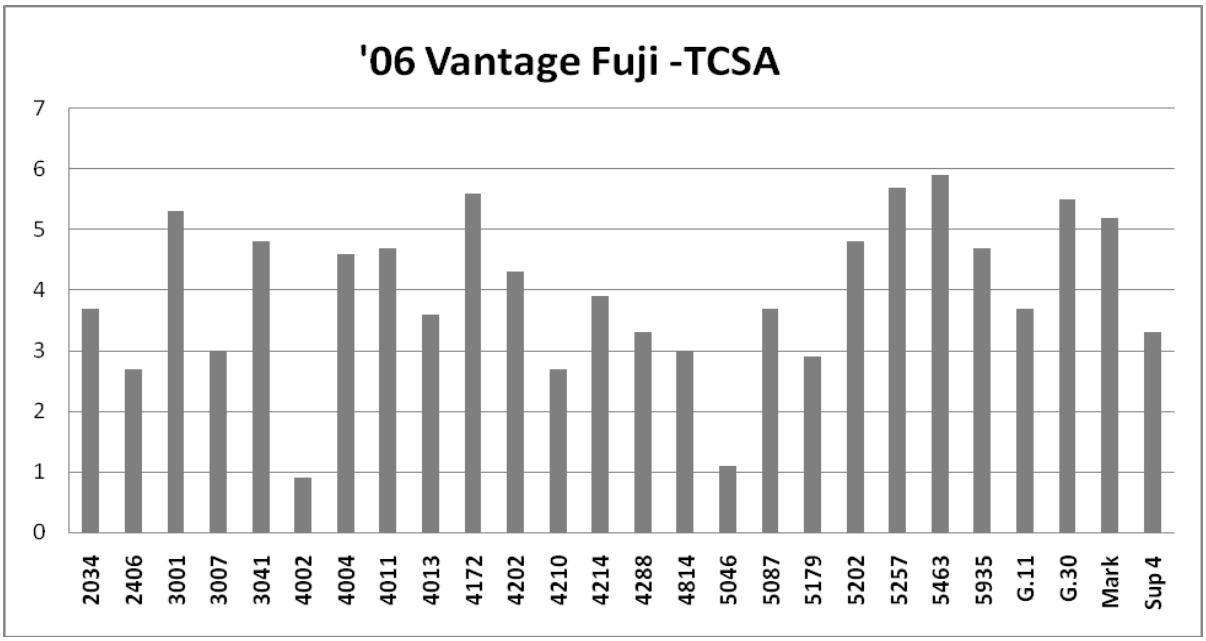
TCSA is trunk cross sectional area in centimeters^2 and is calculated from trunk circumference data. Trees in the ’04 Wapato gala trial that have reached a TCSA of 8 or more have filled their in-row space at 8 feet from the ground and are capable of yields of 40 bins/acre or more. TCSA of 10 indicate trees that have completely filled the fruiting wall and exceeded the maximum height of 12 feet and have yield capacity over 60 bins/acre. 5935 (G.935) is similar in tree size to the M.9 class standards.



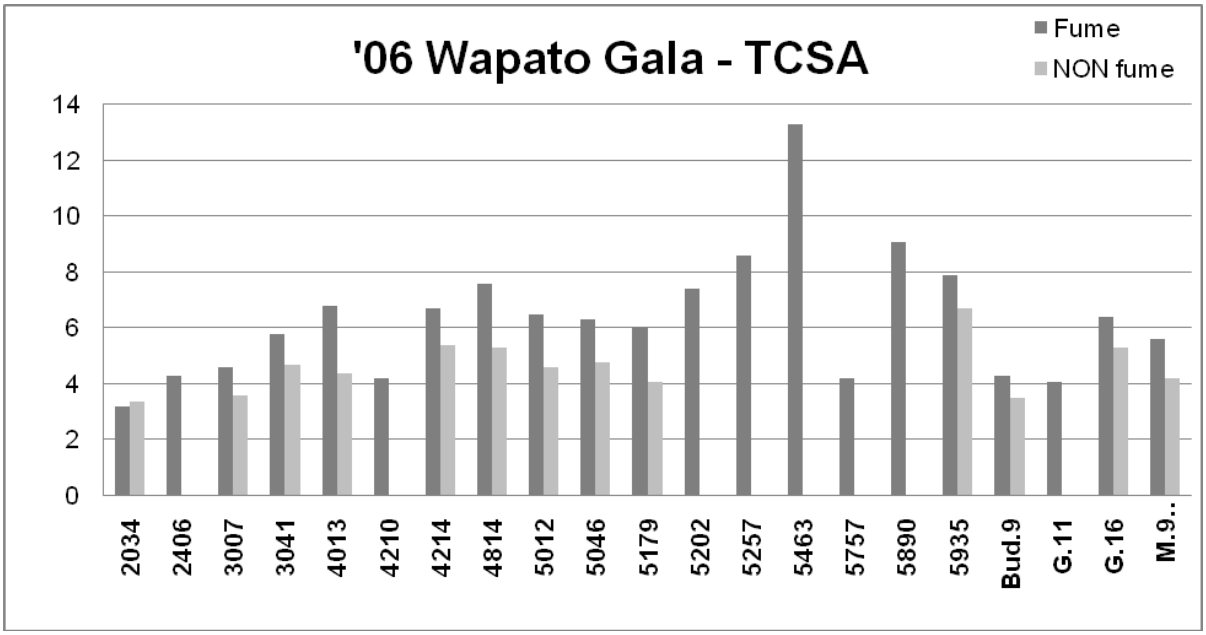
Naches Honeycrisp shows dwarf rootstocks can perform well with this difficult to grow scion. Trees with TCSA above 8 should support crop volumes above 40 bins/acre.



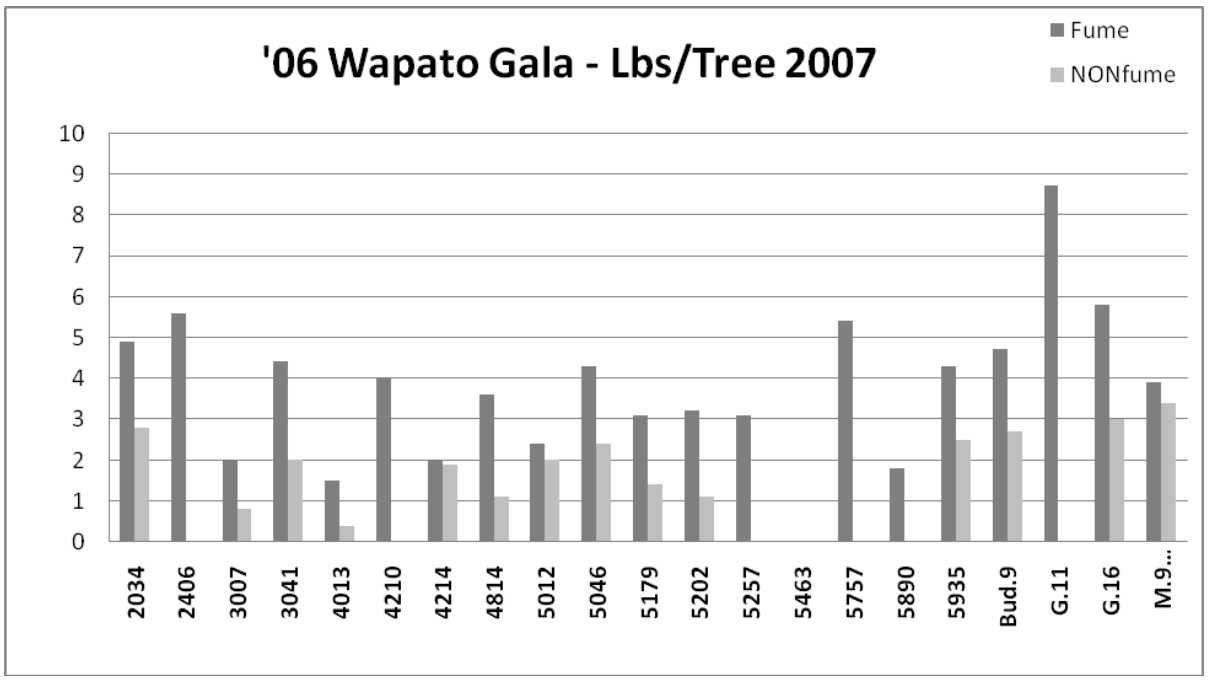
The ’04 Chelan Gala trial is similar to the ’04 Wapato trial. The replant disorder in this site is more debilitating than Wapato. TCSA of 8 or more is capable of 40 bins per acre. The exception to this generalization is Piau 5683. It is seedling size and was removed from the Wapato trial due to over growing and interfering with other plots. Piau has had little to no yield



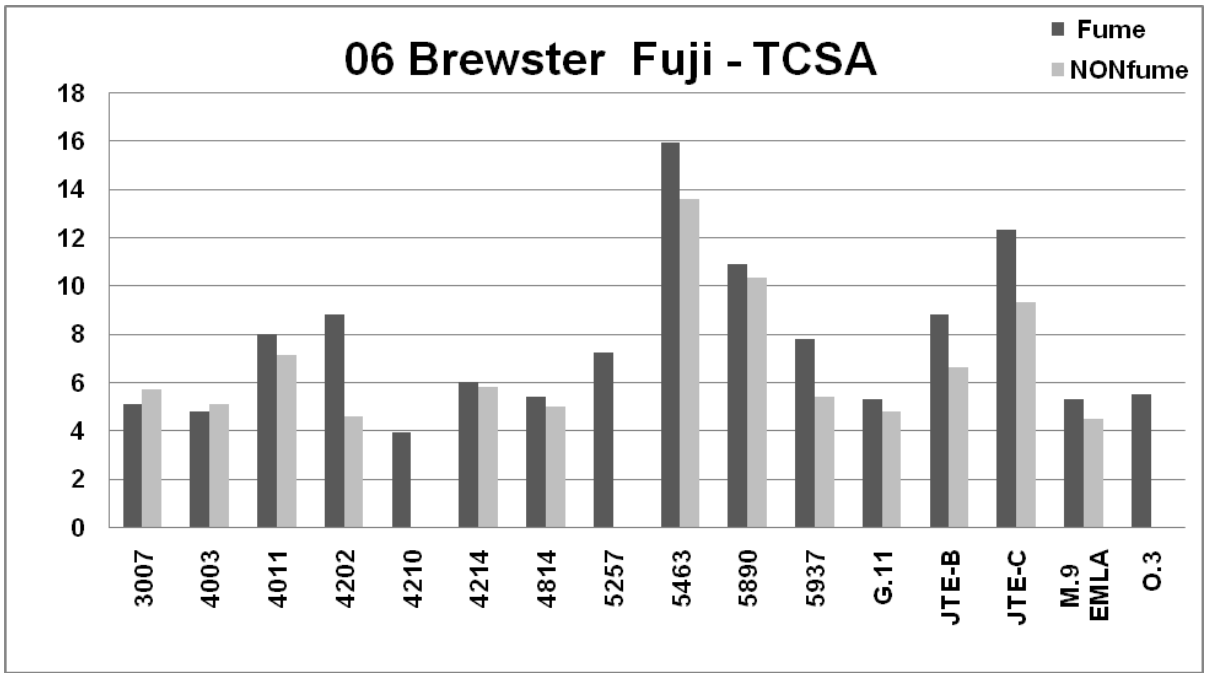
The Vantage trial was fumigated prior to planting. TCSA is a measurement of accumulated dry matter. The management of the canopy at Vantage minimizes development of unnecessary dry matter. First crop of Fuji on third leaf Mark should be about 35 bins per acre in 2008. Rootstocks supporting a TCSA above 6 at the end of next season yield more than 60 bins per acre in fourth leaf.



Many of the ’06 Wapato Gala selections are new to the northwest. There are three groups of tree size in the fumigated plots in the ’06 Wapato trial. Dark bars not reaching 5 are the small group. Dark bars reaching 6 to 71/2 are the medium group. Dark bars above 8 are the large group. 5202 (G.202) is similar in size to 5935 (G.935).



5.5 pounds per tree on a 10'x 3'(1452 trees per acre) spacing is about 9 bins per acre at 870 pounds per bin. The more vigorous trees have less fruit. Nonfumigated plots have less fruit.



Many of the rootstocks in the '06 Brewster trial are new to the northwest. Brewster has severe replant conditions.

FINAL PROJECT REPORT
WTFRC Project Number: N/A – Internal

Project Title: Apple Rootstock Propagation-G.41

PI: **Tom Auvil**
Organization: WTFRC
Telephone/email: 509-669-3060 auvil@treefruitresearch.com
Address: 1719 Springwater Ave.
Address 2:
City: Wenatchee
State/Province/Zip WA 99801

Cooperators: Gennaro Fazio

Total Project Funding: $30,000

Budget History:

Item	**2007**		
Stemilt RCA room rental			
Crew labor			
Shipping			
Supplies			
Travel			
Miscellaneous			
Contingency fund for Propagation of G.41	$30,000		
Total	**$30,000**		

Footnotes:

OBJECTIVES:

1. Evaluate the status of G.41 propagation. Determine what barriers of a technical nature are preventing propagation of commercial volumes of rootstock liners.
2. Generate interest in research project to reduce or eliminate barriers.

Significant Findings:

- Barriers to production:
    - Cost of liners started in stand-alone TC labs are too high for rapid layer bed planting.
    - Tissue Culture production is too low. 500 liners of G.41 have been released to greenhouse after 8 months in Tissue Culture production.
    - Liner production is limited mostly due to poor rooting trait of G.41
- The tissue culture labs being favored to produce G.41 do not have mist bed or greenhouse facilities. Customers purchase the x-plants from the tissue culture lab and arrange to have them shipped to a mist bed/greenhouse facility to be placed into potting mix, then mist bed, and finally into the greenhouse to grow into a liner that can survive outdoor environments. The Customer stands all losses. This format pushes the cost to nearly $3.00 per liner.

Layer Bed Licensees:

- Willow Drive Nursery – All available plant material going back to layer beds. A few G.11 are being grown for finished trees in 2008. Have ordered 65,000 G.41 tissue culture liners; have received 500.
- Treco – G.41 layer bed removed due to mixed plant material from Cornel. Will be re-establishing the G.41 layer bed.
- Willamette – Oregon
- Cummins Nursery –New York

Tissue Culture Licensees

- North American Plants in Lafayette, Oregon. Is developing G.41 and G.935 lines. NAP has greenhouse and mist bed facilities. Tissue Culture production of OHxF 87 is successful and liners cost about $1 each.
- Microplant – Not producing apple rootstocks at present time.
- Phytocel –New York. X-plants are shipped to a nursery. Current price is about $1.25 for a plantlet with two root nubs.

Project solicited:

- Amit Dhingra proposed a project in December 2007 experimenting with an improved recipe, alternative medias, different light media and automated phasing. Plus combinations of the various alternative treatments.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Replant disease of Geneva rootstocks

PI:	Dr. Gennaro Fazio	**Co-PI(2):**	Dr. Mark Mazzola
Organization:	USDA-ARS, PGRU	**Organization:**	USDA-ARS. TFRL
Telephone/email:	315-787-2480/ Gennaro.fazio@usda.ars.gov	**Telephone/email:**	509-664-2280x 245
Address:	630 W. North Street	**Address:**	
City:	Geneva	**City:**	Wenatchee
State/Province/Zip	NY	**State/Province/Zip:**	WA 99801

Cooperators: Tom Auvil, Tim Smith and others at WTFRC

Total project funding request: **Year 1:** $30,000 **Year 2:** 30,000 **Year 3:** $35,230

Budget 1:
Organization Name: USDA-ARS, PGRU **Contract Administrator:** Dianne Emerson
Telephone: 315-787-2329 **Email address:** Dianne.emerson@ars.usda.gov

Item	**2008**		
Salaries[1]	21,000		
Benefits	6,000		
Wages			
Benefits			
Equipment			
Supplies[2]	6,000		
Travel[3]	1,530		
Miscellaneous[4]	700		
Total	35,230		

Footnotes: [1]Technician salary for part-time assistance in propagation budding and maintenance of stoolbeds.
[2]Includes cost for rootstock liners, trees, support system, laboratory supplies etc.
[3]Travel to and from trials.
[4]Includes shipping expenses, communication costs etc.

Objectives

1. To study the relative performance of Geneva dwarfing apple rootstocks compared to commercial controls in replant soils and the study of genetic mechanisms related to tolerance to ARD.
2. In the most recent visits we have come to appreciate the need by a certain segment of the industry to plant liners in place in the orchard either as sleeping eyes or as bench grafts. We would like to modify our existing protocol to discover "nursery in place" properties of rootstocks and how they interact with replant disease when the plants are so young. The question we are trying to answer is: how well do ARD tolerant sleeping eyes and bench grafts do in a replant situation?
3. To set up an early evaluation protocol for newly developed genotypes that screens for components of apple replant disease resistance in the early stages of breeding.

SIGNIFICANT FINDINGS AND DEVELOPMENTS

- **The rootstock B.9 continues to be one of the weakest and least productive rootstocks in the replant experiments that have been planted so far. Malling 9 survival has been compromised by several fire blight events. G.41 and G.935 have performed well in the replant settings of Wapato (conventional) and Chelan (organic). G.4214 had the highest cumulative yields in Wapato.**
- **First data from a graft in place replant experiment with the widest selection of Geneva rootstocks ever tested in WA has been collected in Vantage, WA and from new replant experiments in Wapato and Brewster, WA.**
- **Fumigation's positive effect on season's tree growth treatment has disappeared in the fourth season while the cumulative effect (TCSA and Fruit Yield) is still detectable.**

OBJECTIVE 1. There are three phases within this objective: 1) propagation of rootstocks and tree design, 2) Establishment of replant trials, and 3) Data collection at the sites and analysis.
At the onset of this period we had already established 6 ARD (Table 1) and two non ARD field trials.

Table 1. Locations and rootstocks planted in ARD trials 2003-2006.

Rootstock	Location*	Scion Varieties
G.16	WA, CH, NA	
G.11	WA, CH, BR, VA	Brookfied Gala, Torres Fuji, Aztec Fuji
G 3041	WA, CH, VA	Brookfied Gala, Aztec Fuji
G 5935	WA, CH, NA, VA	Brookfied Gala, Honeycrisp, Aztec Fuji
PiAU-56-83	WA, CH	Brookfied Gala
Pajam 2	WA, CH	Brookfied Gala
M.26 EMLA	WA, CH, NA	Brookfied Gala
Bud 9	WA, CH, NA	Brookfied Gala
Supporter 1	WA, CH	Brookfied Gala
Supporter 2	WA, CH	Brookfied Gala
Supporter 3	WA, CH	Brookfied Gala
4214	WA, NA, BR, VA	Brookfied Gala, Torres Fuji
4003	NA	Honeycrisp
4814	NA, BR, VA	Honeycrisp, Torres Fuji, Aztec Fuji
4210	NA, BR, VA	Honeycrisp, Torres Fuji, Aztec Fuji
G.30	NA, VA	Honeycrisp, Aztec Fuji
5087	NA, VA	Honeycrisp, Aztec Fuji

Rootstock	Location*	Scion Varieties
G 4202	NA	Honeycrisp
4013	NA	Honeycrisp
4213	NA	Honeycrisp
M.9 EMLA	NA, BR	Honeycrisp, Torres Fuji
5757	BR	Torres Fuji
G.202	BR, VA	Torres Fuji, Aztec Fuji
6879	BR	Torres Fuji
MM.106	BR	Torres Fuji
6006	BR	Torres Fuji
7707	BR	Torres Fuji
5257	BR, VA	Torres Fuji, Aztec Fuji
3007	BR, VA	Torres Fuji, Aztec Fuji
4011	BR, VA	Torres Fuji, Aztec Fuji
5935	BR	Torres Fuji
5463	BR, VA	Torres Fuji, Aztec Fuji
4003	BR	Torres Fuji
6001	BR	Torres Fuji
6210	WA	
M.7	BR, WA	Torres Fuji
JTE-B	BR	Torres Fuji
Ottawa 3	BR	Torres Fuji
JTE-C	BR	Torres Fuji
5890	BR	Torres Fuji
2034	VA	Aztec Fuji
2406	VA	Aztec Fuji
3001	VA	Aztec Fuji
4002	VA	Aztec Fuji
4004	VA	Aztec Fuji
4013	VA	Aztec Fuji
4172	VA	Aztec Fuji
4288	VA	Aztec Fuji
5046	VA	Aztec Fuji
5179	VA	Aztec Fuji
5202	VA	Aztec Fuji
4019	VA	Aztec Fuji
Mark	VA	Aztec Fuji
Supporter 4	VA	Aztec Fuji

*** WA=Wapato, CH=Chelan, NA=Naches, VA=Vantage, BR=Brewster**

FINDINGS BY LOCATION (Please refer to poster for more graphs and data):

2004 CHELAN AND WAPATO REPLANT TRIALS

Several M.9 and B.9 trees in these plantings have died. The likely cause for the M.9 was fire blight. The fumigation effect on cumulative tree growth and productivity is still significant in the overall planting; yearly growth was not different between the two treatments: the effect of the fumigation treatment on growth is restricted to the first two growing seasons. When we look at the performance of the individual rootstocks (figures 1 and 2) we notice that some are relatively unaffected by the replant problem and seem to do relatively well in fumigated and non-fumigated soils.

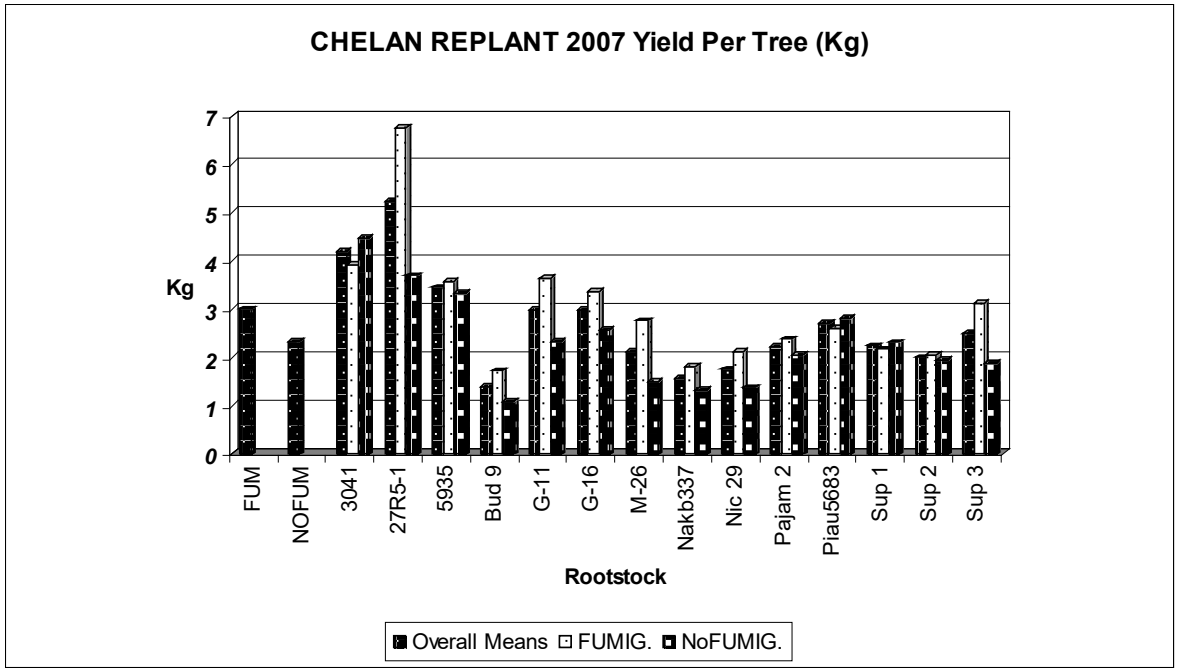


Figure 1. Yield per tree 2007. In this organic planting in Chelan the overall effect of the fumigation is still detectable. Some rootstocks however do not seem to be affected as much (3041 aka G.41, 5935 aka G.935). A mixture was identified in G.41 rootstock. Every G.41 tree in the trial was DNA fingerprinted resulting in the identification of all misidentified trees (roughly 20% of the total). This rootstock was labeled 27R5-1.

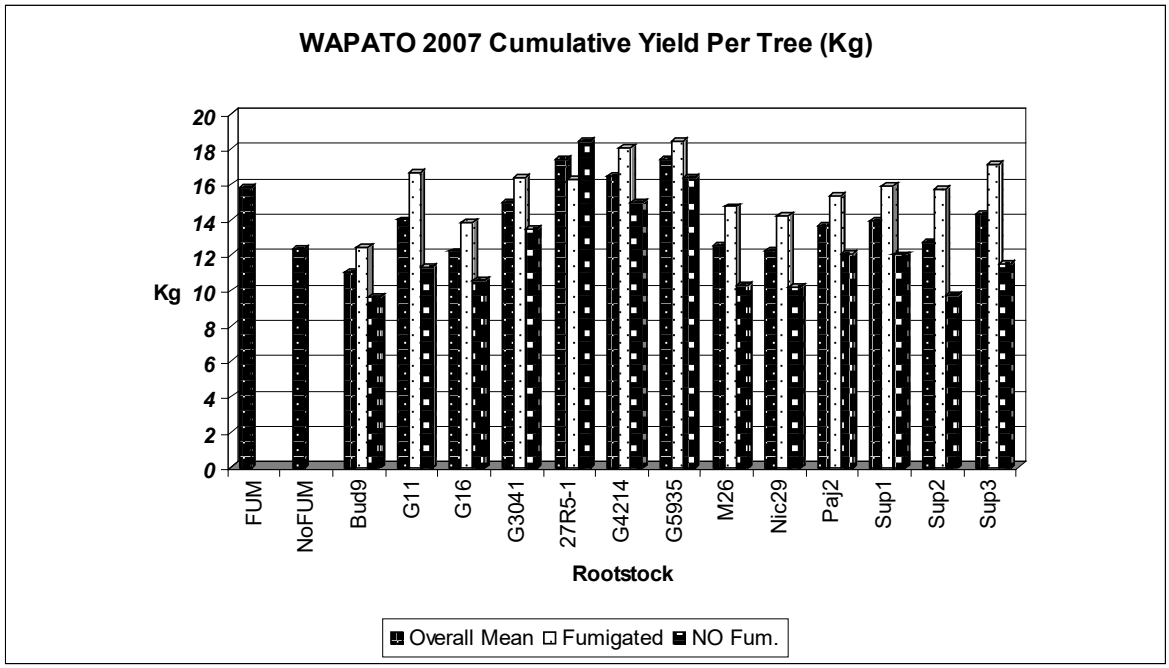


Figure 2. Cumulative yield per tree 2007. In this planting in Wapato the overall effect of the fumigation is still detectable. Some rootstocks however do not seem to be affected as much (G4214, G.41, G.935) the highest yielding rootstocks were G4214, G.935 and 27R5-1. A mixture was identified in G.41 rootstock. Every G.41 tree in the trial was DNA fingerprinted resulting in the identification of all misidentified trees (roughly 20% of the total). This rootstock was labeled 27R5-1.

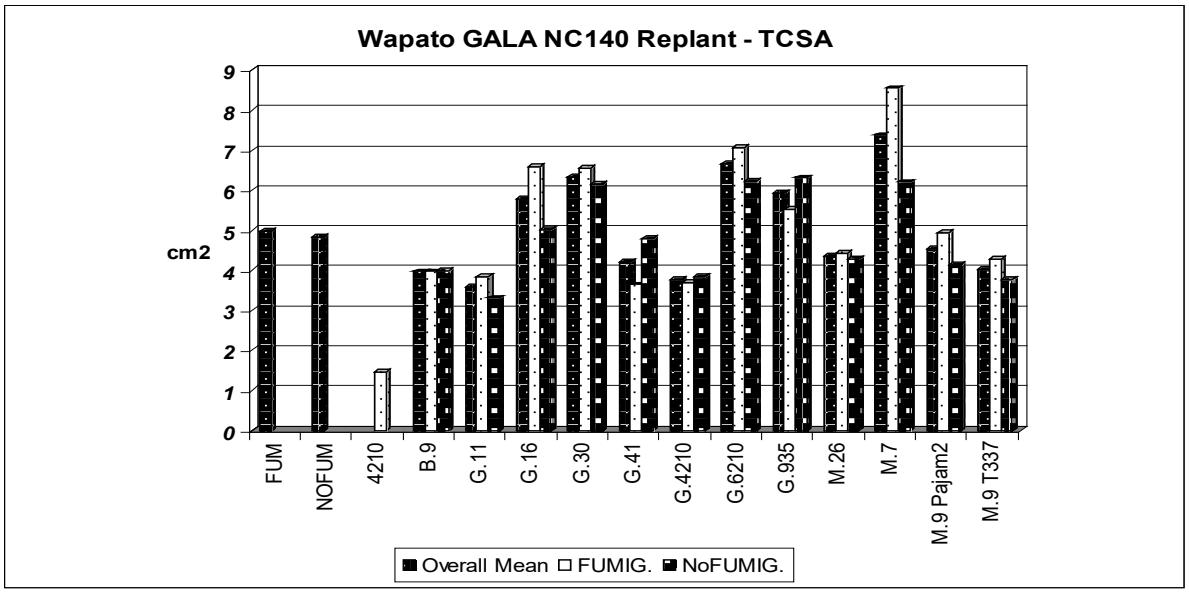


Figure 3. 2006 Wapato Replant Trial NC-140. Average Trunk Cross Sectional Area of rootstocks in this trial – this planting is still too young to draw any conclusions, however overall effect of the fumigation treatment is not as drastic as previous experiments.

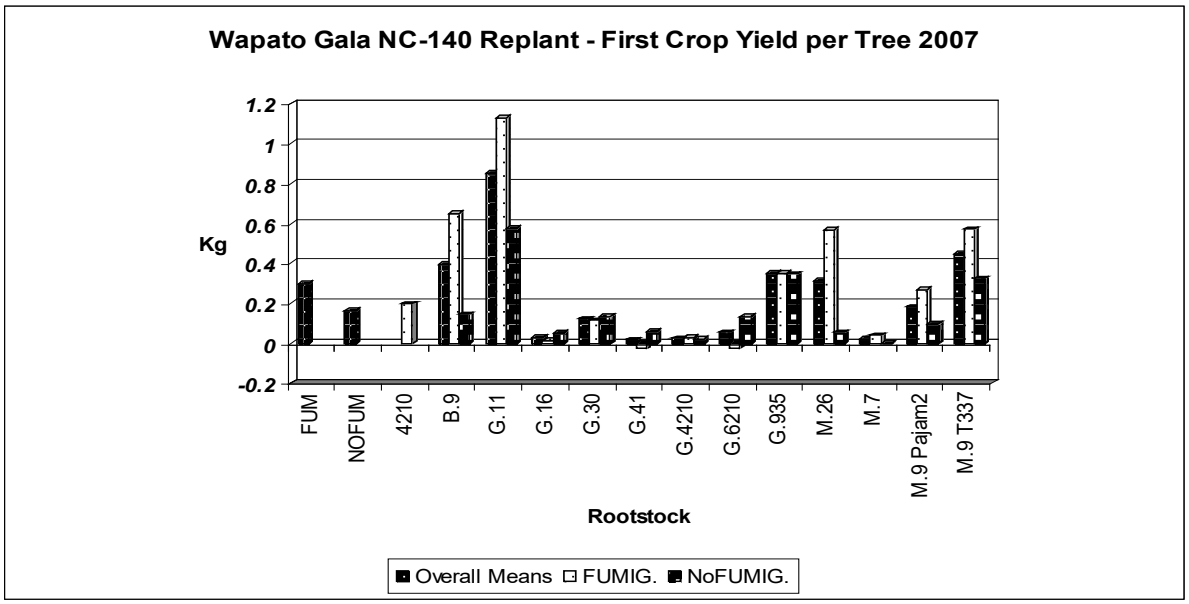


Figure 4. Early Yield is affected by fumigation and rootstock. G.11 had the highest average per tree first crop yield followed by B.9 and G.935.

NACHES REPLANT TRIAL

This year was a very odd year for this planting – all of the rootstocks seemed to be affected by the biennial nature of HoneyCrisp. A regression of this year's low yields based on last year's yields (Reg equation: Number of Fruit 2007= 9.37 - 0.279 Number of Fruit 2006 – P=0.0001) showed that the crop yield of last year had a definite negative effect on this year's yield. The trees that had a low crop last year had a good crop this year and vice versa. No individual rootstock seemed to overcome the biennial nature of HoneyCrisp Overall the effect of the fumigation on the cumulative production of this planting is very evident (Figure 1).

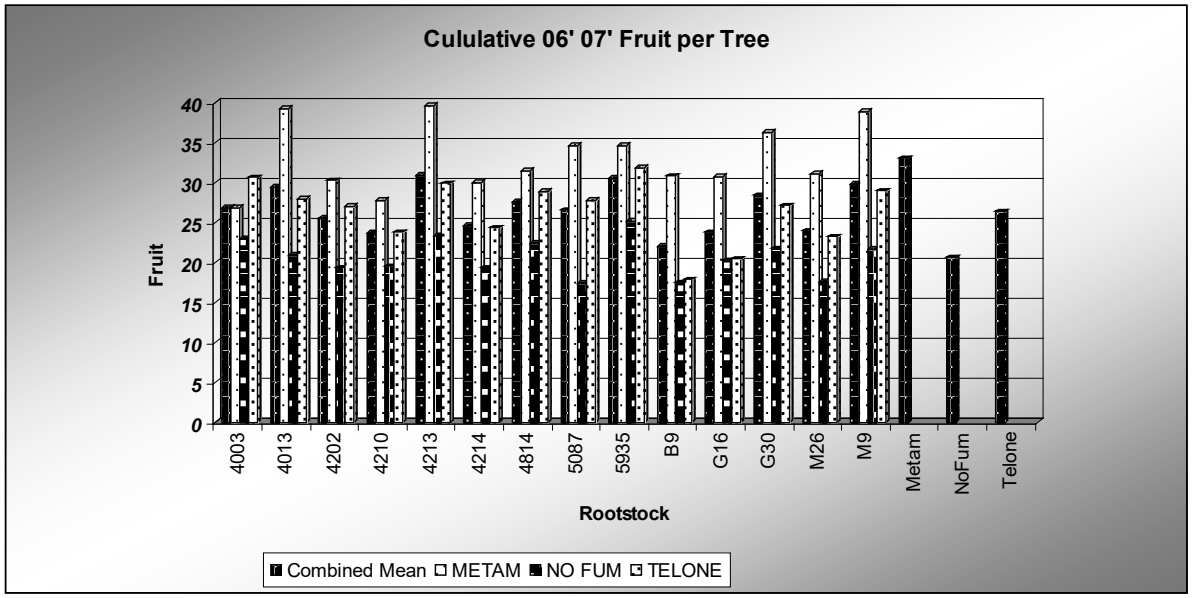


Figure 3. Cumulative number of fruits per tree (Honeycrisp). Overall the Metam Sodium treatment seems to have performed better than other treatments. Productivity of the rootstocks was correlated with their dwarfing category. One rootstock (4003) did not have a significant difference in productivity due to treatment (e.g. the fumigated trees were not very different from unfumigated).

2006 BREWSTER REPLANT TRIAL

The first year of data for the Dwarf and Semi-Dwarf Fuji Replant trials are summarized in Figures 4 and 5. The fumigation effect in the Semi-Dwarf trial is not statistically significant even though it is perceptible. Rootstocks G6001 and M.7 show the greatest differences between treatments. The fumigation effect in the Dwarf trial was significant.

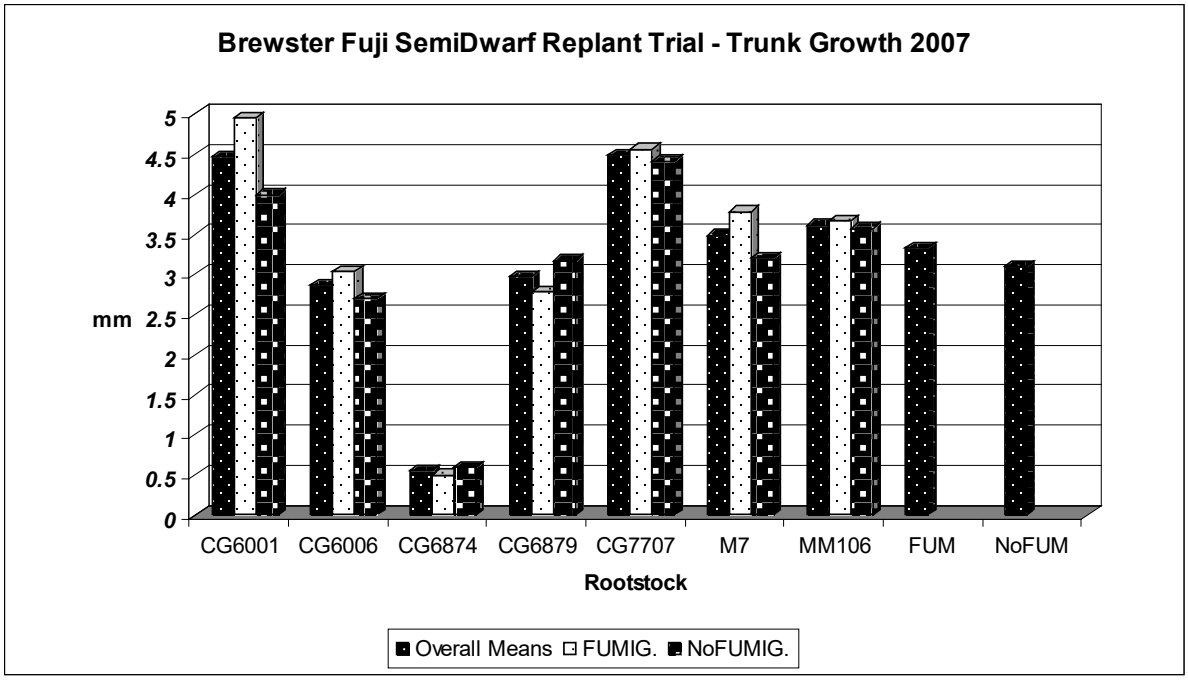


Figure 4. Brewster Semi-Dwarf Trial planted in 2006. The fumigation effect is not statistically significant. CG 6874 grew the least pointing to a possible replant independent problem with this rootstock.

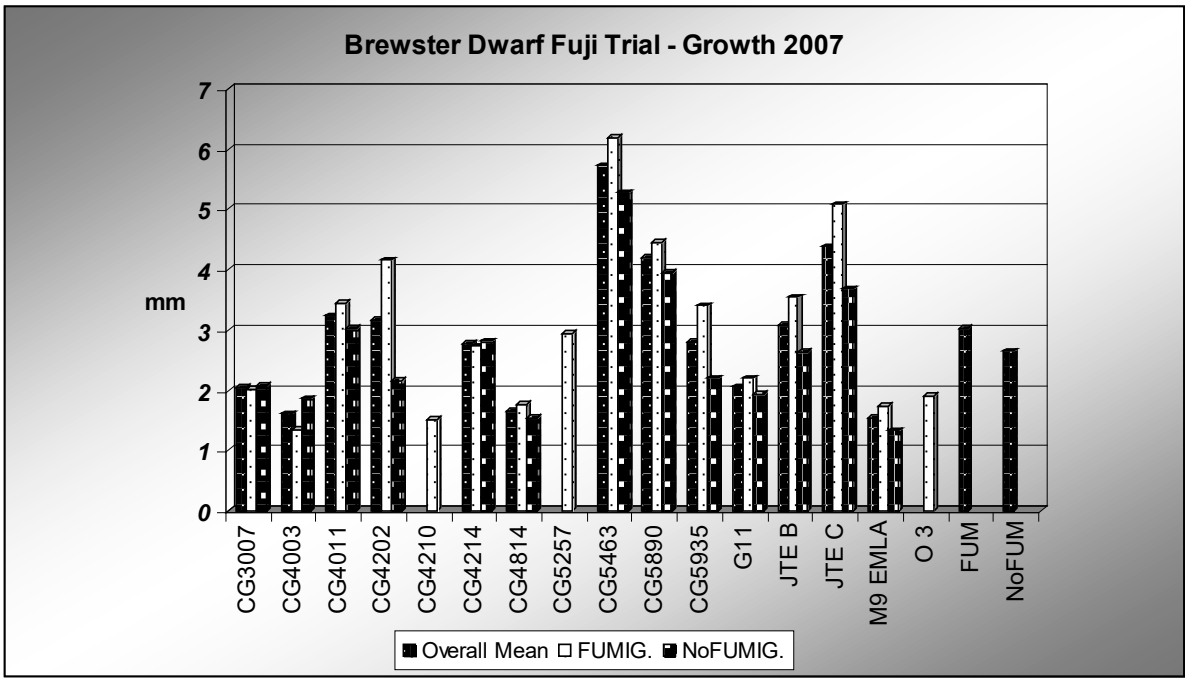


Figure 5. Brewster Dwarf Trail Planted in 2006. The growth of some rootstocks like JTE C, CG4202, is affected by the fumigation treatment. The growth of CG4214, and CG3007 and G.11 is independent of fumigation treatment.

OBJECTIVE 2

We have made progress on Objective 2 by collecting data on BenchGrafts (BG) at the Auvil Fruit Tree Farm (Vantage, WA). We are in the process of bulking up more rootstock material to establish a sleeping eye (SE) experiment in a replant soil. The questions we are trying to answer in this part of the experiment are:

1. Are certain rootstocks better adapted at producing healthy trees from SE or BG in ARD fumigated soil?
2. What are the long term effects on fruit production (yield, quality) from growing trees in place according to different rootstocks?

OBJECTIVE 3

We are going to inoculate seedlings from the breeding program with Rhizoctonia solanii for the first time in 2008 and evaluate the selection potential of this new screening procedure. Lack of base funding has hindered the incorporation of novel selection techniques for replant disease components.

AKNOWLEDGEMENTS

We would like to express heartfelt gratitude to the many growers that are cooperating in this effort by hosting trials as well as the staff members at the WTFRC that have worked very, very,hard to obtain this data. THANK YOU!!!

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-07-703

Project Title: Cultivar improvement via controlled sport induction (CSI)

PI:	Amit Dhingra	**Co-PI(2):**	Bruce Barritt
Organization:	WSU	**Organization:**	TFREC, WSU
Telephone/email:	509 335 3625 adhingra@wsu.edu	**Telephone/email:**	509 663 8181 etaplz@wsu.edu
Address:	PO Box 646414	**Address:**	1100 N Western Ave
City:	Pullman	**City:**	Wenatchee
State/Province/Zip	WA 99164	**State/Province/Zip:**	WA 98801

Cooperators: Fred Bliss and Cameron Peace

Total project funding request: **Year 1:** 26,150 **Year 2:** 36,725 **Year 3:**

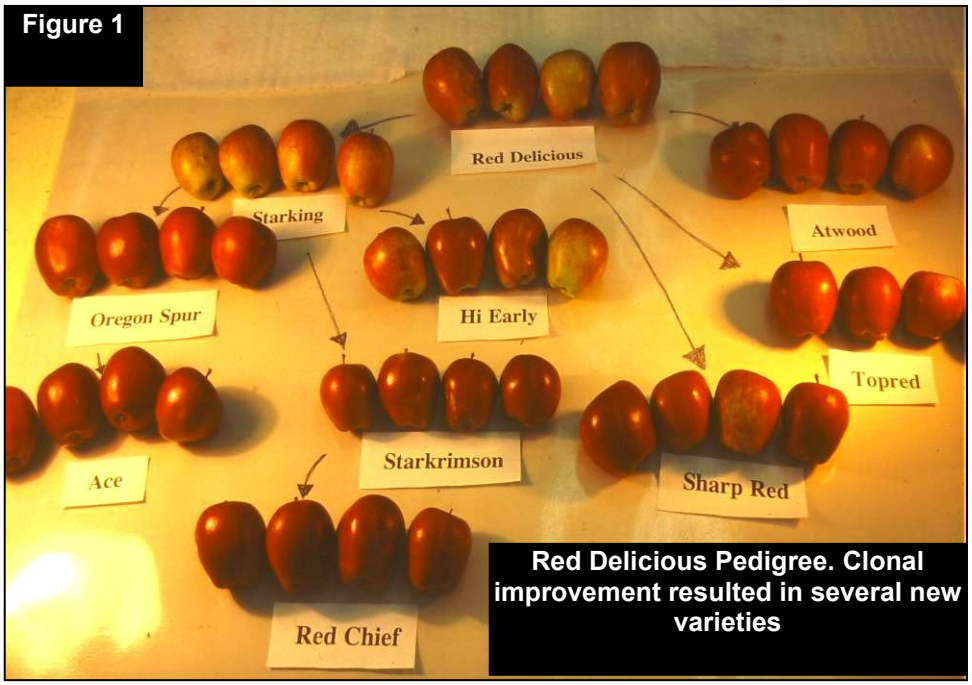
Other Funding Sources: NONE

Budget 1:
Organization Name: WSU **Contract Administrator:** ML Bricker
Telephone: 509 3357667 **Email address:** mdesros@wsu.edu

Item	2007	2008	
Salaries			
Benefits			
Wages	10,000	**15,000**	
Benefits	1,150	**1,725**	
Equipment			
Supplies	13,000	**18,000**	
Travel	2,000	**2,000**	
Miscellaneous			
Total	26,150	**36,725**	

Footnotes:

Naturally occurring sports have been a source of improvement to apple cultivars in the past but it is a chance process that is long-term and unpredictable. Figure 1 illustrates the pedigree of Red Delicious where all the new varieties are sport events. In essence, the new cultivars thus generated were clonal variants.



We had proposed a procedure to accelerate this natural process of sport induction and regulate it by targeting economically important traits. A technology platform is being established for inducing trait-targeted mutations (sports) to increase allelic diversity of the gene (cause) that regulates a trait of interest (effect). As cause and effect or gene-trait relationships continue to be established both in our program and by other programs, the scope of this platform will expand to encompass numerous important traits. Examples of cultivars and traits to be improved include HoneyCrisp without bitter pit, an earlier maturing Cripps Pink and a firmer Gala or a self compatible apple.

Objectives: Proposed objectives of the project were:

1. *Using tissue culture, establish cultures from selected cultivars*:

   Progress: We have used Fuji, Granny Smith, Golden Delicious, Red Delicious, Braeburn, Royal Gala, and HoneyCrisp for establishing tissue culture derived propagules that will form the starting material for sport induction.

2. *Standardize the technique for efficient sport induction*

   Progress: The cultures are being produced to move to this stage.

3. *Perform a pilot experiment with tissue culture material for identifying allelic diversity for the genes that regulate fruit firmness (ACC-synthase and ACC-oxidase)*

Progress: The equipment to perform this experiment is now available and this is the first target gene that we will be utilizing for CSI.

Proposed activities for 2008:

- Expand collection of apple callus tissue to include Cripps Pink, Jonagold, Cameo, Rome, Ambrosia, and Pacific Rose during the 2008 growing season.

- Establish suspension cultures for Red Delicious, Granny Smith, Braeburn, and Fuji by February 2008 and Cripps Pink, Jonagold, Cameo, Rome, Ambrosia, and Pacific Rose by December 2008. Maintain and increase stocks of all apple suspension cultures.

- Perform random mutation experiments and CSI associated with desirable apple traits by the end of 2008.

Deviations from Original Schedule:

Due to budget limitations some key pieces of equipment could not be afforded including the particle gun that is necessary for CSI. However, Washington State University provided $200,000 for extra equipment. The equipment is now in the lab to perform the CSI experiments.

Significant Findings:

- Tissue from Fuji, Granny Smith, Golden Delicious, Red Delicious, Braeburn, Royal Gala, and HoneyCrisp were harvested from the Tukey Orchard in Pullman, WA.

- Callus successfully grown from all collected varieties on Schenk & Hildebrandt (SH) media.

- Majority of callus cultures grew on tissue harvested closest to the core of the apple. Please see results and discussion section for detailed information.

- Suspension cultures successfully established for HoneyCrisp, Golden Delicious, and Royal Gala.

Methods:

After establishing suspension cultures in year one for HoneyCrisp, Golden Delicious, and Royal Gala, we are ready to begin Controlled Sport Induction. We are currently in the process of increasing our stocks of the aforementioned suspension cultures and attempting to derive suspension cultures of Red Delicious, Granny Smith, Braeburn, and Fuji. The method of CSI is a modified version of TILLING, a process that has been used in plants such as wheat, rice, Arabidopsis, and sugarbeet.

Samples of our suspension cultures will also be given to the WSU Nuclear Radiation Center. The samples will be bombarded with gamma irradiation to force random mutations in the plants. Mutated cells will be grown into plants and observations will be made upon the induction of desirable changes of the fruit and plant itself. The desired mutations will be screened using the LICOR equipment acquired via a Genomics Matching Funds Grant from LiCOR Inc.

Results and Discussion:

Controlled Sport Induction is becoming a realistic goal for improvement of apple traits. The major hurdle of optimizing procedures for callus and suspension culture production has been completed.

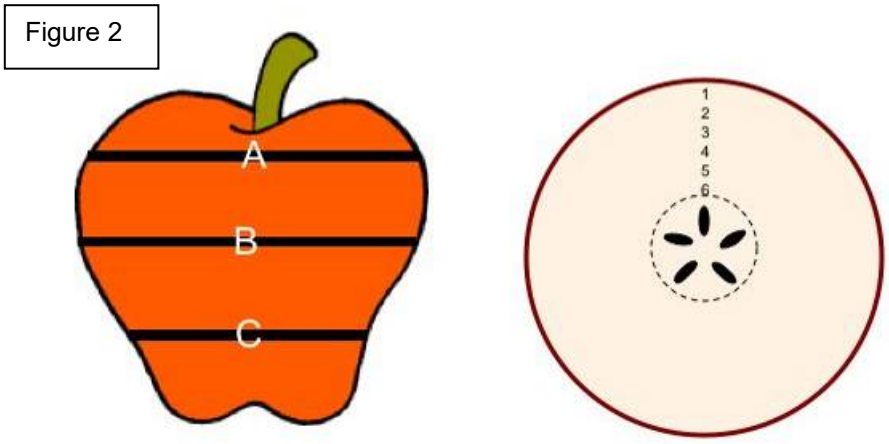
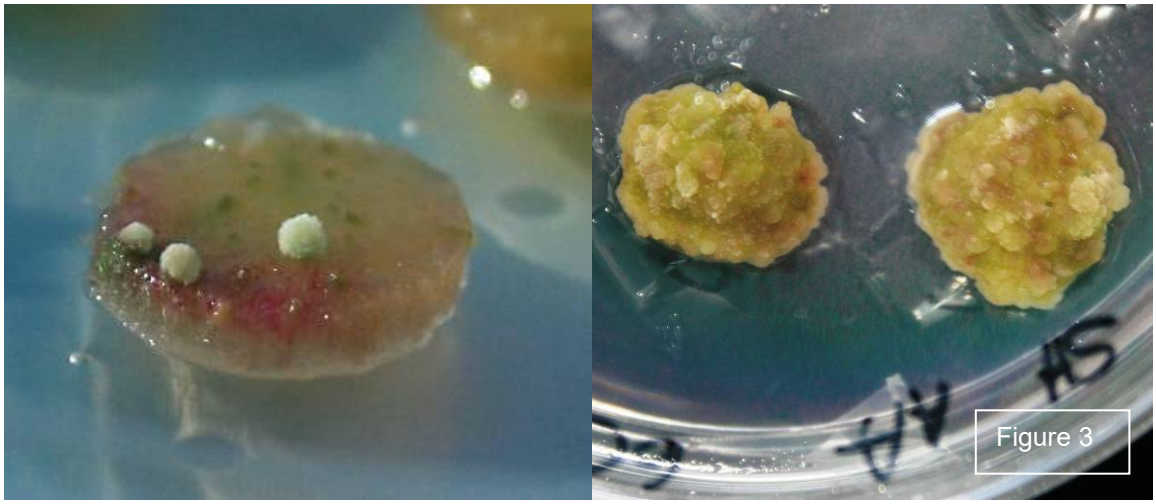


Figure 2 displays the methods used to determine which parts of the apple produce the most viable callus cells. Cores were taken from three parts of the apple; the top (A), middle (B), and bottom (C). The cores themselves were split into multiple sections and discs were cut from each section. Callus was able to grow from sections A, B, and C with no section showing any significant increased callus growth. Tissue nearest to the core (6 and 5), however, generally displayed the highest aptitude for callus growth.



Optimal growth of callus was determined to occur by changing the SH media every three weeks. **Figure 3** displays early callus growth (left) on apple tissue discs after two weeks of growth and a later stage of callus growth (right) after two months of growth. After sufficient callus grew, callus tissue was transferred into liquid media (Figure 4). Cells were shaken to produce individual callus cells. Figure 4 (right) displays cellular growth after 40 days of inoculation.

Single callus cells are required to perform CSI. Now that suspension cultures have been established for some varieties of apple CSI can be utilized to produce apples with more desirable traits. Such traits including longer storage ability, disease resistance, and resistance to browning are not far off in the future. These non-transgenic improvements tailored to Pacific Northwest apples will increase consumer attraction and ultimately contribute to the local economy.

Once this technology and procedures are established improved clonal cultivars can be tested commercially and used directly as this approach involves no transgenic modification. During mutagenesis (sport induction) other mutations, some deleterious, may also be generated but those can be eliminated in the segregating population. The clonal variants will also serve as defined donors or parents of desirable traits for Marker Assisted Breeding. Materials developed using this technology may offer opportunities for new intellectual property in the form of novel clonal variants. The data generated from the activities mentioned above will be leveraged to attract long-term federal funding for continued apple improvement.

Outreach:

1. The work and the ideas underlying this project were featured in the invited presentation at the USApple annual convention in August 2007 to communicate the concepts to the stake holders.
2. The preliminary concepts were presented at the WSHA meeting in 2007 by Scott Schaeffer, current lab manager and future graduate student in the Dhingra lab.
3. This work will be presented at the Annual Rosaceae Genomics Conference in Chile in March 2008 and American Society of Plant Biologist annual meeting in July 2008.

CONTINUING PROJECT REPORT **YEAR**:2 of 3
WTFRC Project Number: AP-06-602

Project Title: Auxin and ethylene dynamics in the abscission zone

PI: Steve van Nocker
Organization: Michigan State University
Telephone/email: 517-355-5191 / vannocke@msu.edu
Address: 390 Plant and Soil Science Building
City: East Lansing
State/Province/Zip MI 48824

Cooperators: M John Bukovac, MSU Horticulture

Total project funding request:	**Year 1:**	**Year 2:**	**Year 3:**
	18,845	18,425	18,966

Other funding Sources

Agency Name: Michigan Agricultural Experiment Station
Amount requested or awarded: Matching (Year 1 only), undefined support for Years 2 and 3.
Notes: This project is included in the PI's MAES 5-year Project. MAES pays partial faculty salary for the PI.

WTFRC Collaborative expenses:

Item	**2008**		
Stemilt RCA room rental			
Crew labor			
Shipping			
Supplies			
Travel			
Miscellaneous	$500		
Total	**500**		

Footnotes: Requesting ~10 h of consulting time with staff (Tory Schmidt) on field data for year 3 ('07-'08).

Budget 1:
Organization Name: Michigan State Univ **Contract Administrator:** Lorri Busick
Telephone: 517-355-5191 x363 **Email address:** busick@msu.edu

Item	**Year 1:** 2007	**Year 2:** 2008	**Year 3:** 2009
Salaries	8,676[1]	8,936	9,204
Benefits	819[2]	693	956
Wages	4,200	3,846	4,456
Supplies	1,800	1,600	4,200[3]
Travel	150	150	150
Miscellaneous	3,200[4]	3,200	
Total	**18,845**	**18,425**	**18,966**

Footnotes: [1]We have obtained matching funds from the Michigan State Agriculture Experiment Station. [2]Will support $^1/_2$ effort by a graduate student (stipend). [3]Costs include production and screening of microarrays. [4]Costs for DNA sequencing in Year 1 and Year 2.

OBJECTIVES

Manipulation of auxin and/or ethylene signaling in apples is a commonly used strategy for regulating crop load and controlling preharvest drop. However, there is practically no information on how these phytohormones participate in the natural process of flower/fruit abscission! The goal of this project is to map auxin and ethylene signaling pathways relevant to flower and fruit abscission through a methodical characterization of auxin and ethylene signaling components in the flower and fruit abscission layers, and analysis of the effects of cultural practices (including application of bloom and postbloom thinners) and environment on the interactions between these components.

To date, we have identified apple counterparts of known components of auxin and ethylene signaling (enzymes involved in biosynthesis, degradation, receptors, transporters, signaling intermediates, and regulatory proteins). We have engineered new tools to study the activity of all of these genes in the flower/fruit abscission layers during natural progression of abscission (pollination/fruit set, fruitlet abscission, wound- or pathogen-induced abscission, and ripening-associated abscission), and in response to exogenous manipulations (bloom thinners, reduction in photosynthate flow to the fruit, postbloom thinning compounds, and biochemical inhibitors of auxin transport and ethylene biosynthesis and perception). We will also compare the activity of these genes between varieties that are difficult to thin (e.g., Fuji) and those that are prone to overthinning (Delicious) in order to understand the biochemical basis for this difference.

The eventual goal of this program is to map auxin/ethylene signaling pathways during abscission to design more effective, easier, cheaper and safer strategies to control fruit load.

Objectives for Year 2 (current year): Complete construction of microarray (done), test efficacy of microarray to detect changes in gene expression in samples collected in Spring '07 and Fall '07 (done), replication of Year 1 sample collections during bloom thinning trials in the Wenatchee area (done), field work and tissue sampling at MSU (done, with some incomplete experiments; see below).

Objectives for Year 3 (coming year): Analysis of gene expression data generated in Fall '07, replication of Year 2 field studies at MSU*, identification of key signaling components, writeup and dissemination of results.

*Year 2 field studies to be replicated (see below for methods):

fruit abscission promoted by wounding
fruit drop in response to reduced photosynthate
natural progression of mature fruit abscission and its influence by phytohormones

Studies proposed for Year 2 now proposed for Year 3 (see below for methods):

pollination/fruit set
postbloom thinning response to NAA, BA, and ethylene
fruitlet competition within a cluster

Schedule of activities and anticipated accomplishments:

Dec '07/Jan '08 Data analysis of Year 1 and 2 experiments.
Apr-May '08 Field work and tissue sampling:
- pollination/fruit set
- postbloom thinning (NAA, BA, ethylene)
- fruitlet competition within a cluster
- Jun '08 Field work and tissue sampling:
  - fruit abscission promoted by wounding
  - fruit drop in response to reduced photosynthate

Sep/Oct '08 Field work and tissue sampling:
phytohormone effects on mature fruit abscission
May-Nov '08 Analyses of expression of key signaling genes identified through microarray analysis.
Nov-Dec '08 Prepare results for publication.

SIGNIFICANT FINDINGS (YEAR 2)

Microarray analysis and verification.

- We completed verification testing of a microarray containing all DNA sequences of the auxin or ethylene signaling genes that we identified. This microarray is now commercially available (CombiMatrix Corp.) and can be ordered by researchers. This will be useful in studies of flowering, fruit ripening, color and aroma production, and other developmental processes important for production and storage.

Field work design and manipulations.

- We sampled flower and abscission zone tissues from bloom thinning trials on Gala in the Wenatchee area. This was in collaboration with the more extensive thinning trials done by WTFRC staff. Treatments included:
    - ReTain (200 ppm)
    - MCP (Nate Reed, AgroFresh)
    - Ethrel (3 pts/acre)
    - CFO/Lime sulfur
    - Retain pretreatment/CFO/Lime sulfur
    - MCP pretreatment/CFO/Lime sulfur
- To dissect the molecular mechanisms of flower abscission promoted by chemical thinners, we dissected abscission zone tissues from flowers from trees treated with lime sulfur. To help evaluate the potential role of ethylene in promoting thinning in response to lime-sulfur, we also analyzed tissues from trees treated with lime sulfur that had also been pretreated with AVG, an inhibitor of ethylene biosynthesis, or MCP, a strong repressor of ethylene sensitivity. When evaluated 2d following application, flowers treated with lime-sulfur were found to generate markedly more ethylene (0.52 ppm) than the control (0.15 ppm) or plants treated with lime sulfur pretreated with AVG (0.31 ppm)(not shown). We predict that the AVG treatment abrogated the effect of lime-sulfur on fruit load, but have not seen the WTFRC data yet.
- We collected abscission zone tissues from trees during the course of natural fruit drop. We were also able to accelerate drop using ethrel and delay drop using NAA or ReTain, and we collected tissues from these trees as well for the upcoming analyses.

Gene expression analyses.

- We identified all potential auxin/ethylene signaling genes that are active in quiescent or activated abscission layers of the flower and mature fruit.
- We identified changes in gene expression in the abscission zone of immature fruit in response to fruit removal, a treatment that reproducibly induces abscission of the remaining pedicel in 4-5 days.
- We identified a regulon - set of similarly regulated genes - that are activated at the earliest stages of abscission zone activation. Intriguingly, this set is enriched in genes encoding carbohydrate-modifying enzymes such as pectate lyase.
- We observed activation of an ACO (ACC oxidase) gene midway through abscission, suggesting ethylene production participates in, but is not an initial promoter, of abscission.

METHODS

Field work was done using Gala at Manson, WA and at the MSU Research Center in E. Lansing, MI. Bloom thinning treatments were carried out at ~50% bloom. Samples were taken prior to full bloom treatments, and ~2h, 1d, 3d, and 8d following full bloom treatments. Abscission zone tissues were dissected from open terminal (king) flowers prior to anthesis by gently bending the pedicel until it separated from the stem at the pedicel/stem junction (incipient abscission zone) and shaving a ~1-mm thick radial section from the basal end of the pedicel using a razor blade. Pedicel tissues were collected by shaving ~1 mm from the new basal end of the pedicel. For the initial, 2h and 1d time points, whole flowers not including the pedicel were also collected, or petals and gynoecium were dissected and collected separately. Tissues were immediately frozen in liquid nitrogen or in a dry ice/ethanol bath and stored at -80 C until analysis.

For study of natural abscission at MSU, samples were taken at 2-d intervals, commencing before the first sound fruit dropped. Ethrel or NAA (Fruitone N) was applied when the first sound fruit began to drop in response to branch shaking, and samples were taken every two days until peak drop. ReTain treatments commenced 3 weeks prior to anticipated peak drop, and sampling dates corresponded to natural abscission (no treatment).

This coming year, we will replicate two studies carried out at MSU in '07 to evaluate gene activity patterns through several distinct circumstances of abscission:

1) Fruit abscission promoted by wounding. Fruits will be subjected to artificial wounding following the period of early fruitlet drop. Non-wounded fruit will be used as a comparison. Samples will be taken at 1d, 2d, and at one additional time point before abscission.

2) Fruit abscission promoted by reduced photosynthate. Cool, cloudy conditions early in the season promote excessive fruitlet drop associated with reduced photosynthate availability. We will attempt to identify the biochemical pathway leading to abscission layer activation under these conditions. We will attempt to induce abscission using two approaches:

i. shading to 50% canopy with shade cloth

ii. treatment with a photosynthesis inhibitor, Terbacil

We will take samples 1d, 2d and at one additional time point before enhanced drop of fruitlets is observed. Non-treated trees will be used as controls.

We will also carry out three studies originally proposed for MSU in '07. However, it was not possible to complete these studies because of field work in Wenatchee (bloom periods at Wenatchee and MSU overlapped substantially in '07).

1) Flower abscission or retention associated with pollination/fruit set. We will analyze the progression to abscission in a set of flowers that are prevented from being pollinated. A matched set of hand-pollinated flowers will be used as a control, utilizing the king flower (most likely to be retained) within each cluster. Three samples will be collected during the time between anthesis and flower abscission.

2) Fruitlet abscission associated with competition within a cluster. We will analyze fruitlets most likely to abscise within a cluster (lateral fruitlets) in comparison with those most likely to be retained (the king fruitlet). Samples will be taken throughout the period of early fruitlet drop.

3) Postbloom thinning by PGRs. Here we will analyze the activity of four postbloom thinners:

i. ethephon (Ethrel)

ii. carbaryl (Sevin)

iii. benzyladenine (MaxCel)

iv. NAA (Fruitone N)

For all studies, we intend to perform many of these manipulations with cultivars that exhibit the extremes of thinning/drop responses seen in the field. For example, for postbloom thinning assays we

will also evaluate both Red Delicious (sensitive) and Fuji (relatively insensitive). For evaluations of preharvest drop we will also evaluate Red Delicious (prone).

RESULTS AND DISCUSSION

To recap, we have developed a model for fruit abscission involving the interaction between two endogenous PGRs, auxin and ethylene. Specifically, at a very early stage in abscission we have found changes in the activity of a number of genes that participate in mediating auxin signal transduction, and this preceded observed changes in the activity of several genes that function in ethylene signaling. Taken together with a variety of studies of the effects of bloom and postbloom thinners, and with very recent findings in basic plant biology, our results allow us to propose a model for the initiation of flower and fruit abscission. In this model, loss of directional auxin transport through the abscission layers triggers enhanced ethylene signaling in abscission layer cells, culminating with activation of genes that promote cell separation.

We found that among the first genes to be activated in the abscission zone upon fruit removal are a subset of genes associated with carbohydrate modification. The presumed role of these genes in abscission is to degrade the cell wall, allowing for cell separation. This was initially confusing, because in the system used for this study, separation of abscission layer cells takes place much later (3-4 d after activation), and previous studies demonstrated that a large variety of cell-wall-modifying genes became active only late in abscission. However, a possible scenario is that the early-induced carbohydrate-modifying genes participate in generating a signaling molecule that acts as an initiator of abscission. Hypothetically, degradation of the cell wall contributes to an extracellular pool of small oligosaccharides, some of which are well-known signaling intermediates in other pathways such as defense response. Analogous to the defense pathway(s), this could result in initiation of ethylene production and coordinated advance of the abscission process. Though highly speculative, this idea is supported by two recent findings: 1) Our observation that an ACO gene is induced at a later stage of abscission, consistent with some of our previous observations and suggesting activation of ethylene signaling, and 2) Recent findings from Michael McManus' lab (Ann Bot, Oct 2007) showing that abscission depends on a mobile signal, generated in the stele of the pedicel, that works in concert with ethylene. In fact, vasculature is considered to be a main route of polar auxin transport; disruption of auxin flow resulting from a variety of cultural manipulations or environmental trauma could somehow act as a trigger to generate this signal within cells of the vasculature. A small oligosaccharide produced in cells of the vasculature would be expected to diffuse to surrounding cortical cells, activating the abscission process, and generating more of the oligosaccharide signal, positively reinforcing the abscission process. Although this idea needs significant development, it suggests that small oligosaccharides could somehow be manipulated for thinning and control of preharvest drop.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-07-706A

Project Title: Functional genomics of flowering in apple

PI:	Herb Aldwinckle	**Co-PI(2):**	Steve VanNocker
Organization:	Cornell University	**Organization:**	Michigan State University
Telephone/email:	315-787-2369 HSA1@cornell.edu	**Telephone/email:**	517-355-5191 x394 vannocke@msu.edu
Address:	630 W. North Street	**Address:**	A390C Plant & Soil Sciences
City:	Geneva	**City:**	East Lansing
State/Province/Zip	NY 14456	**State/Province/Zip:**	MI 48824

Cooperators: M John Bukovac, Michigan State University

Total project funding request: **Year 1:** $53,773 **Year 2**: $42,546

Other funding Sources

Agency Name:
Amount requested or awarded:
Notes:

Budget 1:
Organization Name: Cornell University **Contract Administrator:** Brenda Truesdail
Telephone: 607-255-7124 **Email address:** bmt2@cornell.edu

Item	2007	2008	(type additional year if relevant)
Salaries	14,500	15,370	
Benefits	7,434	8,034	
Wages			
Benefits			
Equipment			
Supplies	3,000	1,000	
Travel			
Miscellaneous			
Total	24,934	**24,404**	

Footnotes:
The salary and benefits requested are for a technician to work 50% time on transferring silencing constructs into apple. During the first year transfers will be made of constructs for already identified genes to develop the technology for use with flowering genes. In the third year, as candidate genes are identified, they will be added to silencing gene transfer pipeline. Supplies are for tissue culture, chemicals, enzymes, plastic ware, and potting supplies.

Budget 2: (*Complete only if funding is split between organizations***)**
Organization Name: Michigan State University **Contract Administrator:** Lorri Busick
Telephone: 517-355-5191 x363 **Email address:** busick@msu.edu

Item	2007	2008	
Salaries	12,854	13,240	
Benefits	885	912	
Wages			
Benefits			
Equipment			
Supplies	5,000	3,390	
Travel	600	600	
Miscellaneous	9,500		
Total	28,839	**18,142**	

Footnotes:
The salary and benefits requested are for 1/2 grad student for first two years. Note that for the grad student, this amount includes tuition/fees ($4215) and stipend ($8640).
Supplies are for molecular studies, and include enzymes, primers, and reagents.
Expenses for travel are for one trip/year in years 1 and 2 from Michigan to New York.
Miscellaneous costs are for DNA sequencing of 454 EST's (partial gene sequences) related to flowering.

PROGRESS REPORT, YEAR 1

Overall Objectives:

1. Genomic census of *FT/TFL* gene family members in apple. We will identify all possible *FT/TFL* gene family members in apple, and determine their DNA sequence. As mentioned above, from previous studies we know that at least five exist in apple. This step is important, because additional genes may exist with even more important functions.

2. Gene expression atlas of the apple *FT/TFL* genes. We will extensively analyze the activity pattern of all *FT/TFL* genes identified through Objective 1, concentrating on flowering. This analysis will include expression in various parts and organs of the plant, changes in expression in response to phytohormones, effects of biotic and abiotic stresses on expression, and temporal control of expression during development (e.g. flower induction). The goal of this approach is to identify those members of the *FT/TFL* family that have the most important role in flowering.

3. Functional analysis. The best way to unambiguously determine the function of a gene is to examine the phenotypic consequences of loss of that gene's activity. In other words, how does flowering occur without that gene? In apple, genes can be repressed through a technique called RNA interference (RNAi), which PI Aldwinckle has several years' experience with other apple genes. We will examine the effect on flowering of suppression of the most important genes identified in Objective 1 and 2.

Revised proposed schedule of accomplishments (for 2 yr project instead of 3 yr):

Rapid progress in the early objectives has enabled us to start some of the 2nd year objectives in the first year, and we will attempt to cover most of the original objectives by Year 2.

Year 1: Identification of *FT/TFL*-related genes. Refinement of silencing methods for flowering genes. Preliminary gene activity analyses and dentification of key flowering genes. Start silencing with selected candidate genes.

Year 2: Continue gene activity analyses and identification of key flowering genes. Silencing with all identified flowering genes. Applications for federal funding to expand studies.

Summary of Significant findings in Year 1

- **In addition to the previously described *MdTFL1-1/-2* and *MdFT*, we found one clear homolog each of the Arabidopsis *MFT* and *BFT* genes. These genes have not been characterized in Arabidopsis, and we are excited about the possibility that the related genes may have flowering-related roles in apple.**

- **One additional *FT*-related gene, and one additional *MFT*-related gene were identified. In addition, preliminary results suggest that there exists a gene in apple related to the uncharacterized, Arabidopsis *ATC* gene. Thus, in apple, we discovered five new *FT/TFL*-related genes.**

- **Only *TFL1-1* and *TFL1-2* were expressed to detectable levels in floral buds. Interestingly, *TFL1-1* showed an expression pattern corresponding with flowering, being expressed weakly at 2-5 weeks, and strongly at 10 and 12 weeks. In**

contrast,*TFL1-2* was expressed at a similar and very low level at all time points observed. This is consistent with our initial hypothesis that a *TFL1* gene plays an evolutionarily conserved role to govern inflorescence architecture (ie, whether an inflorescence develops determinately or indeterminately) in apple.

- **While *TFL1-1* appeared to be expressed specifically in the inflorescence, *TFL1-2* was expressed to highest levels in seedling and leaf tissues. Based on these results, we now hypothesize that *TFL1-1* and *TFL1-2* have become functionally specialized in apple, with *TFL1-1* devoted to regulating inflorescence architecture, and *TFL1-2* devoted to maintenance of juvenility. This suggests that juvenility and inflorescence architecture can be regulated independently in apple – a tremendous opportunity for improving production.**

- **Artificial miRNA (amiRNA) technology was used to design amiRNAs targeting *MdTFL1-1*, *MdTFL1-2*, or both *MdTFL1-1* and *1-2*, while minimizing the risk of suppressing off-targets. This means that these genes can be silenced individually and their distict roles dissected.**

- **Initial transformation experiments with the amiRNA silencing constructs for TFL1-2 were done in Aldwinckle's lab, and transformed plants are now being selected.**

Procedures and Results

1. Genomic census of *FT/TFL* gene family members in apple. To date, three members of the *FT/TFL* gene family have been identified by various research groups: *MdTFL1-1 and MdTFL1-2*, homologs of Arabidopsis *TFL1*, and *MdFT*, a homolog of Arabidopsis *FT* (Kotoda and Wada, 2005; Esumi et al, 2005). However, based on our preliminary analyses of public sequence databanks for apple, it was apparent that there exist at least two additional related genes in apple. Our goal was to use various techniques to identify all possible *FT/TFL* gene family members in apple, and determine their DNA sequence as an initial step in their characterization. We took three approaches to carry this out:

a. Bioinformatics analyses. Our analysis of the ~300,000 currently catalogued apple expressed sequence tags (EST), sequenced cDNAs, and genomic clones (http://genomics.msu.edu/fruitdb/analyses/apple.html; Park *et al*., 2006) identified several sequences phylogenetically included in the *FT*/*TFL1* family. In addition to the previously described *MdTFL1-1/-2* and *MdFT*, we found one clear homolog each of the Arabidopsis *MFT* and *BFT* genes These genes have not been characterized in Arabidopsis, and we are excited about the possibility that the related genes may have flowering-related roles in apple (see below).

b. Gene identification through analysis of chromatin-marked transcribed regions (CMTRs). A chromatin enrichment strategy under development in our lab is being utilized as an advancement to EST sequencing to identify chromosomal regions that are transcriptionally active during the flowering process. In this technique, partial genomic DNA sequence for thousands of genes that are active during flower initiation can be easily generated, and used to quickly identify *FT/TFL*-related genes. This technique eventually will generate ~30 million base-pairs of apple DNA sequence corresponding to much of the euchromatic gene space of apple. We are now optimizing this approach for apple, and intend to complete this step before the end of the first year.

c. *Direct gene identification using molecular techniques*. We cloned known *FT/TFL1*-family member genes (above) from apple, and used these DNAs as molecular probes in genomic DNA gel

blotting to identify any additional *FT/TFL* gene family members in apple. We cloned all *FT/TFL1* family genes from Arabidopsis and utilized these DNAs as probes as well. This approach identified one additional *FT*-related gene, and one additional *MFT*-related gene. In addition, preliminary results suggest that there exists a gene in apple related to the uncharacterized, Arabidopsis *ATC* gene. Thus, in apple, we discovered five new *FT/TFL*-related genes. We are currently completing sequencing of these genes.

2. Gene expression atlas of the apple *FT/TFL* genes.

The second objective is to establish the spatial and temporal pattern of regulation of apple FT/TFL-related genes during flowering, focusing on *TFL1-1* and *TFL1-2*. As a first step, we carried out transcriptional profiling of developing shoot/inflorescence apices of apple. To provide tissue for RNA extraction and document developmental landmarks to accompany these transcriptional profiles, a morphological study of Gala vegetative and floral buds was conducted. To target either vegetative or floral buds for comparison, we exploited the fact that bud development can be influenced by growth regulators and is also dependent on shoot architecture (Foster *et al*., 2003; M. J. Bukovac *pers comm*.). Treatment with GAs was expected to maintain the buds in their vegetative state, whereas the growth regulator B-Nine (2, 2, dimethyl hydrazide, a GA inhibitor) promotes floral bud formation. Apical buds on short lateral spurs on well exposed branches, which have a high probability of developing into inflorescences, and apical buds on elongated spurs, which are more likely to remain vegetative (M. Bukovac *pers comm*.) were targeted for collection. To study the apices that were expected to develop vegetatively, we dissected apical sections from elongated spurs from trees treated with GA4+7. To collect floral apical tissues, we collected buds from short spurs treated with B-Nine. 'Gala' trees growing at the Clarksville Horticulture Experiment Station located at Clarkesville, Michigan were used. For each collection, branches were removed and apices were dissected with the aid of a microscope. For each bud type (vegetative or inflorescence), 20 apices were observed under the scanning electron microscope to record their stage of development and determine the percentage of floral and vegetative buds, and 40 apices of each type were stored at -80^{0}C for molecular analysis. Buds were collected at 2, 4, 5, 6, 8, 10, and 12 weeks after full bloom and subjected to transcriptional profiling.

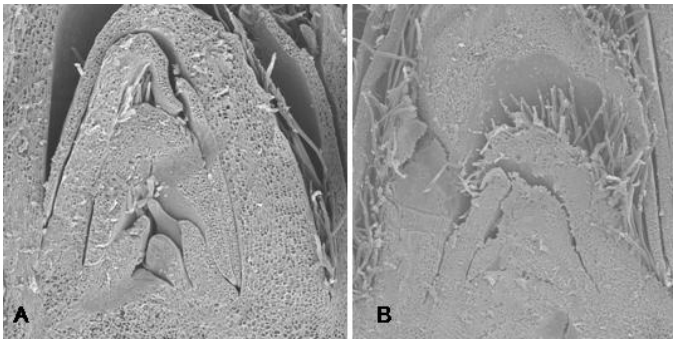


Fig. . Scanning micrograph of a apical bud expected to be floral, (A) and a bud expected to be vegetative,at 12 weeks after full bloom. (Dissected apices were fixed in 4% glutaraldehye in 0.1 M phosphate buffer, dehydrated using an ethanol series, and critical point dried using liquid CO_2 and the Blazer's Critical Point Drier. The fixed tissue was mounted on stubs and coated with 20 nm gold particles and osmium tetroxide. The samples were observed under a JSM 6400 Scanning Microscope to

We utilized PCR to evaluate expression of all known *FT/TFL*-related genes during flower initiation and inflorescence development. In summary, we found that only *TFL1-1* and *TFL1-2* were expressed to detectable levels in floral buds. Interestingly, *TFL1-1* showed an expression pattern corresponding with flowering, being expressed weakly at 2-5 weeks, and strongly at 10 and 12 weeks. In contrast, *TFL1-2* was expressed at a similar and very low level at all time points observed (not shown). This is consistent with our initial hypothesis that a *TFL1* gene plays an evolutionarily conserved role to

govern inflorescence architecture (ie, whether an inflorescence develops determinately or indeterminately) in apple.

To characterize these genes further, we analyzed their expression in various organs and parts of apple, including seed, hypocotyls, cotyledons, shoot and leaves. The preliminary results of these experiments are exciting. While *TFL1-1* appeared to be expressed specifically in the inflorescence, *TFL1-2* was expressed to highest levels in seedling and leaf tissues. A role for *TFL1-1* in repressing juvenility was previously suggested by Kotoda et al. (2006) based on the precocious induction of flowering resulting from its antisense expression. We suggest that those effects resulted from an off-target effect on the *TFL1-2* gene. Based on results from the first year of this project, we now hypothesize that *TFL1-1* and *TFL1-2* have become functionally specialized in apple, with *TFL1-1* devoted to regulating inflorescence architecture, and *TFL1-2* devoted to maintenance of juvenility. This idea is enticing, because it suggests that juvenility and inflorescence architecture can be regulated independently in apple – a tremendous opportunity for improving production.

An incidental but exciting result relates to our analysis of expression of one of the apple *MFT*-related genes. We found that this gene was strongly active in immature fruit and seed tissue (not shown). This is exciting because it has recently been shown that the related Arabidopsis gene, *FT*, acts cell-non-autonomously in flowering induction, and in fact may comprise the elusive flowering hormone termed *florigen*. There is a wealth of data showing that developing seeds act to inhibit flowering in apple, and we are assessing the possibility that *MFT* might participate in this flowering repressive pathway as a mobile, flowering inhibitor.

3. Functional analysis.

This objective is to evaluate flowering-related role(s) of the most important genes identified in Objective 1 and 2, by suppressing their expression in transgenic apple and examining the effects on flowering. To assess the relative contribution of *MdTFL1-1* or *MdTFL1-2* activity to juvenility and/or inflorescence architecture, we are generating and analysing the phenotype of plants in which either gene has been selectively downregulated in transgenic apple (cv. Gala) through artificial miRNA (amiRNA) technology (Schwab *et al*., 2006). This approach exploits previously determined parameters of target site selectivity for natural plant miRNAs to design miRNA sequence specific for a single gene or a group of related genes. The miRNA sequence is cloned into a natural pre-miRNA and expressed constitutively or inducibly in transgenic plants. We collaborated with investigators at the Max Planck Institute/Tubingen to incorporate apple expressed gene sequence data compiled by our group into WMD2, a web-based tool for the automated design of amiRNAs maintained by Detlef Weigel's lab (http://wmd2.weigelworld.org/cgi-bin/mirnatools.pl). This allowed us to design amiRNAs targeting *MdTFL1-1*, *MdTFL1-2*, or both *MdTFL1-1* and *1-2*, while minimizing the risk of suppressing off-targets. Oligonucleotides will be incorporated into the miR319a backbone and expressed under control of the CaMV 35S promoter.

Initial transformation experiments with the amiRNA silencing constructs for TFL1-2 were done in Aldwinckle's lab, and transformed plants are now being selected.

Literature Cited:

Esumi, T., Tao, R., Yonemori, K. (2005) Isolation of *LEAFY* and *TERMINAL FLOWER 1* homologs from six fruit tree species in the subfamily Maloideae of the Rosaceae. Sexual Plant Reproduction 17(6): 277-287

Foster, T., Johnston, R., Seleznyova, A. (2003) A morphological and quantitative characterization of early floral development in apple (*Malus* x *domestica* Borkh.) Annals of Botany 92: 199-206

Kotoda, N., Wada, M. (2005) *MdTFL1*-like gene of apple, retards the transition from the vegetative to reproductive phase in transgenic Arabidopsis. Plant Science 168: 95-104

Park, S., Sugimoto, N., Larson, M.D., Beaudry, R., van Nocker, S. (2006) Identification of genes with potential roles in apple fruit development and biochemistry through large-scale statistical analysis of Expresssed Sequence Tags. Plant Physiology 141: 811-824

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Discussion of Results

The progress so far in identifying five new genes potentially critical to regulation of flowering in apple is very promising. Also the use of the new artificial microRNA technique for silencing genes very specifically should enable us to determine exactly which genes are doing what in controlling flowering time and flower structure. The rapid progress has allowed us to start functional analysis in the first year, rather then the second as we first planned.

Thus we should be able to understand flowering control well enough to be able to design strategies to address many practical problems related to flowering, including overbearing,, biennial bearing, asynchronous flowering, and season of flowering. This will eventually result in great benefits for apple production in terms of quality and packout.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-07-702

Project Title: Establishing trait – gene relationships and gene discovery in apples

PI:	Amit Dhingra	**Co-PI(2):**	Bruce Barritt
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Address:	PO Box 646414	**Address:**	1100 N Western Ave,
City:	Pullman	**City:**	Wenatchee
State/Province/Zip	WA 99164	**State/Province/Zip:**	WA 98801

Cooperators: Fred Bliss and Cameron Peace

Total project funding request: **Year 1:** 21,690 **Year 2:** 21,690 **Year 3:**

Budget 1:
Organization Name: WSU **Contract Administrator:** ML. Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	2007	2008	2009
Salaries			
Benefits			
Wages	6,000	**6,000**	
Benefits	690	**690**	
Equipment			
Supplies	11,000	**11,000**	
Travel	2,000	**2,000**	
Sequencing	2,000	**2,000**	
Miscellaneous			
Total	21,690	**21,690**	

Footnotes:

Empirical knowledge of desirable trait-gene relationships is vital for effective marker assisted selection in the breeding program and as a target for rapid crop improvement via controlled sport induction. This is of renewed importance as WSU searches for the next apple breeder who will have a larger application of genomics related research in the program. Specific objectives as outlined in the proposal were:

Objectives:

1. *Prioritization of a subset of apple traits of greatest economic and immediate importance*.

   Progress: Three traits critical to fruit quality, crispiness, juiciness and firmness were selected by the "think-tank" that comprises of industry group, Bruce Barritt (current apple breeder) genomics researchers and Fred Bliss (Consultant to WTFRC). All subsequent objectives and activities are based on improving these traits.

2. *Identification and grouping of contrasting genotypes to be used for the study based on available knowledge in the breeding program*.

   Progress: In order to perform gene discovery experiments for the traits listed in objective 1 samples have been collected from the following genotypes: HoneyCrisp (crisp not firm), Fuji, Pink Lady (firm, not crisp, not juicy but dry), Red Delicious and Golden Delicious (mealy, no texture, no firmness, disintegration of character, soft). The rationale behind selecting these genotypes is that this group represents full range of extremes in fruit characters and presents the phenotypic differences needed for our investigations. Two sets of tissues have been harvested for each genotype. The peel and the cortex represent contrasting sites of action physiologically. Thus we have taken very thin peel and cork bored cortex samples for our experiments.

3. Perform side by side expressed genomic comparisons using a method termed Differential Display (DD).

   Progress: We have initiated performing initial experiments where the isolation and processing of nucleic acids derived from different fruit tissues is being standardized to get consistent and reproducible results from differential display experiments.

Upcoming Goals and Activities:

- Sample collection began post-bloom in 2007 in the Tukey Orchard. Samples were collected every 3-4 week till harvest. We are now collecting samples from fruit in storage. This is being done to charter the course of development and identifying genes that play important role during the physiological progression of the fruit on the tree and during post-harvest storage. In 2008, another set of samples will be collected both at Tukey Orchard and in Wenatchee. Core and peel samples have been harvested from HoneyCrisp, Red Delicious, Golden Delicious, Fuji, so far and the same genotypes will be used during the 2008 growing season.

- We plan to expand on the differential display experiments on the samples collected. It will help us to locate genes that are expressed at different stages of fruit growth and under different environmental conditions.

Significant Findings:

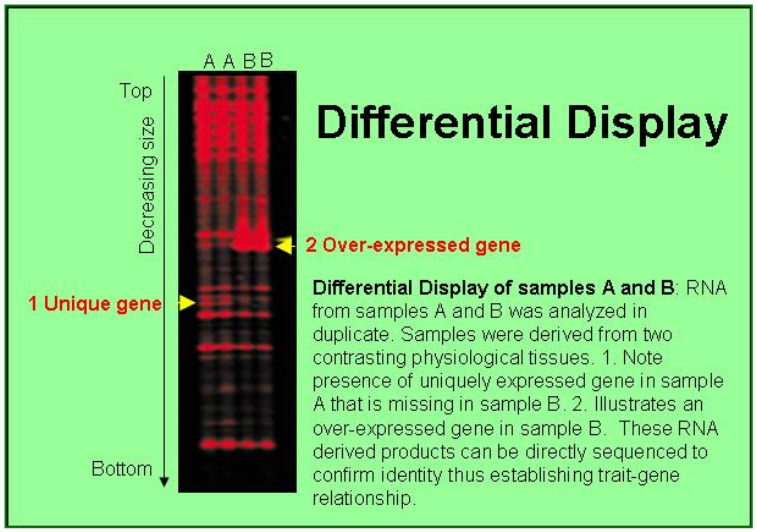
- We have normalized sample collection and are in the process of optimizing downstream experiments. The technique of differential display utilizes RNA (Ribonucleic acid) that is the functional output of the DNA in the genes. RNA by nature is very labile and standard RNA isolation methods are very unsuitable to extract best quality RNA. We found that grinding samples while completely submerged under liquid nitrogen helps in retaining the high quality of RNA that is vital for the success of these experiments. The equipment to perform specialized grinding called Freezer Mill (worth $ 12,000) has been procured from leveraged funds provided by the Agriculture Research Center and the Department.
- As mentioned above the quality of RNA is the most vital part if these experiments were to succeed and provide reliable information. Another piece of equipment to assess RNA quality called the Bioanalyzer (worth $10,500 from WSU funds) has also been procured in the lab. We have nearly optimized RNA isolation and quality parameters.

Thus the limitations to access the RNA have been resolved and we are now at the stage of proceeding with the experiments to identify the genes responsible for differences in important traits like crispiness, juiciness and firmness using the above listed genotypes.

Methods:

Sample Collection: The normalized protocol for collecting peel and core samples will be performed at the Tukey Orchard in Pullman, WA. In addition this season we will be expanding our sample collection to fruits grown in Wenatchee. Samples will be collected every month at both sites. These samples will be transferred in liquid nitrogen for return to the laboratory. By grinding samples in the Spex SamplePrep 6870 freezer mill we will produce high quality RNA ready for analysis.

RNA will be isolated from the ground tissue using a Qiagen RNA extraction kit or other improved protocols. This season's material has been processed with the Qiagen kit. Differential display can then be performed using the isolated and quality tested RNA**.**



Results and Discussion:

At this point we have started the differential display experiments to identify important genes that participate in fruit development of different genotypes. Over the past one year since the initiation of this project the lab has expanded in its technical capacity with sate of the art equipment procured via leveraged funding from WSU and other sources like some companies. In terms of personnel capacity a high-quality graduate student supported by non-WTFRC funds has taken on this project and this project has also been instrumental in attracting one undergraduate student. Over the next few weeks, the first sample of genes will be in our hands to perform downstream assessments. Validation of the data will be performed with samples collected this year.

Overall, the identification of genes responsible for Pacific Northwest apple traits is necessary for the local economy to remain competitive in the world market place. As a juggernaut in the apple industry, we can use this information to improve the quality and attractiveness of our products. Traditional breeding programs and the non-traditional Controlled Sport Induction (Dhingra) method can exploit this knowledge to introduce new traits to existing varieties. New apple varieties could be developed tailored to the Northwest needs by selecting plants expressing the genes necessary for a given trait.

Outreach:

4. The work and the ideas underlying this project were featured in the invited presentation at the USApple annual convention in August 2007 to communicate the concepts to the stake holders.
5. The preliminary concepts were presented at the WSHA meeting in 2007 by Scott Schaeffer, current lab manager and future graduate student in the Dhingra lab.
6. This work will be presented at the Annual Rosaceae Genomics Conference in Chile in March 2008 and American Society of Plant Biologist annual meeting in July 2008.

CONTINUING PROJECT REPORT **PROPOSED DURATION**: 2 years

Project Title: Functional genomics and marker development for apple sensory qualities

PI: Yanmin Zhu
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Collaborator: Cameron Peace,
Organization: Department of Horticulture and Landscape Architecture, WSU
Telephone/email: 509-335-6899 cpeace@wsu.edu

Budget 1:

Organization: USDA, ARS		**Contract Administrator:** Charles Myers, Extramural Agreements Specialist	
Telephone:(510) 559-6019		**Email:**cwmyers@pw.ars.usda.gov	
Item	**Year 1: 2007**	**Year 2: 2008**	
Salaries	$33,000	33,000	
Benefits	10,000	10,000	
Wages			
Benefits			
Equipment			
Supplies	10,000	10,000	
Travel	1,500	1,500	
Miscellaneous	500	500	
Total	55,000	55,000	

The **salaries and benefits** are for hiring a postdoc dedicated to this project.
The **supplies** include common reagent for molecular genetics study and gene profiling analysis.
The budget for **travel** includes the cost for visiting Malus germplasm repository at Geneva, New York, for identify the phenotypic extremes on related fruit quality.

Objectives:

1. Continue to apply the tested ethylene molecular markers for ACS1 and ACO1 in segregation populations in the WSU Apple Breeding Program to select for low ethylene production.

2. Test and apply a reported apple fruit peel red color marker in the existing WSU segregation population for selection of red color development capacity.

3. Identify potential candidate genes regulating apple firmness and crispness.

4. Elucidate relationships between expression of apple AAT (alcohol acyl transferase) genes and cultivar differences in volatile ester production.

Proposed schedule of accomplishment (August 2007-July 2008):

1. Apply markers for ethylene production and peel red color to 5000+ seedlings of the Honeycrisp X Pinklady segregation population. The expected results could be 20% retention based on the criteria of low ethylene production and red skin color.

2. Based on physiological characterization of firmness and crispness, select fruit tissues at specific developmental stages for gene expression profiling by microarray analysis. Analyze microarray data and select 20-50 potential candidate genes for preliminary validation of regulatory activity for fruit firmness and crispness.

3. Characterize the developmental and cultivar differences in the expression level of the four known AAT genes and their relationship to volatile ester production.

Significant findings

1. Both ethylene gene markers have been tested and show good correlation with fruit firmness at harvest and after 60 days cold storage. The markers are being utilized to genotype 5,000 seedlings of diallel crosses between Pink Lady, Honeycrisp and CrimsonCrisp for ACS1 and ACO1 allelotypes:
   Most of the leaf tissues were collected in a 96-well format, high throughput DNA isolation are being carried out.

2. Expression analysis of AAT and ACS gene families for their potential role on apple aromatic volatile ester production. Distinct gene expression patterns were observed for the members in both gene families, differential responses among the gene family members were also observed. The findings suggest roles for AAT1, AAT2, ACS1 and ACS3 that could account for differences in volatile ester emission between Granny Smith and Golden Delicious.

3. Comparative gene expression profiling to identify genes controlling fruit firmness and crispness:
   Samples for molecular analysis for Honeycrisp and Pink Lady fruit were collected during maturation and ripening following physiological characterizations for each sample (firmness, crispness). Distinguishable characteristics for crispness and firmness from both cultivars were identified. The collected samples are being used for RNA isolation for large scale gene expression analysis. Nimblegen array format and microarray experiment design are being implemented. A postdoc dedicated to this part of work and following array data validation has

been identified. Additionally, preliminary fruit quality phenotype for 177 Pink Lady X Honeycrisp trees has been completed, and fruit from these trees will be useful for gene expression validation at later stages.

Methods

1. Physiological characterization of textural attributes and volatile ester production: Systematic characterization of apple fruit firmness, crispness during ripening on selected cultivars will be carried out using the Mohr Digitest instrument, and volatile production using GS-MS. Weekly measurement will be started 6 weeks before projected ripening date for characterization of crispness development, and carry over to postharvest storage for evaluation of firmness retention and volatile production. In addition to fruit firmness and crispness, analyses of respiration, ethylene production, and starch index will be conducted. Such physiological study is critical for selecting tissues at specific developmental stage for gene expression analysis.

2. Comparative gene expression analysis on genes and pathways regulating fruit textural attributes: selected tissues based on physiological study will be used to isolate RNA for comparative gene expression profiling (microarray analysis), during fruit ripening and between cultivars.

3. Candidate gene approach for identifying the genes on volatile production capacity: There are at least four acyl alcohol transferase (AAT) encoding genes in apple genome which are expressed fruit tissue. Based on sequence polymorphism at 3' end of coding region, gene-specific primers has been designed for different gene/allele amplification. The correlation between volatile production and specific gene expression will be analyzed by GC-MS, RT-PCR, real-time PCR. Genome walking will be performed to obtain the regulatory region of the selected gene(s) to look into the sequence signatures in promoter regions for their different expression levels between cultivars.

4. Test and apply available molecular markers: Fruit ethylene production markers and fruit skin color marker will be applied in segregation population to select low ethylene production and red peel individuals.

Result and Discussion

1. Test and apply available molecular markers:

Functional molecular markers for ethylene biosynthesis gene ACS1 (major effect) and ACO1 (minor effect) were tested within the WSU breeding program. For 60 currently existing elite cultivars genotyped, very few belong to the extreme high ethylene genotype (ACS1-1/1) category. Of the cultivars remaining, roughly half belong to the intermediate ethylene production group (ACS1-1/2) and the other half to the low ethylene production group (ACS1-2/2) (Table 1). Similar distribution applied to 35 advanced selections from the WSU breeding program. This genotypic data may prove to be essential to allow the breeder to make informative selections of breeding parent combinations and to predict the percentage of low ethylene progeny. There is a good correlation between ethylene synthesis gene genotypes and fruit firmness (see Figure 1). The independent effect of ACO1 was also demonstrated in this wide selection of breeding parents (data not shown here).

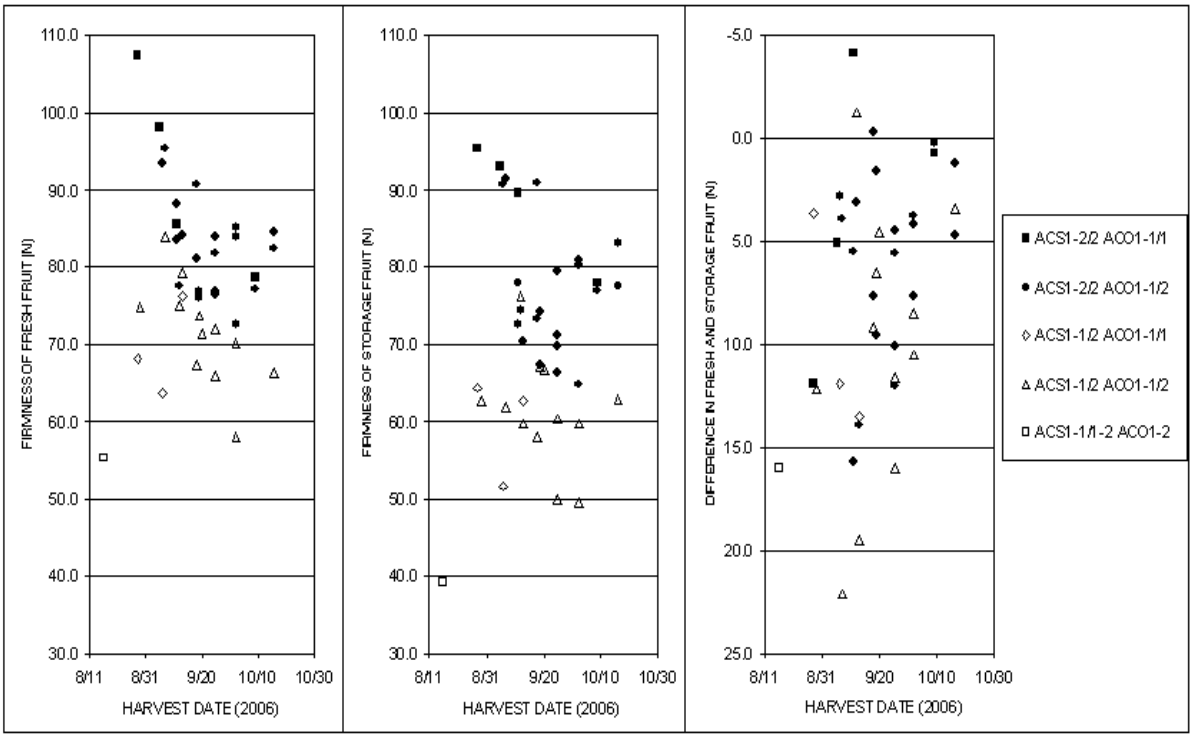
Table 1. Distribution of elite apple breeding parents and advanced selections for their allelotypes on ethylene production genes ACS1 and ACO1.

ACS1-1/1 (high C_2H_4)	**ACS1-1/2 (medium C_2H_4)**	**ACS1-1/1 (low C_2H_4)**	
Fortune	Delorgue Sunrise 3 WSU selections		**AC01-1/1 (high C_2H_4)**
Hampshire Hatsuaki Monidal NY75414-1 3 WSU selections	Arlet, Autumn Gold Braeburn, Cameo Coop 15, Creston, Elliot Empire, Enterprise Ginger Gold Golden Delicious Granny Smith Honeycrisp, NJ 109 NY 79507-72, Orin Pink Lady, Pristine Shizuka, Silken, Suncrisp, Sundowner 6 WSU selections	Ambrosia BC 8S-26-50, BC 8S-31-56 BC SPA493 Chinook, CrimsonCrisp CQR10T17, CQR12T50 Gala, GoldRush, Huaguan NY 632 Pacific Queen, Pacific Rose Pinova, Sansa, Senshu, Shinsekai, Sonja, Splendour Sundance, Zestar 17 WSU selections	**AC01-1/1 (medium C_2H_4)**
	Delicious NJ90 Runkel 2 WSU selections	**Delblush (Tentation)** **Fuji** **Pacific Beauty** **Sabina** 4 WSU selections	**AC01-1/1 (low C_2H_4)**

Categorized breeding parents based on ethylene gene allelotypes clearly indicate that these elite breeding parents are closer to lower ethylene producing genotype and away from high ethylene producing genotype. This means conventional breeding guided solely by phenotype result in similar trend to those obtained using a genotyping approach.

Figure 1 (see next page). Fresh fruit firmness (Newtons) at harvest (A), firmness after 60 days cold storage (B) and the difference in firmness (fresh minus stored) (C) for 40 samples displayed by their ACS1 and ACO1 genotypes across harvest dates.

Distinguishable separation of fruit firmness for low ethylene production genotype (solid symbols) from medium or high (blank symbols) illustrates a good correlation between fruit firmness and ethylene gene allelotypes. This study supports the use of ethylene production gene markers for marker assisted selection. Correspondingly, 5,000 seedlings of diallele crosses between Pink Lady, Honeycrisp and CrimsonCrisp are being genotyped to determine their ACS1 and ACO1 allelotypes.



2. Candidate gene approach to identify genes related to fruit volatile production capacity

Along the same principle of developing ethylene gene functional markers, elucidation of the genetic control mechanisms and identification of genes that regulate volatile ester biosynthesis through functional genomic approach may lead to the generation of functional molecular markers.

Alcohol acyltransferase (AAT) catalyzes the last step of volatile ester biosynthesis, and ethylene purportedly regulates AAT gene expression. In this study, expression patterns of apple (*Malus* x *domestica* Borkh.) AAT genes and ethylene biosynthesis genes of 1-aminocyclopropane-1-carboxylate synthase (ACS) were investigated in two apple cultivars with high ('Golden Delicious') or low ('Granny Smith') volatile ester production. The results (Figures 2, 3) indicate: 1) Differential AAT expression may contribute to phenotypic variation in volatile ester biosynthesis; 2) ACS3 expression may play a role in induction of AAT expression in early fruit development; 3) ACS1 expression correlates with AAT expression levels and volatile ester production; 4) Postharvest 1-MCP treatment resulted in selective inhibition of AAT and ACS genes.

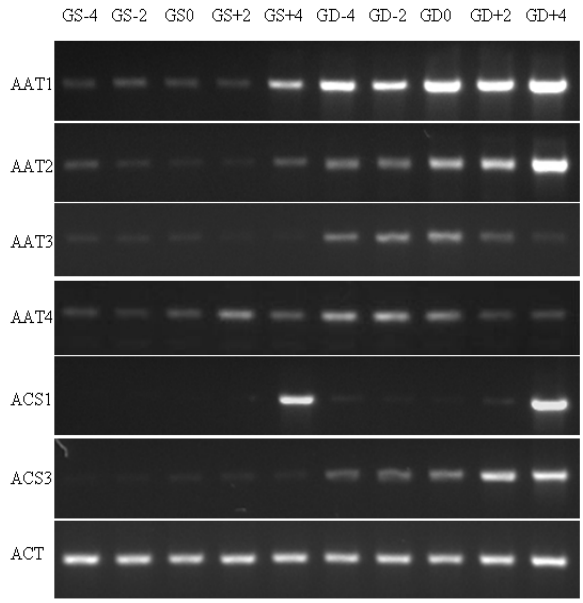


Figure 2. Expression patterns for AAT ACS genes in 'Granny Smith' and 'Golden Delicious' apple peel tissue at different ripening stages. Fruit harvested at two-week intervals beginning 128 (GD-4) or 149 (GS-4) days after full bloom (DAFB) for 'Golden Delicious' (GD), 'Granny Smith' (GS), respectively. Top axis label indicates cultivar and weeks prior to (-), at (0), or after (+) physiological maturity was attained.

All four AAT genes express stronger in 'Golden Delicious' than in 'Granny Smith'. AAT1 and AAT2 are the predominant genes expressed in fruit tissues. AAT1 and AAT2 expression increased as ripening progressed and was consistent with total ester production between two cultivars (data not shown). AAT3 and AAT4 transcript levels decreased at or after the onset of ripening. ACS1 expression increased at the onset of ripening while ACS3 expression was detected throughout the harvest period.

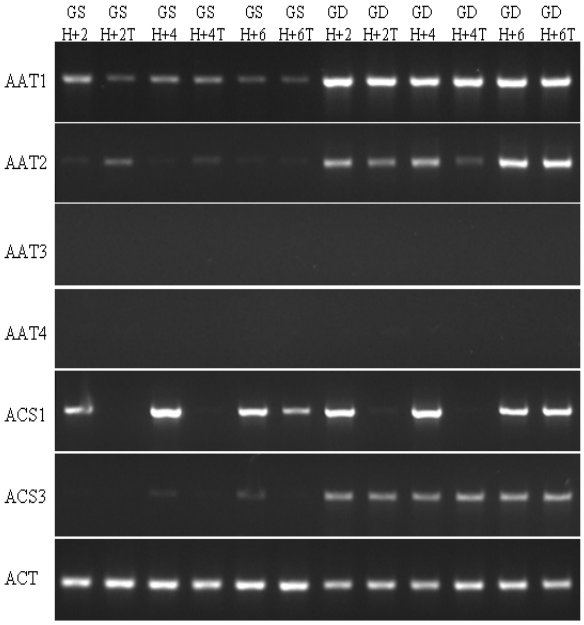


Figure 3. Expression patterns of four AAT genes in 'Granny Smith' and 'Golden Delicious' apple peel tissue during postharvest ripening and after 1-MCP treatment. Fruit were harvested 156 (GD0) or 177 (GS0) days after full bloom (DAFB) and held up to 6 weeks at 20 ºC. Top axis label indicates cultivar, weeks after harvest (H+2, H+4 and H+6), and "T" indicates treatment at harvest with 1-MCP. Postharvest 1-MCP exposure had little impact on expression of AAT1 and ACS3 genes, but substantially suppressed the transcript level for ACS1 in both cultivars, and AAT2 in 'Golden Delicious'.

3. Physiological characterization of firmness and crispness of both Honeycrisp and PinkLady, collect fruit tissues at specific developmental stages for gene expression profiling by microarray analysis.

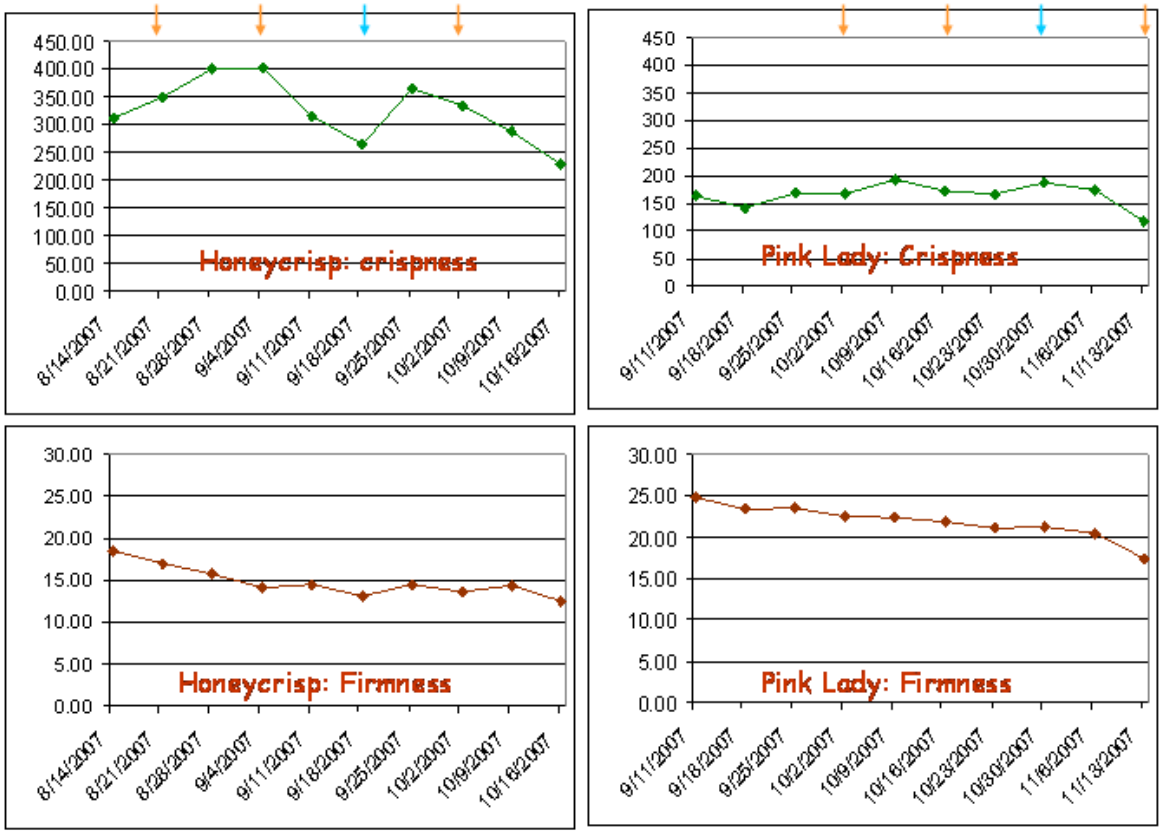


Figure 4. physiological characterization of fruit texture attributes of Pink Lady and Honeycrisp during ripening.

Fruit of two apple (*Malus* X *domestica* Borkh.) cultivars, 'Pink Lady' and 'Honeycisp', were harvested from commercial orchards near Wenatchee, WA. Fruit with uniform size and appearance were harvested weekly beginning approximately six weeks before and continued four weeks after commercial maturity as defined by starch pattern index, fruit firmness, internal ethylene concentration (IEC), and respiration rate as described previously. Fruit firmness was measured on pared fruit surfaces using a Mohr Digitest instrument (Mohr and Associates, Richland, WA). Cortex tissues were collected and stored in -80ºC freezer for upcoming gene expression analysis.

FINAL PROJECT REPORT
WTFRC Project Number: AP-07-707

Project Title: A new approach to understand and control bitter pit in apple

PI: Elizabeth Mitcham
Organization: University of California

Telephone/email: 530-752-7512
Address: Dept. of Plant Sciences,

City: Davis
State/Province/Zip CA 95616

Other funding Sources: None in 2007, we just received a small grant for California Tomato Research Board for 2008

Total Project Funding: $10,000

Budget History:

Item	**Year 1:** 2007		
Salaries	4,445		
Benefits	580		
Wages			
Benefits			
Equipment			
Supplies	3,300		
Travel	1,600		
Miscellaneous	75		
Total	**10,000**		

INTRODUCTION

For many years, bitter pit in apple fruit has been studied; however, its mechanism remains unknown. This disorder is responsible for a reduction in quality and loss of commercial value of apple fruit, and the incidence can be quite high in some seasons (>15%). After an extensive literature review and in agreement with other scientists, we proposed a new approach to better understand and control bitter pit in apples. Our hypotheses are that the levels of growth regulators may regulate the amount of calcium that is translocated to the fruit, as well as how the fruit tissue regulates the availability of calcium for important structural functions such as membrane stabilization. High levels of gibberellins during fruit growth and development may change xylem function and calcium uptake to the fruit, as well as induce changes in membrane permeability, which is responsible for increasing weight loss and bitter pit incidence (SAURE, 2001; SAURE, 2004).

Bitter pit is believed to be similar to a disorder in tomato fruit, blossom end rot (SAURE, 2001). Based on this similarity, in the first part of the project we used greenhouse tomato plants as a faster and more controlled model to test our hypothesis. Next, we tested our hypothesis in a commercial apple orchard.

OBJECTIVES

1. Explore the relationship between growth regulators and xylem function as related to fruit susceptibility to bitter pit.
2. Explore the relationship between gibberellin levels late in the growing season and membrane permeability as related to water stress and fruit susceptibility to bitter pit.
3. Determine the cellular location of calcium in the apple fruit and the relationship to bitter pit susceptibility.
4. Develop management strategies to predictably reduce fruit susceptibility to bitter pit under commercial conditions.

METHODS

This work was done in 2007 at the University of California, Davis. Tomato plants (cv. Ace) were grown in 5 liter pots with organic substrate in a greenhouse environment. At full bloom, three to six fully opened flowers were selected on each plant, tagged and pollinated. One day after pollination, the plants were treated with 300ppm of gibberellins (GA4+7), 300ppm of a gibberellin inhibitor (Apogee), 500ppm of growth inhibitor (VBC30053) or water (control). Each treatment included four replications with one plant each. The treatments were applied by weekly spraying the plants with each solution containing also 0.05% polysorbate 20 (Tween® 20). On the day of the first treatment, 20g of slow release fertilizer (24-4-9 NPK) was added to each pot and from this point on, the plants were irrigated with deionized water only. The evaluations were conducted at 12, 24, 31, 38, 45, and 52 days after pollination for blossom-end rot incidence, membrane permeability, xylem function, fruit weight and diameter. At 32 days after full bloom, we evaluated dry matte, and calcium levels in the fruit.

Similar treatments were also applied to twenty year old Granny Smith apple trees growing on 111 rootstock in a commercial orchard in Stockton, California. The treatments were 1) water applied after full bloom (AFB) + before harvest (BH), 2) 300ppm of gibberellin (4+7) AFB, 3) 150ppm of gibberellin (4+7) BH, 4) 300ppm of gibberellin inhibitor (Apogee) AFB + BH, 5) 500ppm of growth inhibitor (VBC30053) AFB, and 6) 100ppm of growth inhibitor (VBC30053) BH. Each treatment included four replications with two trees each. The treatments were applied weekly, starting two weeks after full bloom and/or four weeks before harvest and were applied for six and four weeks, respectively. Plants were sprayed with 3.9 liters of the respective solution containing also 0.05% of polysorbate 20 (Tween® 20). Commercial calcium sprays were not applied to trees in this experiment. Fruit were harvested at commercial maturity and stored at 0°C (32F) for two months.

Seven weeks after full bloom, the fruit were evaluated for xylem function, weight, dry matter, and total levels of calcium in the blossom-end region. At harvest, the fruit were evaluated for bitter pit and water core incidence, dry matter, calcium content, flesh firmness, starch content, titratable acidity, and total soluble solids. After two months of storage, the fruit from each treatment were analyzed for bitter pit incidence and severity, then divided into groups with and without bitter pit and analyzed for,weight loss, total soluble solids, titratable acidity, dry matter content, and total calcium content (still being processed). We also selected one fruit with initial symptoms of bitter pit and another sound fruit without any symptoms to investigate calcium localization in the cells using electron microscopy analysis. Postharvest application of the growth regulators was tested by dipping fruit for 5 minutes in growth regulator solutions before storage.. All solutions included 0.05% polysorbate 20 (Tween® 20) as a surfactant except one water only control. The treatments were: 1) water, 2) 150 ppm GA (4+7), 3) 300ppm Apogee, 4) 100ppm VCB30053 and 5) water without surfactant. Fruit were allowed to dry before storage and evaluated after two months at 0C (32F).

SIGNIFICANT FINDINGS

Tomato experiment

The results in the tomato experiment showed that growth regulators indeed play a role in blossom-end rot development. The plants treated with gibberellin had higher incidence of blossom-end rot (BER), whereas plants treated with the growth inhibitor VBC30053 had significantly lower incidence of this physiological disorder (Figure 1A). Membrane permeability also increased in fruit tissues treated with gibberellins and decreased in fruit treated with the growth inhibitor, VBC30053 (Figure 1B), matching the BER incidence. The application of gibberellin inhibitor (Apogee) or VBC30053 increased the number of functional xylem elements in the fruit compared to fruit treated with gibberellin and water (Figures 1C). Calcium concentration was consistently higher in fruit of plants treated with VBC30053 or Apogee (Figure 1D). The dry matter content was reduced in fruit treated with VBC30053 (data not shown). VBC30053 treatment also increased fruit diameter and weight (data not shown). The biggest change observed with VBC30053 treatment was an increase in total fruit weight and the average fruit weight per plant (data not shown).

Apple preharvest experiment

Seven weeks after harvest, our fruit evaluations showed the effects of treatments applied after full bloom. As expected, gibberellin treatment decreased the number of functional primary xylem elements, whereas the growth regulator VBC30053 increased the number of functional xylem elements (Figure 2A). Growth regulator treatments did not result in differences in fruit diameter (data not shown) or fruit weight as was observed in the tomato experiment (Figure 2B). Growth regulator treatments did not have a great influence on the dry matter content of apple fruit early in the growing season (data not shown). However, gibberellin treatments reduced whereas Apogee and VBC30053 treatments increased the total level of calcium in apple fruits at this stage of development (Figures 2C and 2D).

At harvest, apples treated with gibberellins after full bloom showed the highest incidence of bitter pit (Figure 3A). Gibberellin treatment after full bloom or before harvest, as well as VBC30053 treatment before harvest reduced the incidence of water core in the apple fruit at harvest compared with untreated control fruit. However, fruit treated with Apogee or VBC30053 after full bloom had increased amounts of water core (Figure 3B). Fruit treated with VBC30053 after full bloom had much greater dry matter content (Figure 3C). The levels of calcium in the fruit at harvest were quite similar among the treatments with a slightly higher concentration in the fruit treated with Apogee (Figure 3D).

Fruit treated with VBC30053 after full bloom had the highest total soluble solids followed by fruit treated with gibberellins after full bloom. When VBC30053 was applied before harvest, total soluble solids were similar to untreated control fruit (data not shown). Fruit treated with VBC30053 after full bloom or before harvest or gibberellins before harvest had elevated levels of titratable

acidity compared with untreated control fruit (data not shown). The growth regulator treatments did not have much effect on fruit firmness or starch content at harvest (data not shown).

After two months of air storage, bitter pit incidence was observed in all treatments. However, fruit treated with Apogee after full bloom or VBC30053 before harvest both showed a reduction of approximately 14% in bitter pit incidence (Figure 4A). Bitter pit severity was similar for all treatments (Figure 4B). During storage, fruits treated with VBC30053 after full bloom lost significantly less weight than fruit from other treatments (Figure 4C). After two months of storage, the highest levels of dry matter, total soluble solids, and titratable acidity were observed in fruit with bitter pit and fruit treated with VBC30053 after full bloom (Figures 4D, 5A and 5B). Preliminary electron microscopy analysis for calcium localization inside the apple fruit cells have shown differences in precipitates within storage organelles between fruit with bitter pit and fruit without bitter pit that may be calcium sequestered in these organelles (Figure 7). We will confirm this with additional testing in the near future.

Apple postharvest experiment

The severity of bitter pit was the same among the various dip treatments (Figure 6B); however, the incidence of fruit with bitter pit was much lower in fruit dipped in Apogee or VCB30053, although the levels of bitter pit remained very high (Figure 6A). Fruit dipped in water without surfactant had the highest incidence of bitter pit.

RESULTS & DISCUSSION

Many have reported that fruit with the same levels of calcium can have different incidence of bitter pit or blossom-end rot. In this case, the importance may not be the total level of calcium, but where it is located in the tissue. A number of studies have shown that growth regulators can influence calcium balance and distribution in different tissues. Our results with tomato clearly showed that gibberellins increased the incidence of blossom end rot and also decreased fruit calcium content and the number of functional xylem elements early in fruit development, and increased membrane permeability, an early indicator of susceptibility to blossom-end rot. Growth inhibitors such as Apogee and VBC30053 had the opposite effect, controlling blossom end rot, increasing fruit calcium content, and increasing the number of functional xylem elements early in fruit development. Reductions in electrolyte leakage were mainly observed with VBC30053. In addition, VBC30053 decreased fruit dry matter content while significantly increasing fruit size and diameter.

Our results with apple had similar trends, but were not as dramatic in the ability to control bitter pit. While bitter pit incidence was reduced by treatment with Apogee after full bloom or VBC30053 at harvest, there was still incredibly high bitter pit incidence. Early in fruit development, treatment with Apogee or VBC30053 after full bloom increased fruit calcium content; however, at harvest, only fruit treated with Apogee had slightly higher calcium content. As with tomato fruit, treatment with Apogee or VBC30053 increased the number of functional xylem elements while treatment with gibberellins decreased functional xylem elements. The postharvest dip data also showed promise for control of bitter pit and should be further explored. Our preliminary data with electron microscopy indicates that a lot of calcium is stored in the vacuole of fruit with bitter pit as compared to fruit without bitter pit. This may explain why some fruit in one lot have bitter pit and others do not. We plan to increase our observations of calcium localization and learn how to manipulate this to our advantage for fruit quality.

Of course there are large differences between tomato plants and apple trees, the most obvious being one is a woody perennial. The length of time required to grow and mature a Granny Smith apple is considerably longer than for a tomato fruit. Also, bitter pit develops late in the development of apple fruit, most commonly after cold storage, while blossom end rot in tomato develops approximately 2 weeks after full bloom. We found that the timing of application of growth regulator treatments had a great influence on fruit response. Early applications of VBC30053 at the concentrations used had a profound influence on fruit drop and leaf health that may have affected fruit quality at harvest. We need to do much more work with various concentrations and timings to

determine the optimum performance of growth inhibitors for reduction of bitter pit incidence. Application with calcium sprays is another option that should be explored. The postharvest dip treatments also show tremendous promise and have the advantage of no potential affects on the tree and reduced application costs. We would like to test the growth inhibitor dip treatments together with calcium solutions to see if we can influence where in the cells the applied calcium is stored to improve our ability to reduce bitter pit. Our preliminary analysis of calcium localization in fruit cells with electron microscopy appears to be a promising technique to allow a better understanding calcium deficiency disorders.

In addition, it is important to continue investigating the mode of action of growth regulators in reducing blossom end rot and bitter pit as well increase our understanding of the mechanisms involved in calcium disorder development. With this understanding, we can better develop short-term and long-term solutions to control bitter pit in apple to reduce the economic impact of producing unmarketable fruit.

REFERENCES

SAURE, M,C. Blossom-end rot of tomato (*Lycopersicon esculentum* Mill.) – a calcium – or a stress – related disorder? Scientia Horticulturae 90:193-208, 2001.

SAURE, M.C. Calcium translocation to flesh of fruit: its mechanism and endogenous control. Scientia Horticulturae 85:1-25, 2004.

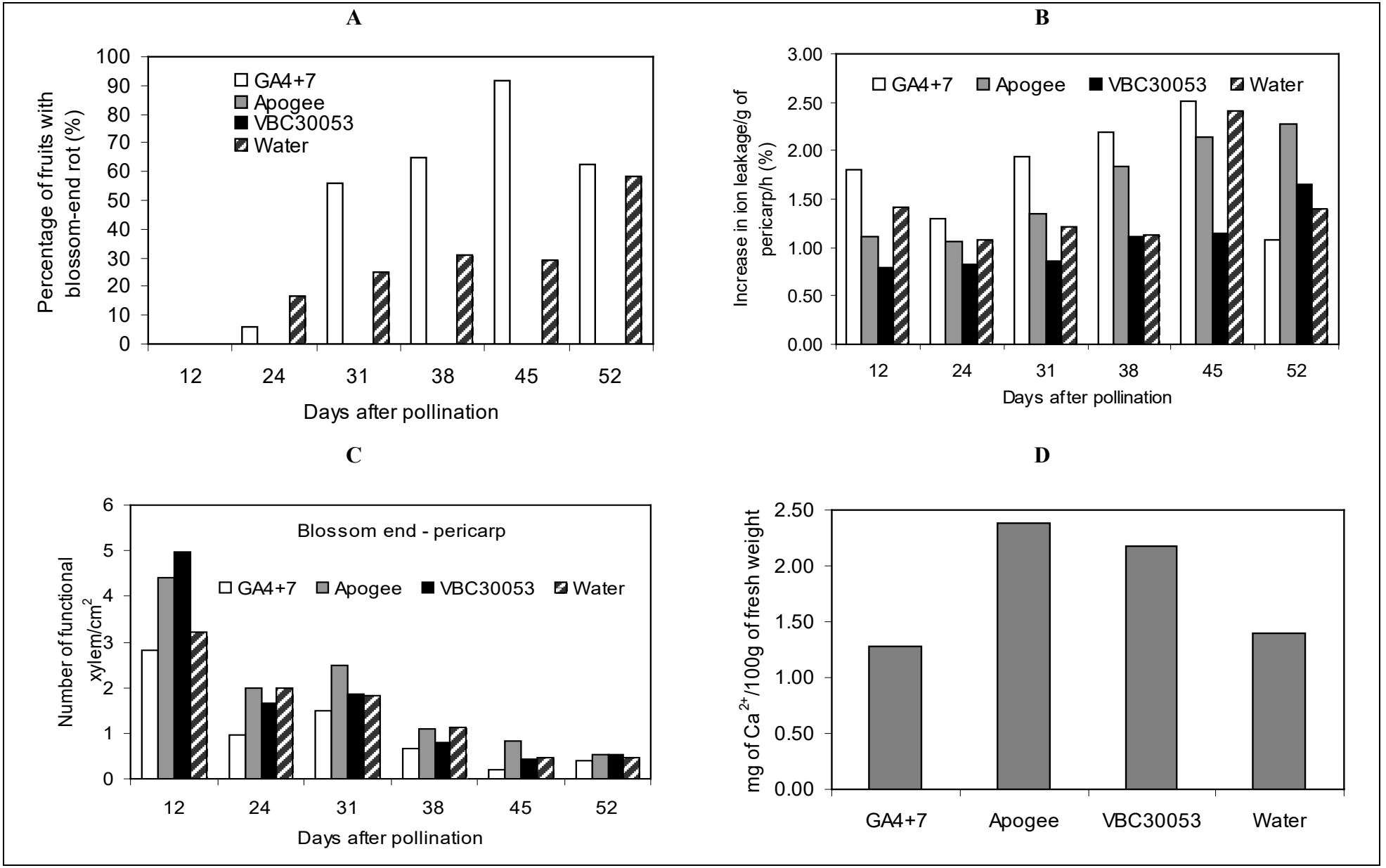
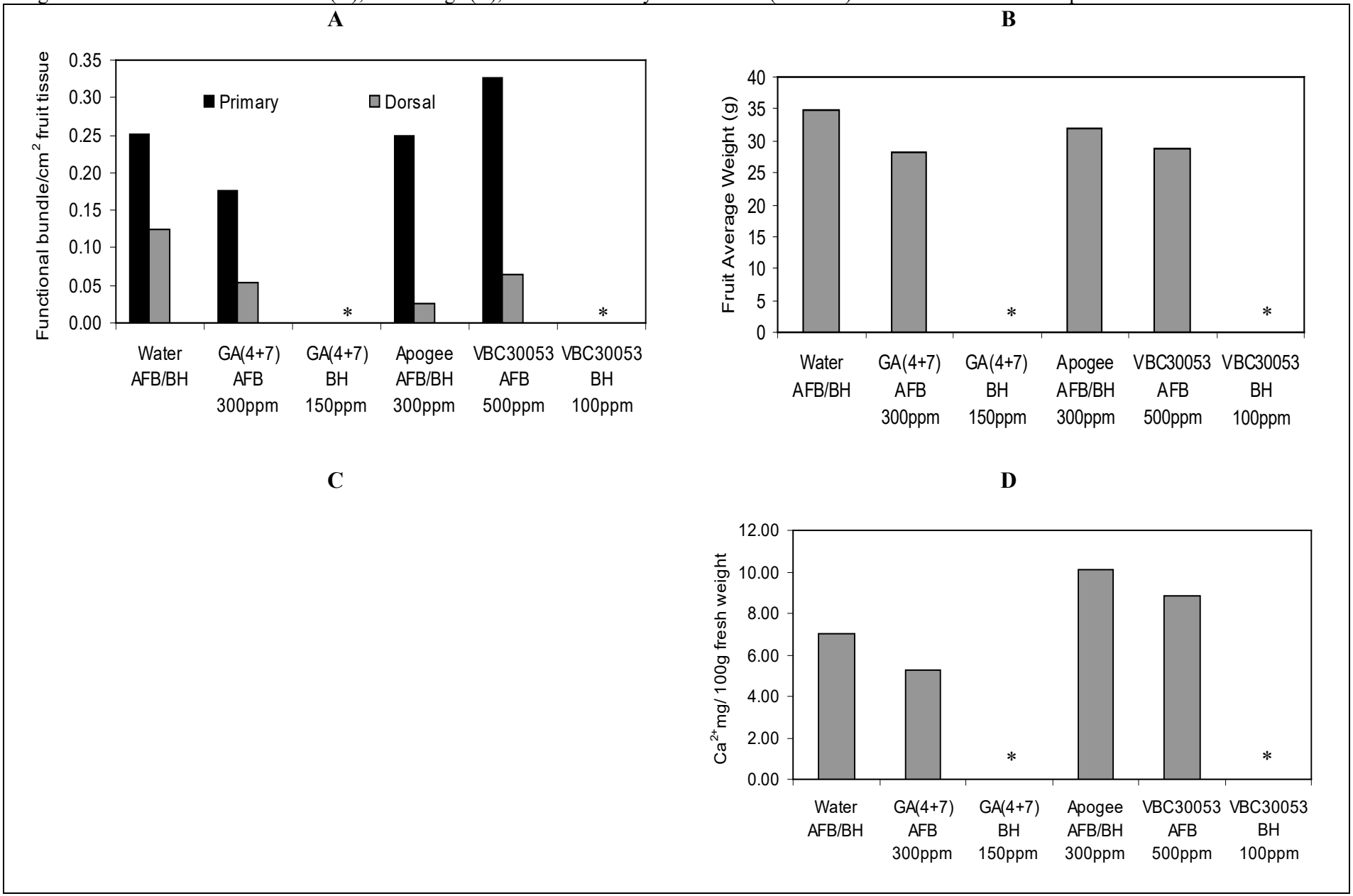

A
Percentage of fruits with blossom-end rot (%)
GA4+7
Apogee
VBC30053
Water
Days after pollination
B
Increase in ion leakage/g of pericarp/h (%)
GA4+7
Apogee
VBC30053
Water
Days after pollination
C
Blossom end - pericarp
Number of functional xylem/cm2
GA4+7
Apogee
VBC30053
Water
Days after pollination
D
mg of Ca2+/100g of fresh weight
GA4+7
Apogee
VBC30053
Water

Figure 1. Blossom-end rot incidence (A), ion leakage (B), and functional xylem elements (C and D) obtained in the tomato experiment.



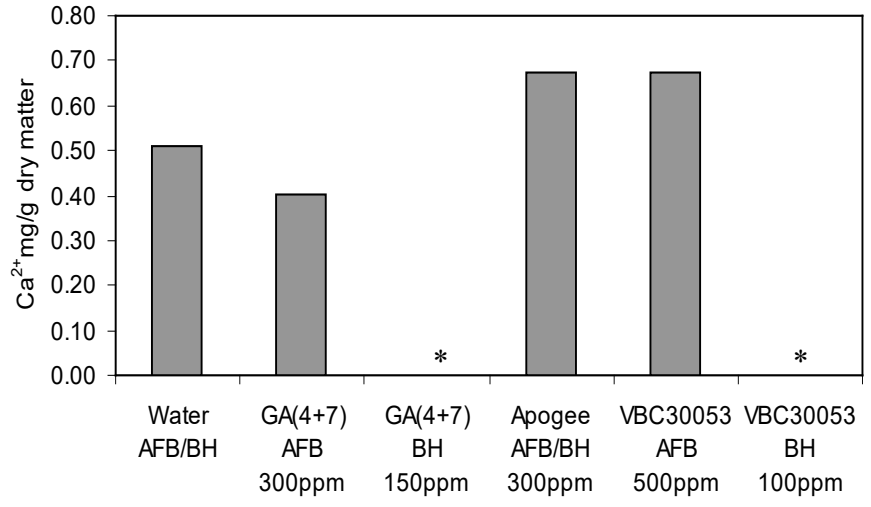
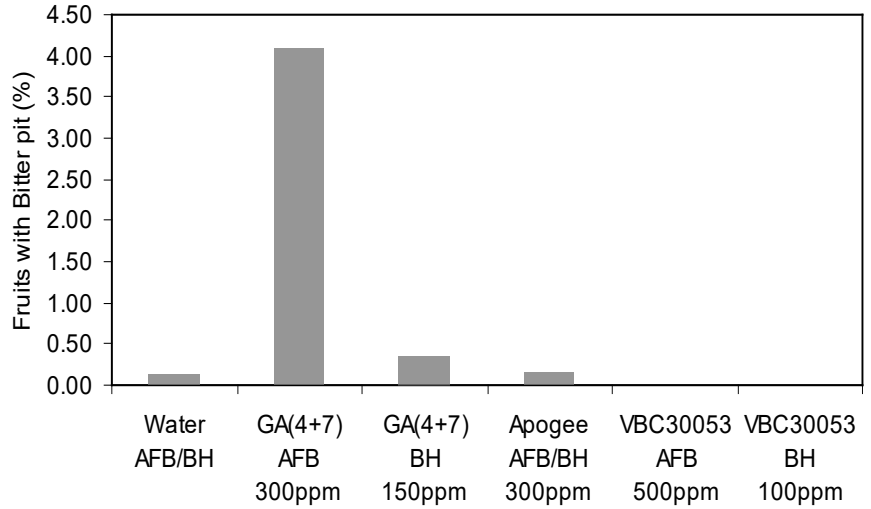
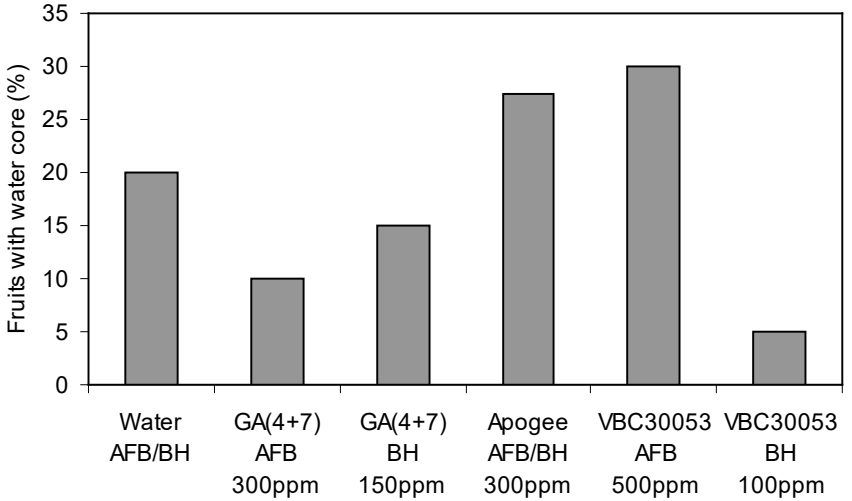


Figure 2. Functional xylem elements (A), avg. fruit weight (B), and calcium content (C and D) of apple fruits 7 wks after full bloom. * = treatments not yet applied.

A



B



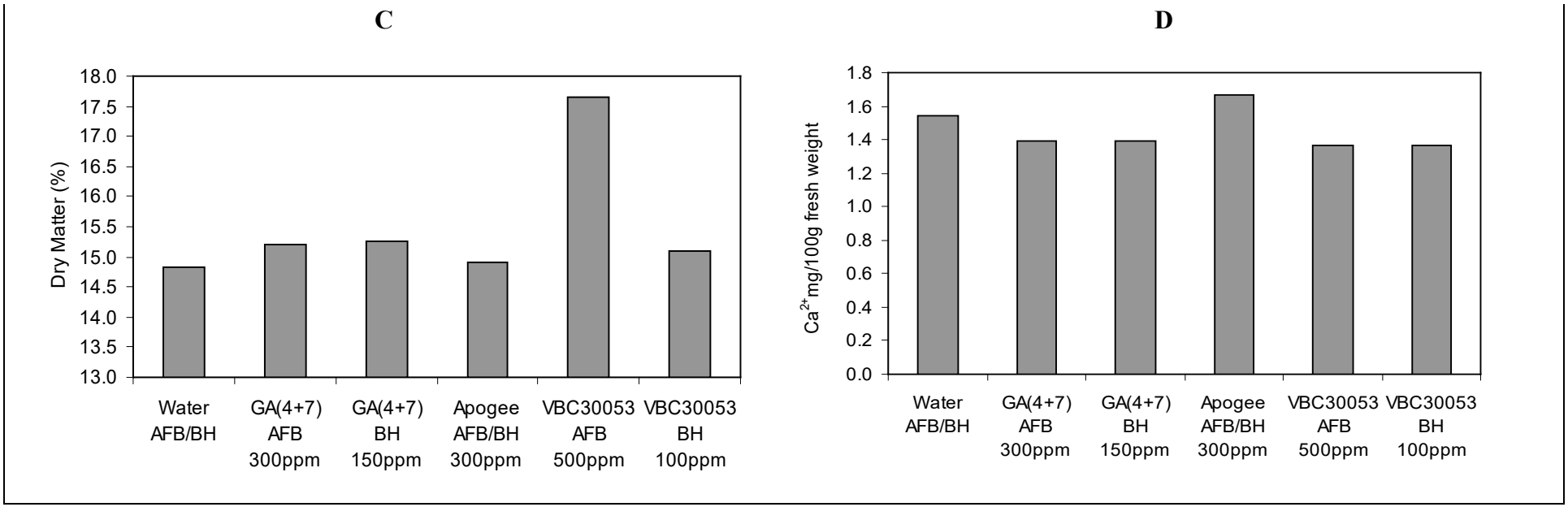
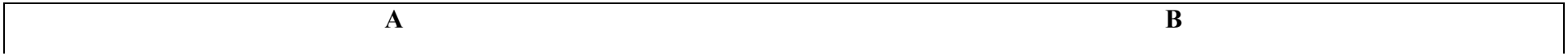


Figure 3. Bitter pit (A) and water core (B) incidence, fruit dry matter (C), and calcium content (D) in apple fruit at harvest.



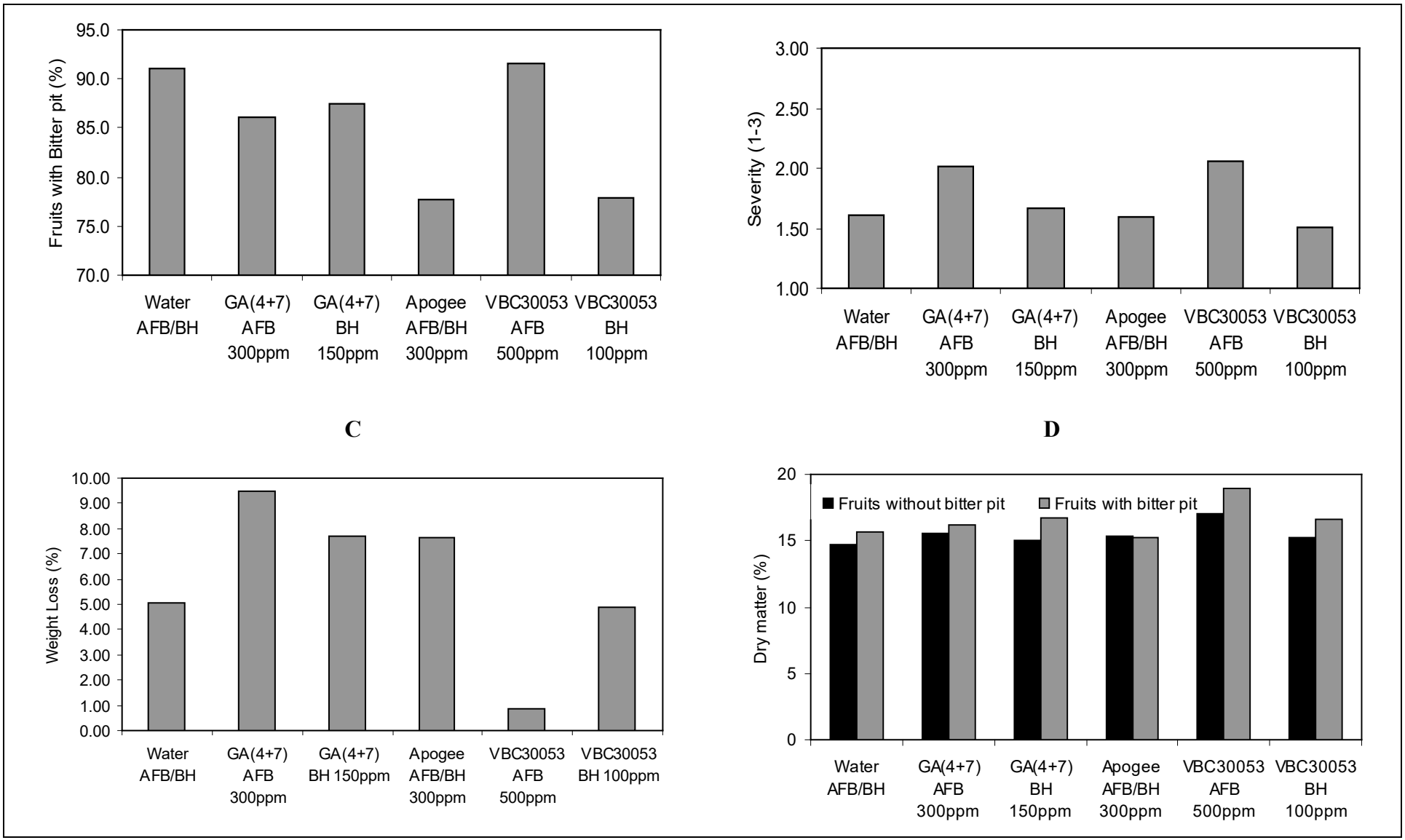


Figure 4. Bitter pit incidence (A) and severity (B), weight loss (C), and dry matter (D) of apple fruits stored for two months at 0ºC (32ºF).

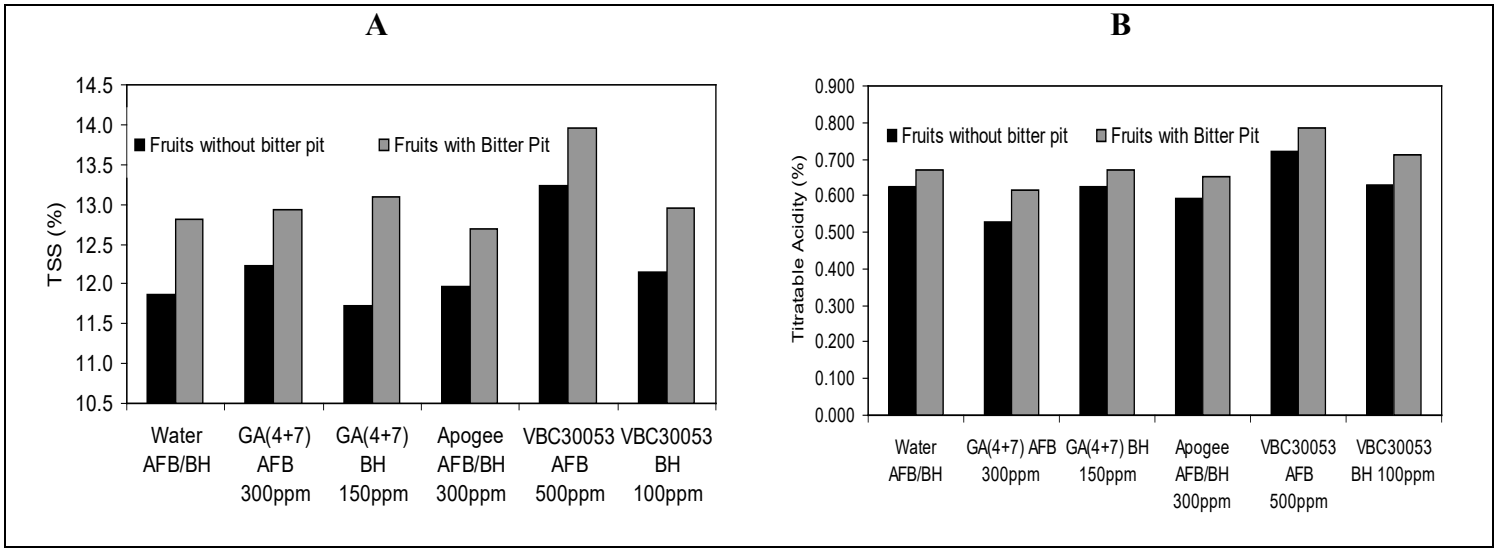


Figure 5. Apple experiment - analysis of apple fruits after two months of cold storage at 0ºC (32ºF).

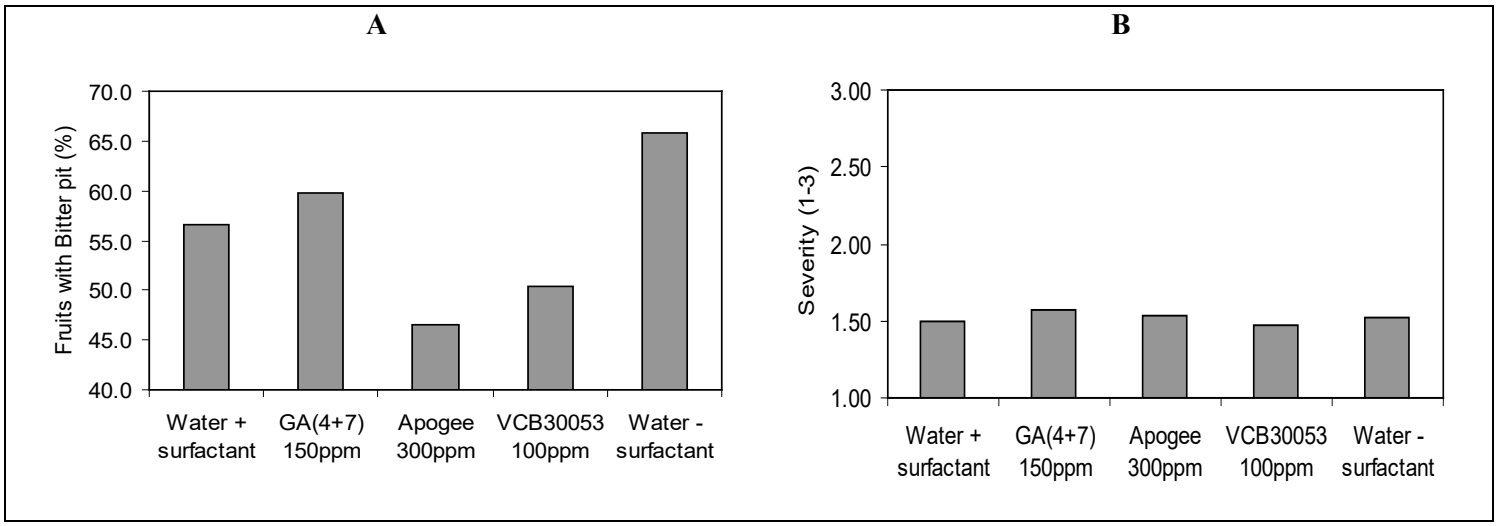


Figure 6. Bitter pit incidence (A) and severity (B) in apple fruits treated after harvest with growth regulators and stored at 0ºC (32ºF) for two months.

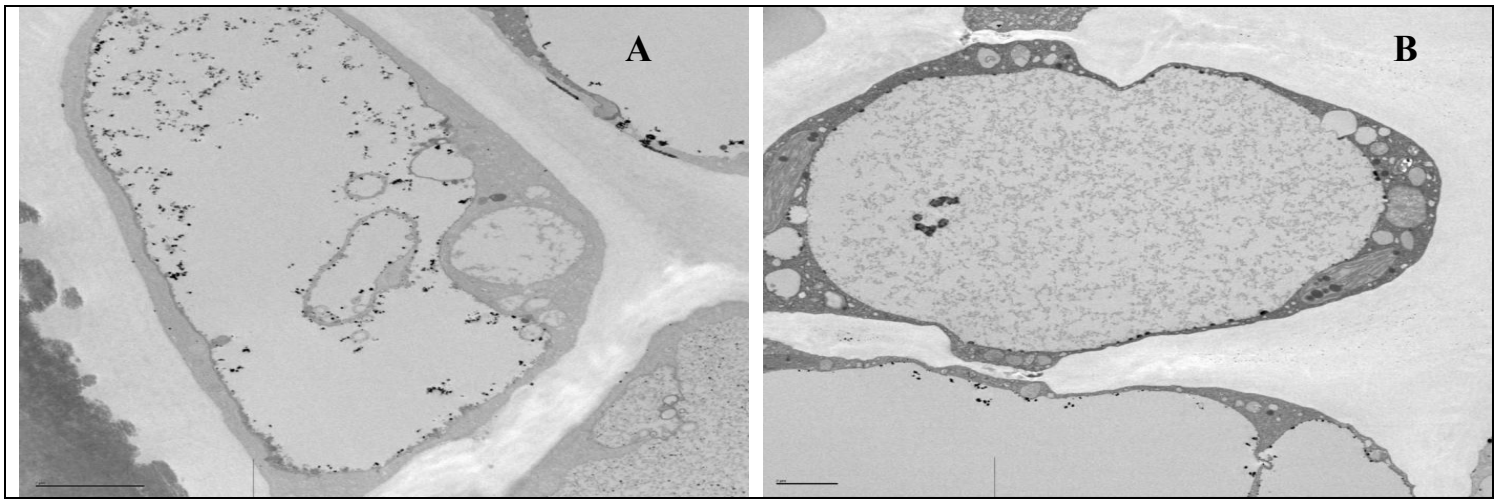


Figure 7. Comparison of bitter pitted fruit (A) and sound fruit (B) cells, looking for calcium localization inside storage organelles (vacuole).

FINAL PROJECT REPORT
WTFRC Project Number: AP-06-605B

Project Title: Testing of a sticker for ethylene release from apples

PI:	Mark Riley, PhD	**Co-PI(2):**	Robert Klein, PhD
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Cooperators: Dr. James Mattheis, USDA/ARS

Other funding Sources

Agency Name: USDA, SBIR phase I, awarded in 2006
Amount awarded: $80,000
Notes:

Total Project Funding: WTFRC $43,060 + USDA $80,000 = $123,060

Budget History:

Item	**Year 1:** 2006	**Year 2:** 2007	**Year 3:**
Salaries	11283	11283	
Benefits	372	372	
Wages			
Benefits			
Equipment			
Supplies	9000	6500	
Travel	1000	1000	
Miscellaneous			
Total	**23905**	**19155**	

Objectives: The goal of this research program is to develop a simple and inexpensive device to provide a noninvasive means to determine ethylene release from apples. This objective will be met through design of a sticker based device that presents a gradual color change indicative of the amount of ethylene released by an individual apple. Specifically, the proposed device is a flat, inexpensive, thin permeable membrane sandwich in the form of a patch or "sticker" that self-adheres to the surface of the apple. The sticker detects the emissions of ethylene from an individual apple (rather than the atmosphere around many apples) and consequently displays a color change indicating ripeness on the external surface of the detector. Ethylene is a demonstrated fruit ripeness indicator and its release correlates with ripening. The stated objective would be met by addressing the following aims.

Specific aim 1: Develop a stable and reproducible device using ethylene sensitive reagents integrated into membranes.

Specific aim 2: Tune the responsiveness of the device for fast (hours) to slow (days to weeks) response to ethylene.

Specific aim 3: Test the devices in the laboratory, orchard, and packinghouse.

Timeline:

Year 1: 1/06 – 12/06

Improve stability of device based on results of small field trials from Fall 2005.
Begin development of slow responding device by modifying reagents and supports.
Perform field trials in the orchard and packinghouse

Year 2: 1/07 – 12/07

Improve stability of device based on results of small field trials from Fall 2006.
Improve time of response of devices.
Perform moderate scale field trials in the orchard and packinghouse

Significant findings: Over the past year, we have made substantial progress in the development and testing of this device. To summarize accomplishments of 2007:

1) Improved the device performance. Advances were made in the device in how the reagents are encapsulated and interface with the adhesive. This greatly diminished the impact of varying humidity (elevated or reduced), improved storage capabilities, and decreased temperature sensitivity. This encapsulation did, however, decrease the sensitivity to ethylene levels. Detection limit is approximately 1.0 ppm ethylene.
2) Developed and modified device prototypes for orchard use; made improvements based on orchard findings.
3) Assessed potential reagent toxicity. Reagents cause less of a biological response than do copper or zinc in soluble forms.
4) Performed laboratory and orchard trials. Laboratory testing was done in conjunction with development and focused on improving stability. Orchard trials were performed by Ines Hanrahan, Ph.D., on a variety of apple cultivars during the July-October 2007 harvest season. Briefly, the device performed reasonably well with good stability and response to ethylene levels ranging from 0.1 to more than 10 ppm in the orchard. Unfortunately, no useful correlation could be found between ethylene release (and sticker color) and other measures of fruit maturity. Full color development was obtained after normal harvest time. These studies are summarized below.

Research and discussion: Our goals for 2007 activities were to continue the progress made in the previous year in improving device stability and sensitivity, to develop a prototype with separate components with variable response to ethylene, to run additional trials in the orchard and packing house, and to explore opportunities for manufacture of the device.

Device development
Significant efforts were made in altering the reagent composition to improve stability, in providing a means to adhere the sticker to fruit surfaces, and improving contrast for ease of assessment in the field. The major advances were produced through adding hygroscopic materials to the reagents, drying the reagents to a lower final water content, and providing a more air tight seal preventing air contact from outside the fruit surface. These efforts are summarized briefly below.

We evaluated a large number of potential support materials with the goal of providing an easy to use, easy to apply to the fruit, and stable approach for detection of ethylene. Support materials that received significant attention include:

Evaluated included polymeric membranes, papers (filter paper, printer paper), and glass filters.

Particulates evaluated include a wide range of materials including TiO_2, SiO_2, $ZnSO_4$, $CaCl_2$, $NaSO_4$, metal oxides, metal chlorides, perlite, and earthstone.

We performed extensive trials on membranes including but not limited to: Nylaflo, GH polypro, polyethersulfone, Tuffryn, nitrocellulose, polypropylene, Biodyne, glass, Durapore (wetted and non wetted), Gore-tex, nylon, tyvek (wetted, non-wetted, food grade), and Whatman filter numbers 1 and 50. For most of this work we focused on utilizing the commercial membrane material, called "Durapore" from Millipore, a PVDF film, as this provides the greatest stability against unintended color development. Membranes were loaded with reagents, allowed to dry, and then exposed to ethylene and other volatiles. For nearly all membranes, no significant benefit was observed over use of Durapore. Wetting of normally hydrophobic membranes was a challenge as our reagents begin as an aqueous solution. Wetted Durapore and the glass membrane showed the most promise.

Due to the high cost of the Durapore membrane, an important part of recent experimentation has involved testing other brands and other membranes. Of the numerous membranes tested, the two that were of most interest from a commercial perspective were Biotrace PVDF and GLA-5000 PVC Membrane Filter, both made by Pall Corporation. However, both of these membranes are highly hydrophobic, making use of a wetting agent necessary. In order to find the best wetting agent, several were tested, including ethyl alcohol, methyl alcohol, and isopropyl alcohol. After thorough experimentation, it was determined that neither of the new membranes was a suitable substrate for reagents, as both reacted (with no ethylene exposure) on the bench as much as or more than Durapore. Figure 1 shows an image of stickers using the wetter Durapore on apples in the laboratory.

Additional studies were run to identify solvents for improved wetting characteristics on membranes including a non-wetted Durapore which has a greater potential stability. Ethanol turns the solution green. All chlorides reduce the sensitivity of the solution. Palladium chloride may not be substituted for palladium sulfate. Sodium sulfate and magnesium sulfate may not substitute for sulfuric acid.

Our prior plans for utilizing a white powder instead of a membrane system led to the development of an easy to apply material (similar to "white out"). This method was tested in the spring and found to have lower sensitivity to ethylene and greater interference due to increased humidity. The approach was set aside and not used in field trials.

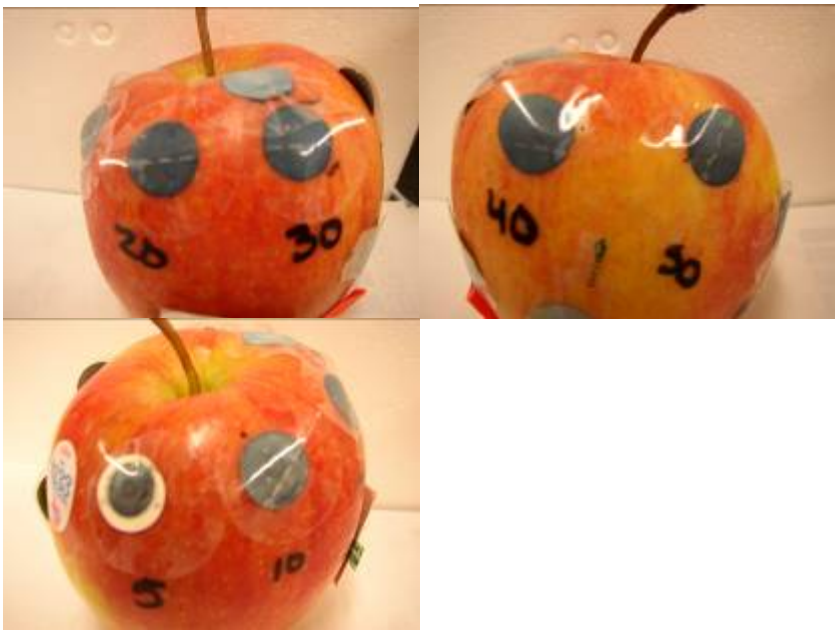

Figure 1: Stickers on apples after improvements in the drying process.

Toxicity tests were performed on the chemical reagents using human cell cultures. It was found that the Pd had an LD50 of approximately 0.5 mM and the Mo had an LD50 of approximately 0.7 mM. More testing needs to be performed, however, these cell response levels place the reagents in a similar category as exposure to soluble Fe, and less hazardous than soluble Cu or Zn. This is encouraging as Fe is considered to be of minimal health concern at the dosage present in our stickers. Further testing is required in order to satisfy requirements for FDA review. We continue to asses mechanisms of cellular response in order to validate these measurements.

Orchard tests - Performed by Ines Hanrahan, Ph.D.

During the July-October 2007 growing season the WTFRC and RediRipe jointly evaluated field performance of ethylene stickers when placed on apples. The objectives were to ensure reliable color change of the stickers under various field conditions and to relate sticker color change to fruit ethylene emission and common harvest maturity parameters.

General trial procedures
Stickers were shipped from Arizona via FedEx overnight and typically arrived in Yakima, WA, in the late morning of the following day. Upon arrival stickers were inspected for color change and signs of damage. Apart from the first set (10% of stickers had turned color) stickers arrived without discernable color change. For transport stickers were kept in the folders provided by Riley's lab and care was taken to avoid any contact with ethylene, such as ripe fruit in the car and office. Typically, stickers were applied to fruit within 24 hours of arrival. Across all trials stickers were placed on fruit of similar size and maturity on the north facing side of the apple (Figure 2).

Figure 2: Position of stickers on single fruit, and within the tree

Positive controls were placed on leaves (upper and lower side), bark and posts approximately one foot away from any fruit (Picture 2). Stickers typically did not change color.

Figure 3: Negative controls on a leaf and a post (bark not shown)

Responses of stickers to changing relative humidity have been a concern in the past. The first two sets of stickers tested in 2007 were split into 4 groups: orchard with or without overhead irrigation, fruit indoors and outdoors. No differences in response patterns were observed (Tables 1-5). The last two sets of stickers experienced 5 separate rain events. About 80% of the stickers remained functional and retained adhesiveness. The position of the stickers on the fruit was inconsequential. We tested all horizontal and vertical. In order to be consistent, we chose to apply stickers around the equator of the fruit facing north.

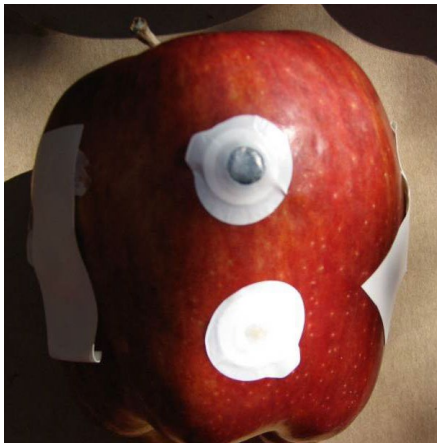

Figure 4: Sticker response to ethylene 24 hours after application

Summary of trial results:
Assessments of ethylene release and sticker color development were performed over the course of the July-October 2007 apple harvest season in WA. Raw data and more details can be provided if needed. Below we summarize the more relevant results.

First we assessed the correlation between IEC (internal ethylene concentration (in ppm)) and the ethylene release rate (in uL / kg –hr). There is a strong correlation ($R^2 = 0.97$) which demonstrates that quantifying the amount of ethylene released by the fruit can be a reliable indicator of the internal ethylene levels (Figure 5)

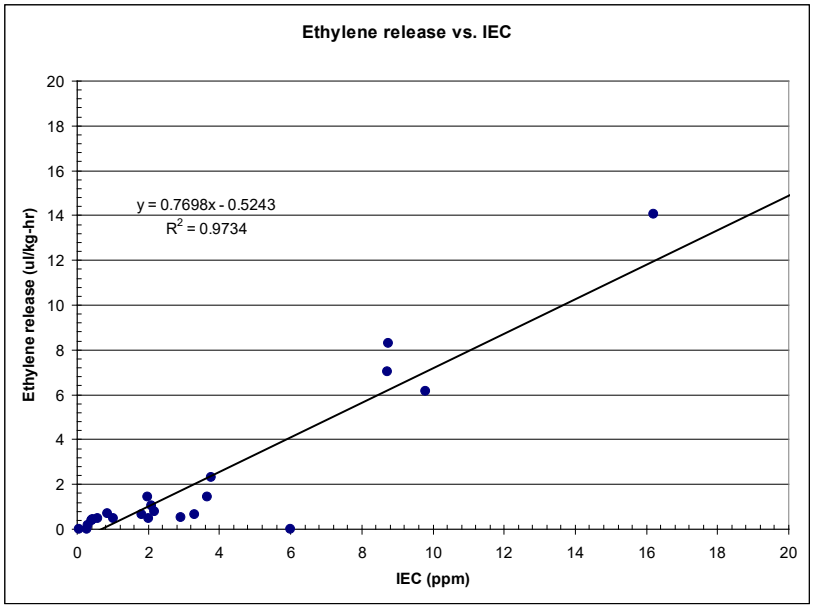

Figure 5: Correlation between IEC and ethylene release rate as measured for the variety of apples shown in Figure 6.

Measurements were performed from August to October. One test performed on August 16, showed a good correlation between sticker color and IEC and ethylene release is shown below (Table I).

16-Aug	wt(kg)	time	ppm	ul/kg/hr	Color?	IEC
Pacific outside for 6 days	0.237	40	3.079	38.39	XXXX	49.4
	0.228	42	2.074	25.60	XXXX	27.7
	0.258	43	0.187	1.99		3.3
	0.26	45	0.227	2.29	XX	1.5
	0.261	46	0.221	2.18		3.5
Pacific indoors for 6 days	0.183	67	4.088	39.41	XXXX	59.9
	0.201	73	0.136	1.10		2.1
	0.226	75	0.123	0.86		1.8
	0.247	77	1.321	8.21	XX	15.2
	0.197	79	5.186	39.39	XXXX	47
Pacific harvested today	0.238	76	0.232	1.52		3
	0.226	77	0.322	2.19		3.6
	0.235	78	0.32	2.06		2.7
	0.227	79	0.427	2.81		5.3
	0.213	80	0.376	2.61		5.5
Imperial harvested today	0.206	81	0	0.00		0.3
	0.208	89	0.075	0.48		1.1

0.189	65	0.075	0.72	1
0.173	66	0	0.00	0.2
0.19	67	0	0.00	0.4

XXXX	stickers had turned blue
XX	lighter color

Later in the season, after improvements in device stability, multiple quantitative assessments of sticker color were performed. A summary of one data set is shown below. Sticker color related to ethylene released had a correlation coefficient of 0.59 when sticker color is compared with a 1-8 scale (1 being white, 8 being a dark blue). Ethylene released at a level as low as 0.1 ppm could be detected with the device. The results shown in Figure 6a are for only fruits that were not damaged; Fuji's are also not shown here. Figure 6b shows a breakdown of all cultivars tested in this set. There appear to be some cultivar-specific effects.

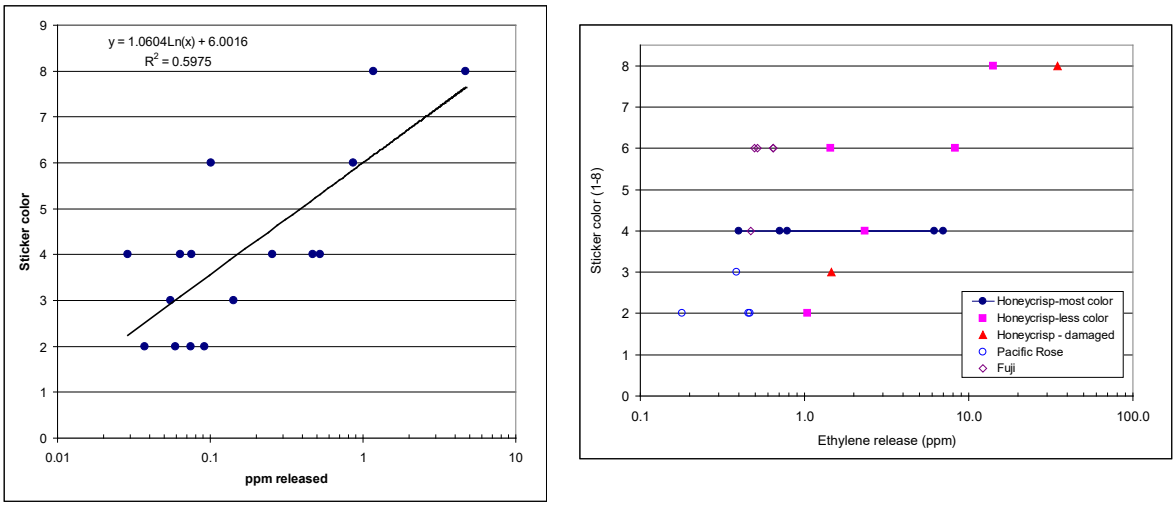

Figure 6: Sticker color development as a function of ethylene release in ppm. This shows a variety of response behaviors based on the fruit cultivar. Measurements performed on 9/28/2007-3 days after application, 2nd pick timing.

An aspect of this work which we believe is critical to assessing the utility of the sticker device is the connection between ethylene release and other standard measures of fruit maturity. These are shown in Figure 7 for the late September test. In short, there appears to be no significant correlation between ethylene release and fruit weight, firmness, starch, color, or SSC.

Conclusions from Dr. Hanrahan:

> Based on our observations, the stickers do pick up ethylene emitted by apples. Environmental factors, especially relative humidity, did not interfere with proper readings in our study. Once apples start to emit ethylene, it appears to dissipate evenly across the fruit surface. Hence, the actual position of the stickers on the fruit does not interfere with the ethylene readings. For the purpose of repeatability we suggest placing stickers around the equator, on the north-facing side of the fruit.
>
> Besides the Honeycrisp trial or when fruit was not intended for storage, commercial harvest always preceded any kind of sticker reaction. Hence, with the current sensitivity the stickers would not be suitable as harvest prediction tool for apples in Washington State. Replicating RediRipe's benchtop tests under field conditions to establish reliable sticker readings in the ethylene range found in apples around commercial harvest timing (0-1ppm) would be of utmost importance.

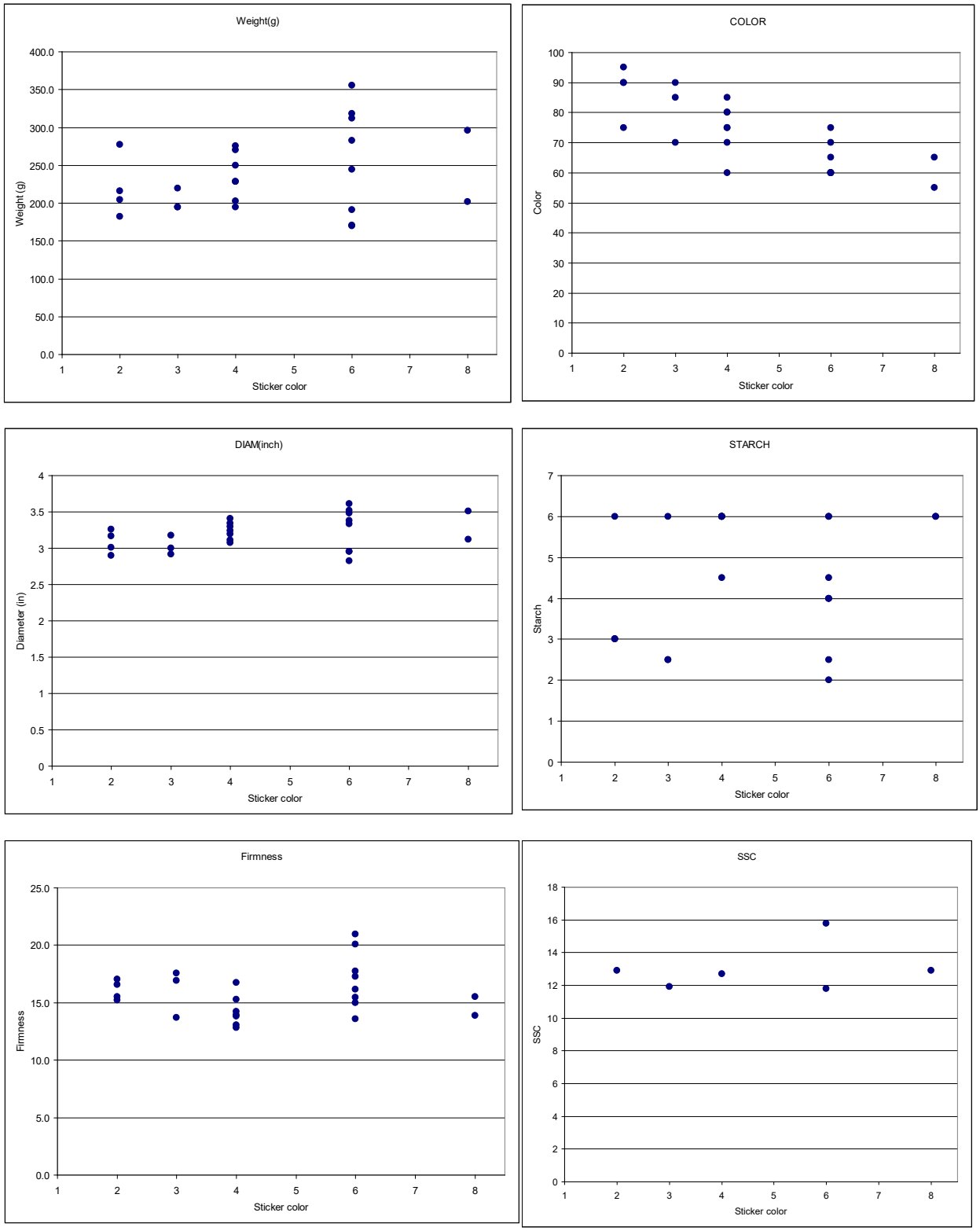

Figure 7: Maturity data with development of sticker color.

Following are suggestions for sticker utility improvement from Dr. Hanrahan:

Without direct negative control, correct reading of the stickers in the field is difficult, even with available color scales on paper. In order to simplify the reading, color gradients could be placed directly next to the sticker and the sticker itself should have a bigger surface area.

Ethylene is indicative of physiological maturity, which does not always correlate well with commercial maturity. Other factors such as background color, starch movement/amount of soluble solids/firmness are widely used by the fruit growing community instead. Current research suggests that starch depletion as well as background color are the closest related to actual maturity. To date researchers were unsuccessful when trying to relate ethylene production, or maybe more precisely the onset of ethylene production to commercial maturity.

It seems like the most practical use for the sticker would be in any kind of scenario that requires presence/absence readings of ethylene such as:

- After treatment of fruit with ethylene synthesis or action inhibitors (i.e. ReTain, Smartfresh), for example: determine exact ReTain timing in the field; assess if and when a certain lot of fruit has 'awaken' after storage and Smartfresh treatment in the warehouse
- To verify when to start preconditioning of fruit such as winter pears
- Document effectiveness of ethylene applications in the field and post-harvest for example to assess: return bloom on apples, stem loosening of sweet cherries prior to mechanical harvest, or preconditioning of winter pears.

Another use would be to explore how each apple variety reacts. For example, Gala apples generally produce about 1ppm right around commercial maturity, hence would be a candidate for the sticker, given reliable sensitivity in that range. We do not know a lot about newer varieties, and one would have to look at ethylene curves closely. For example, some variety might have an ethylene peak much smaller than the climacteric a certain time before commercial maturity, for example Honeycrisp.

Discussion

In the past several years we have developed a simple device that responds to ethylene released by individual fruits. We have improved the device formulation with much of the progress occurring as a result of orchard trials performed in 2006 and 2007. The results indicate that the Durapore stickers are reasonably well able to respond to 1 ppm ethylene while requiring 24-48 hours for full color development. The missing element here is the inability to correlate sticker color with other indices of fruit maturity. It appears that utilizing this device for apple orchard assessment provides minimal advantage to the grower for the cultivars and conditions tested here. We are willing to address other applications of the device, including those suggested by Dr. Hanrahan.

We wish to thank the Washington Tree Fruit Research Commission and its Manager James McFerson, PhD, for their encouragement and generous support.

FINAL PROJECT REPORT
WTFRC Project Number: 3055-7938

Project Title: Identifying disease prevention benefits of apple consumption

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Cooperators: Dr. Ines Hanrahan and Mr. Tom Auvil, WTFRC
Stemilt Growers, Inc., Wenatchee

Other funding Sources

Agency Name: No other funding sources for this project
Amount awarded:
Notes:

Total Project Funding: $94,617

Budget History:

Item	**Year 1:** 2005-06	**Year 2:** 2006-07	
Salaries	19,320	20,286	
Benefits	1,789	1.862	
Wages	8,000	8,000	
Benefits	880	880	
Equipment			
Supplies	16,000	16,000	
Travel	800	800	
Miscellaneous	0		
Total	**46,789**	**47,828**	

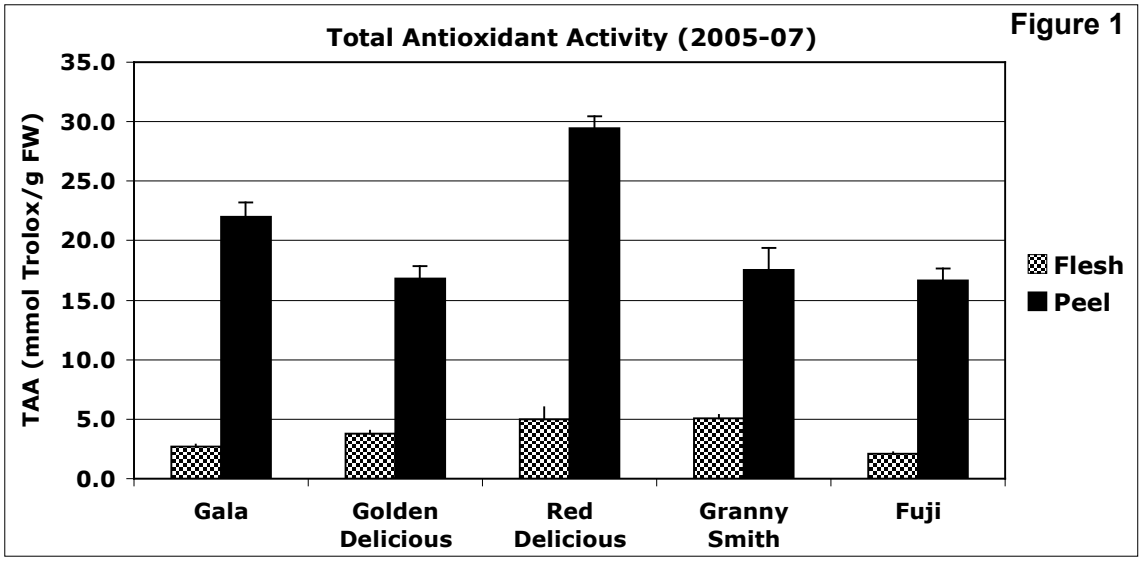
SIGNIFICANT FINDINGS:

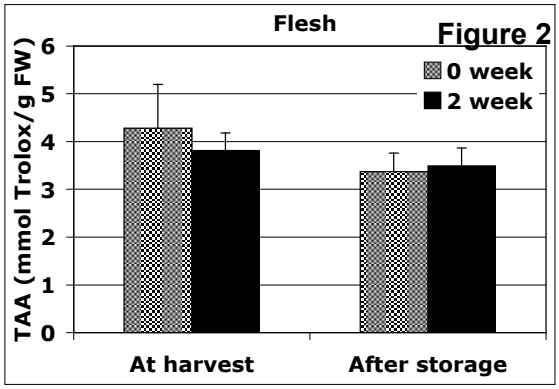
- We determined that **anti-oxidant activity** was consistently and many fold higher in peel tissue than flesh tissue of Gala, Golden Delicious, Red Delicious, Granny Smith, and Fuji apples, with the highest activity in Red Delicious peel. There was little loss in anti-oxidant activity after two-week shelf-life periods and CA storage.
- We determined that **vitamin C** (ascorbic acid) concentrations were also many fold higher in peel than flesh tissue of these varieties, however, they decreased significantly after two-week shelf-life periods at harvest and after CA storage.
- We determined that the concentrations of **phenolic compounds** were many fold higher in peel than flesh tissues, and showed no decline after two-week shelf-life periods either at harvest or after CA storage. Therefore, certain phenolic compounds (i.e. flavonoids) are robust and contribute significantly to the long-lasting anti-oxidant activity of apples that are un-refrigerated or are stored for significant periods of time.
- To quantify **flavonoids** in apples we developed a novel high-performance liquid chromatography (HPLC) method to analyze the glycosides and aglycones of quercetin, kaempferol, phoretin, naringenin enantiomers, and ellagic acid.
- Using this HPLC method, we determined that Gala, Golden Delicious, Red Delicious, Granny Smith, and Fuji apples contained all of the **flavonoids** measured, and that generally there were higher concentrations of them in peel than in flesh tissues, with little loss after two-week shelf-life periods at harvest and after CA storage.
- We recently acquired the instrumentation to separate, identify, and quantify 26 different phenolic phytochemicals using a modified liquid chromatography–mass spectrometry–electrospray ionization (LC/MS/ESI) method.
- We determined that extracts from the peel and flesh tissues of Gala and Red Delicious apples were active, in a dose-dependent manner against *in vitro* cells lines of colorectal, breast, and prostate **cancers**.
- We developed and validated an *in vitro* **anti-inflammatory** assay in canine chondrocytes (i.e. cartilage) using biomarkers for nitric oxide (NO), sulphated glycosaminoglycans (sGAG), and (prostaglandin E3) PGE_2 to quantify the anti-inflammatory activity of Gala and Red Delicious extracts.
- We developed and validated an *in vitro* **anti-adipogenesis** assay to quantify the inhibitory activity of Gala and Red Delicious extracts to the accumulation of triglycerides in a pre-adipocyte cell line.

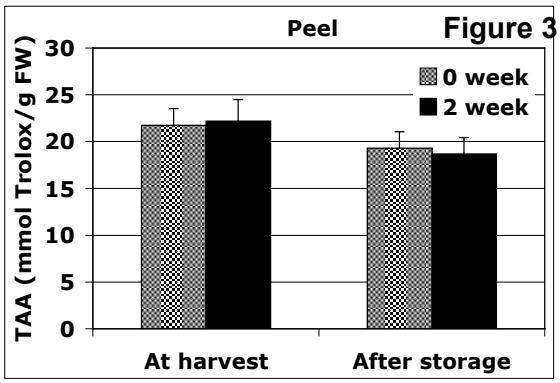
RESULTS AND DISCUSSION:

Fruit sampling and maturity. During 2005 and 2006 we received freshly harvested Gala, Golden Delicious, Red Delicious, Granny Smith, and Fuji apples from orchards in north-central Washington, with sub-samples stored in commercial CA storage until 2006 and 2007, respectively. Upon receipt of either freshly harvested or CA-stored fruit, some of the apples were separated into peel and flesh tissues, which were immediately frozen and powdered in liquid nitrogen and stored in an ultralow temperature (-80^{o}C) freezer for later analysis. Another sub-sample of fruit was left out in the laboratory at room temperature for two weeks before freezing. The average fruit weight and maturity (firmness, soluble solids, and starch index) of each sample was determined. The maturity data will be correlated with biochemical assays and disease inhibition models in scientific publications.

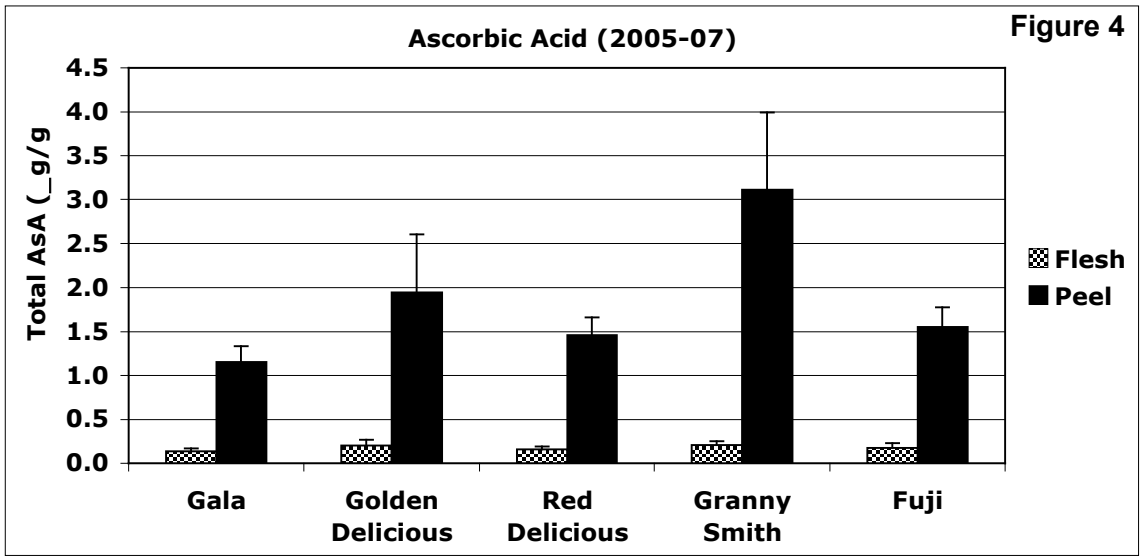
Anti-oxidant activity. We tested peel and flesh tissues of all varieties for anti-oxidant activity using an established ABTS method (1, 2). Peel tissue had approximately 5X higher total anti-oxidant activity (TAA) than flesh tissue, and Red Delicious peel had the highest activity (Fig. 1). There were no significant losses in activity during CA storage or after a two-week, non-refrigerated shelf life (Figs. 2-3). (Error bars in all graphs indicate standard error of the mean.)

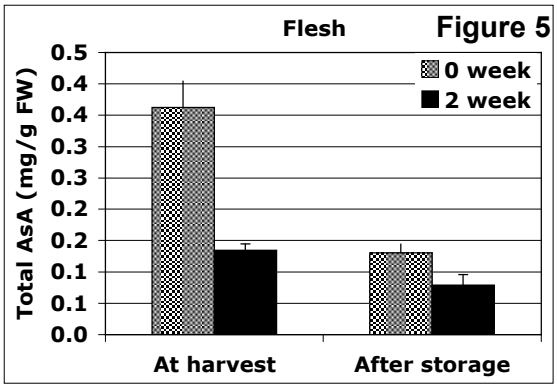


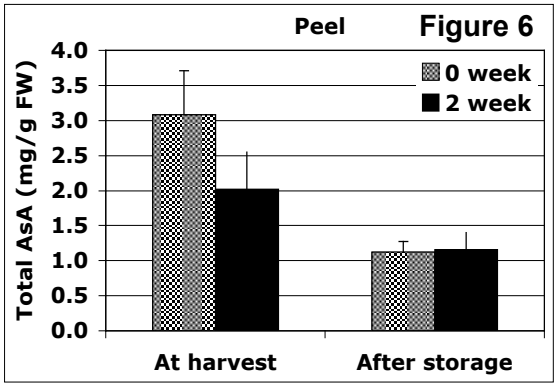




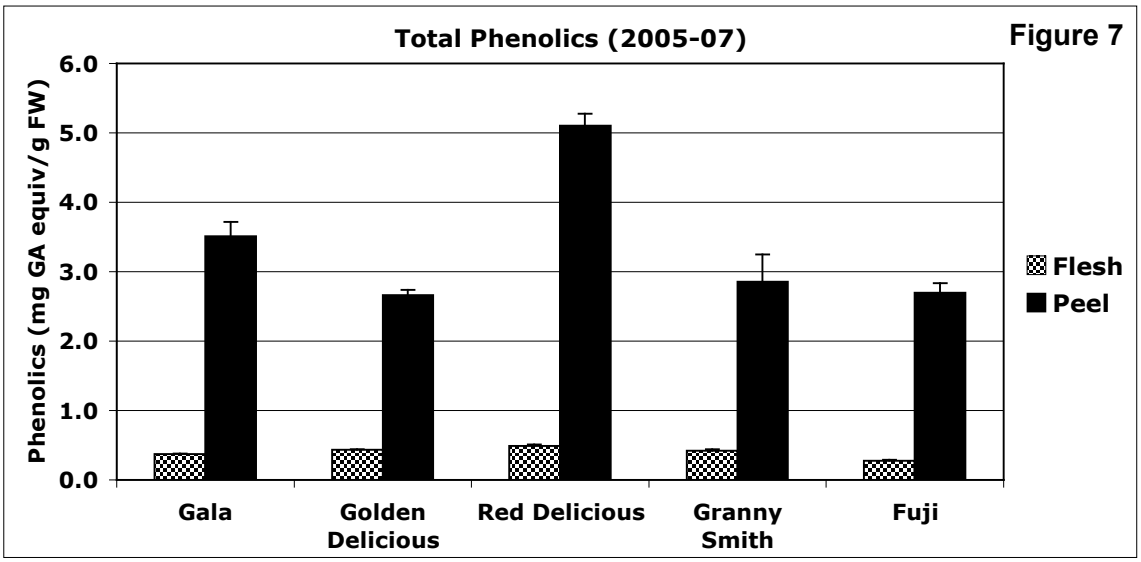
Vitamin C. Concentrations of total ascorbic acid (AsA), measured with a method adapted for apples (3), were up to 15X higher in peel than in flesh tissue of these varieties (Fig. 4), however, they decreased significantly after two-week shelf-life periods both at harvest and after CA storage (Fig. 5-6). Granny Smith peel had the highest concentration of vitamin C (Fig. 4).

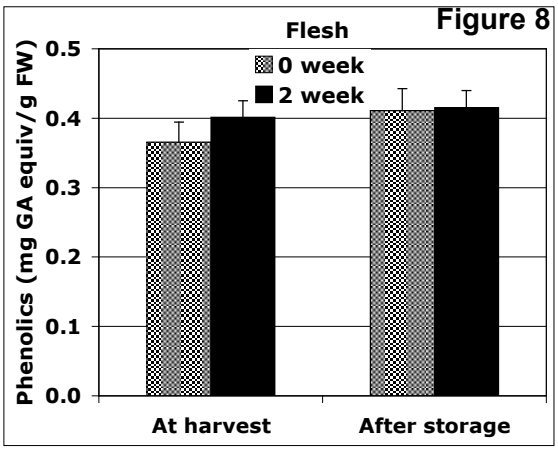


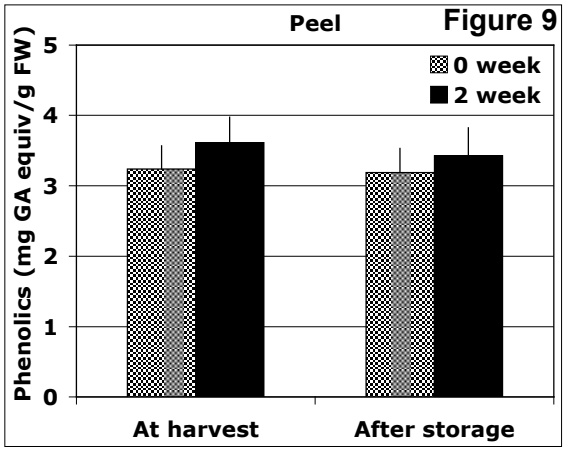




Phenolics. Concentrations of total phenolic compounds, measured as gallic acid (GA) equivalents using Folin-Ciocalteau reagent (4), were nearly 10X higher in peel than flesh tissue, and showed no decline after two-week shelf-life periods either at harvest or after CA storage (Figs 7-9). Phenolic phytochemicals, probably flavonoids, are robust and contribute significantly to the long-lasting antioxidant activity of apples that are either left un-refrigerated or are stored for months in CA storage.

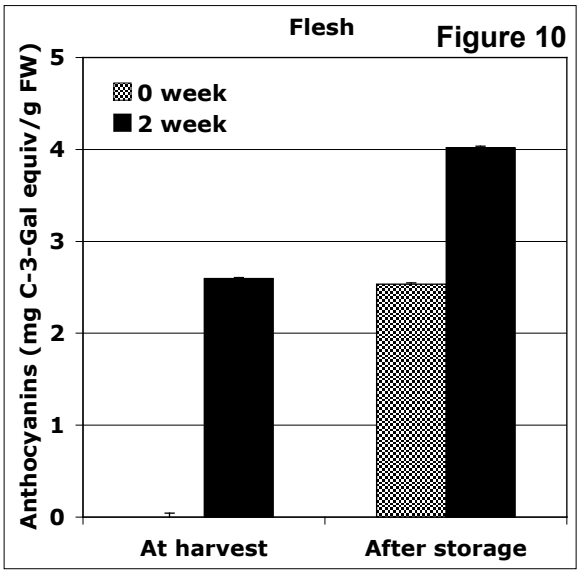




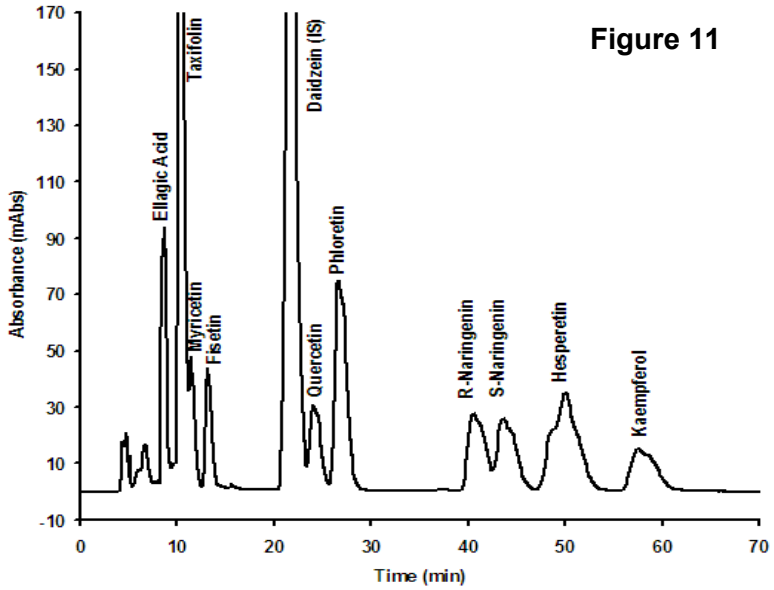


Anthocyanins. Total anthocyanins (a class of flavonoids), extracted with 1% HCl in methanol, were expectedly found in the peel tissue of Gala, Fuji, and especially Red Delicious apples (not shown). There was only a slight loss in anthocyanins (cyanidin-3-galactoside equivalents) in peel tissue after CA storage, and no loss after the two-week shelf-life period (not shown). Despite the

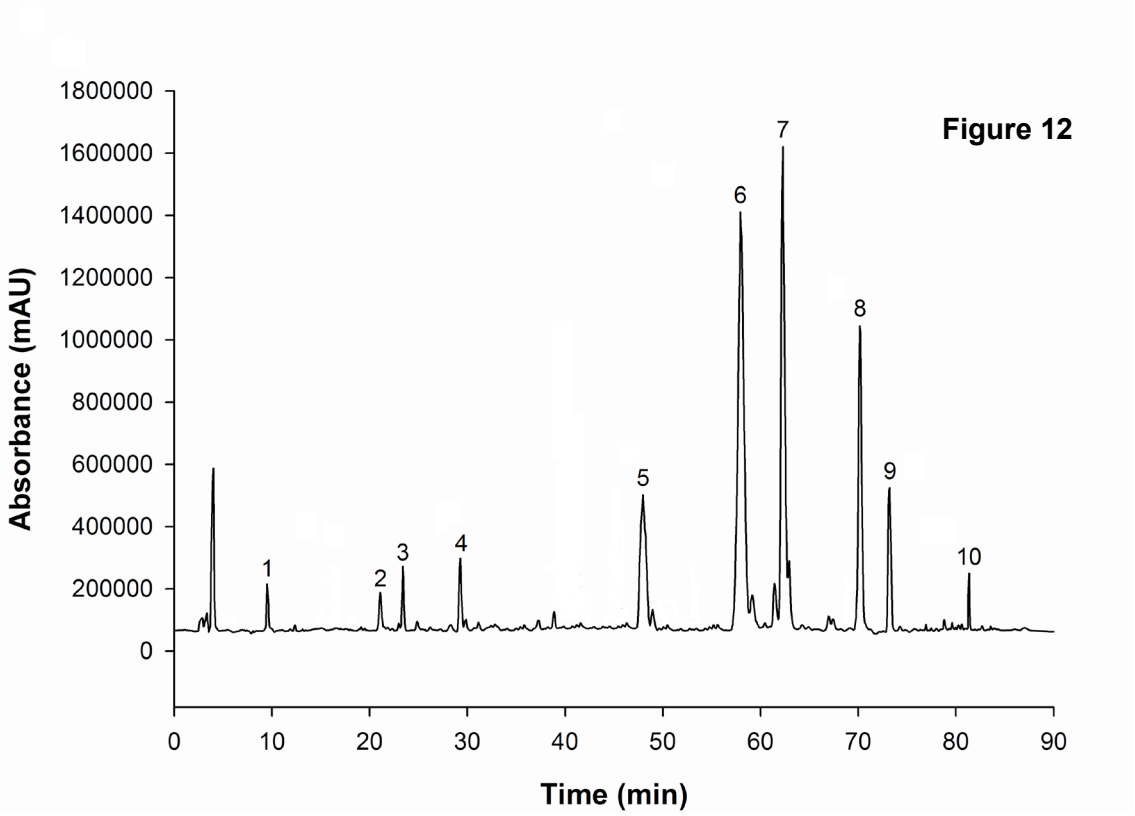
small quantities of anthocyanins in flesh tissue, there was surprisingly an increase after the shelf-life period both at harvest and after CA storage (Fig. 10).



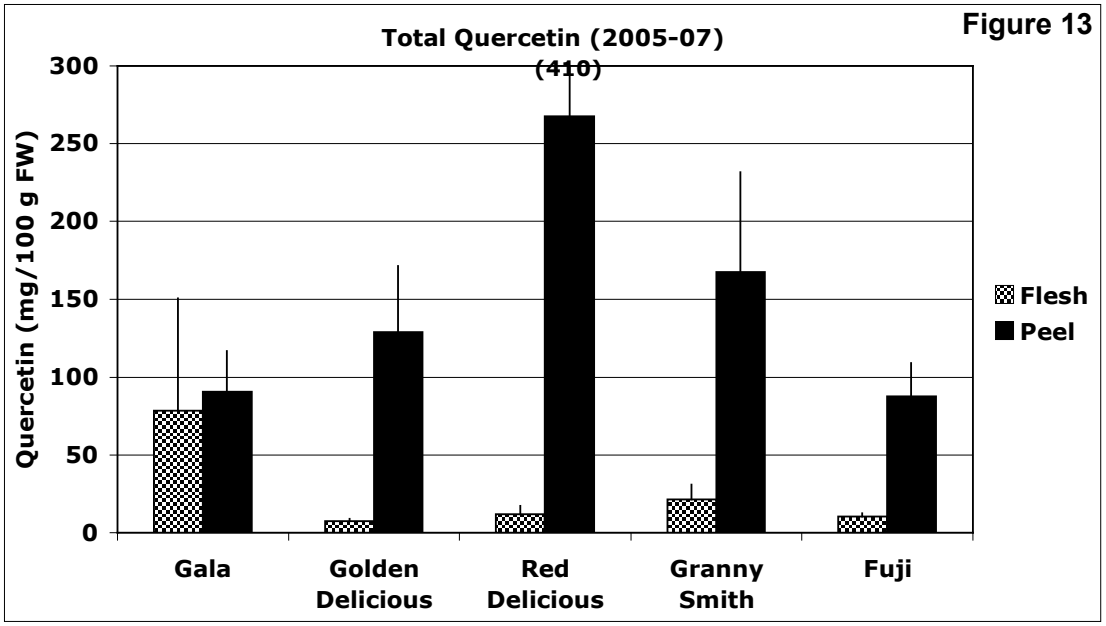
Flavonoids. To quantify flavonoids in apples we developed a novel high-performance liquid chromatography (HPLC) method to simultaneously analyze several of these important phytochemicals, including the glycosides (i.e. with attached sugars) and aglycones (i.e. without sugars) of quercetin, kaempferol, phoretin, naringenin enantiomers, and ellagic acid (Fig. 11).

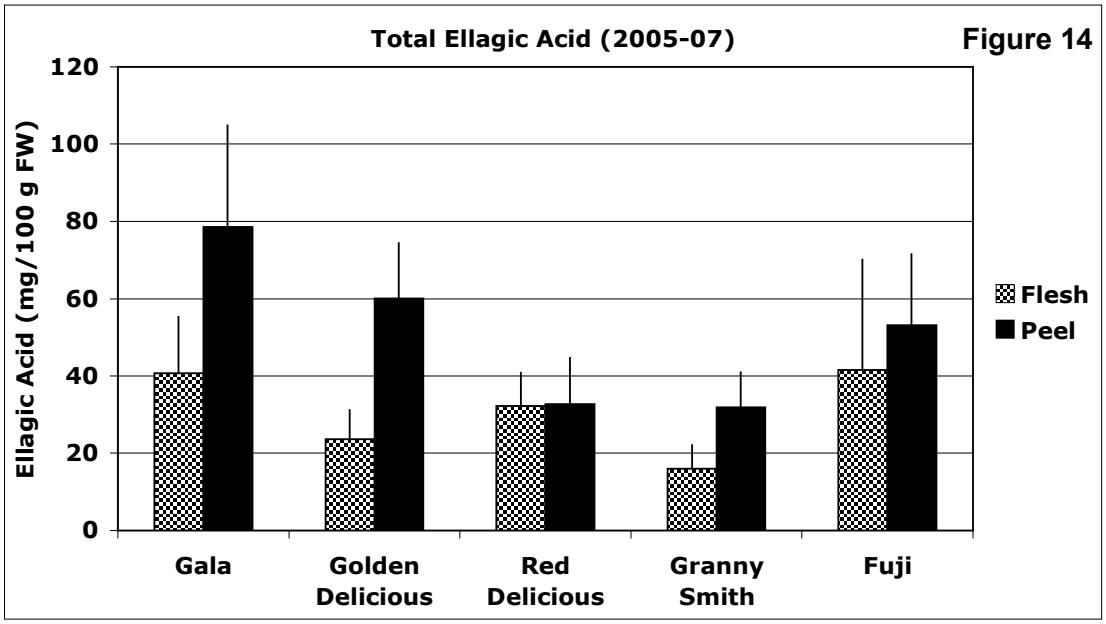


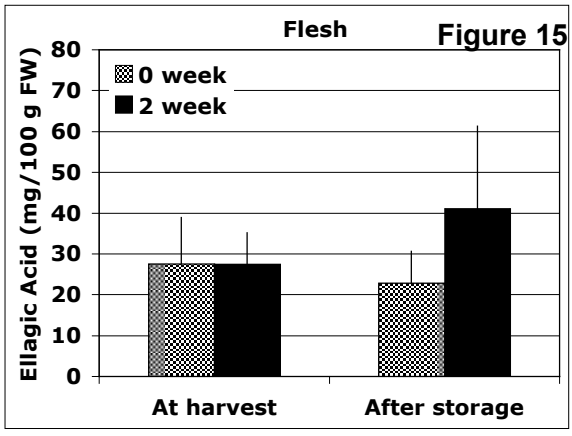
We recently acquired the instrumentation to separate, identify, and quantify 26 different phenolic phytochemicals using a modified liquid chromatography–mass spectrometry–electrospray ionization (LC/MS/ESI) method. We adapted a previously published methodology (5) with this instrument in order to detect nine different phenolic compounds in apple tissue (Fig. 12). [Graph legend: 1=gallic acid, 2=catechin, 3=chlorogenic acid, 4=epicatechin, 5=quercetin-3-rutinoside (rutin), 6=quercetin-3-rhamnoside (quercitrin), 7=phloridzin, 8=daidzein (internal standard), 9=quercetin, and 10=phloretin]

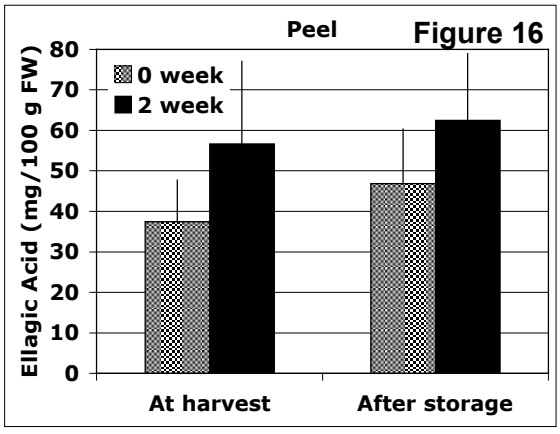


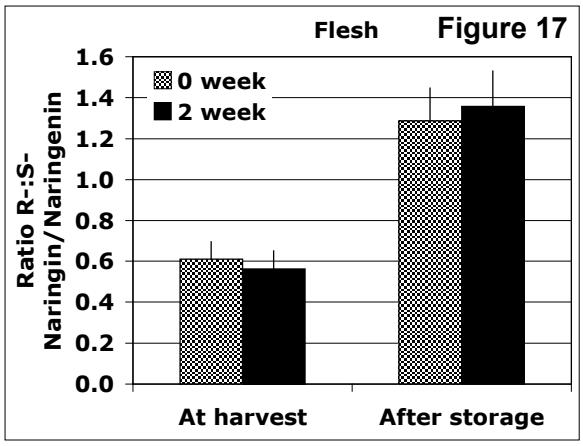
Using the HPLC method of analysis, we generally found higher concentrations of specific flavonoids in peel tissue than in flesh tissue, although in some cases variability was high resulting in large error bars. Quercetin, a predominant apple flavonol (6), was greater in peel tissue of most varieties, with highest concentrations in Red Delicious peel despite the large variability (number in parenthesis indicates maximum value of error bar) (Fig 13). Ellagic acid, primarily found in raspberries and strawberries (7), was measured in significant concentrations in both flesh and peel tissues of these apple varieties (Fig. 14), and may even increase following a two-week shelf-life period (Figs 15-16). Naringenin, known as a citrus flavonone found primarily in grapefruit (8, 9), is unique among the measured flavonoids because it exists naturally as a chiral compound with mirror image enantiomers designated R and S. Both the glycoside and aglycone forms of R- and S-naringenin were found in small concentrations in both the flesh and peel tissues of all varieties, and after a two-week shelf-life period and CA storage (not shown). Their occurrence in apple has not been previously reported. Interestingly, there was inter-conversion between R- and S-enantiomers from harvest to storage, with freshly harvested fruit having relatively more S-naringin/naringenin and fruit from CA storage having relatively more R-naringin/naringenin (Fig. 17). These differences in enantiomer composition could be important to the bioactivity of these enantiomers in human tissue because differences in pharmacokinetics have been shown for them (10). There were also differences in the relative amount of glycosides (with sugars) and aglycones (with sugars) among these flavonoids, with glycosides usually decreasing relative to aglycones following CA storage (Fig 18). These flavonoids are variously known to possess anti-oxidant, anti-cancer, and anti-inflammatory properties, which may aid in the prevention of heart, respiratory, and neurological diseases, diabetes, allergies and infections, and generally promote immune system responses (11, 12).

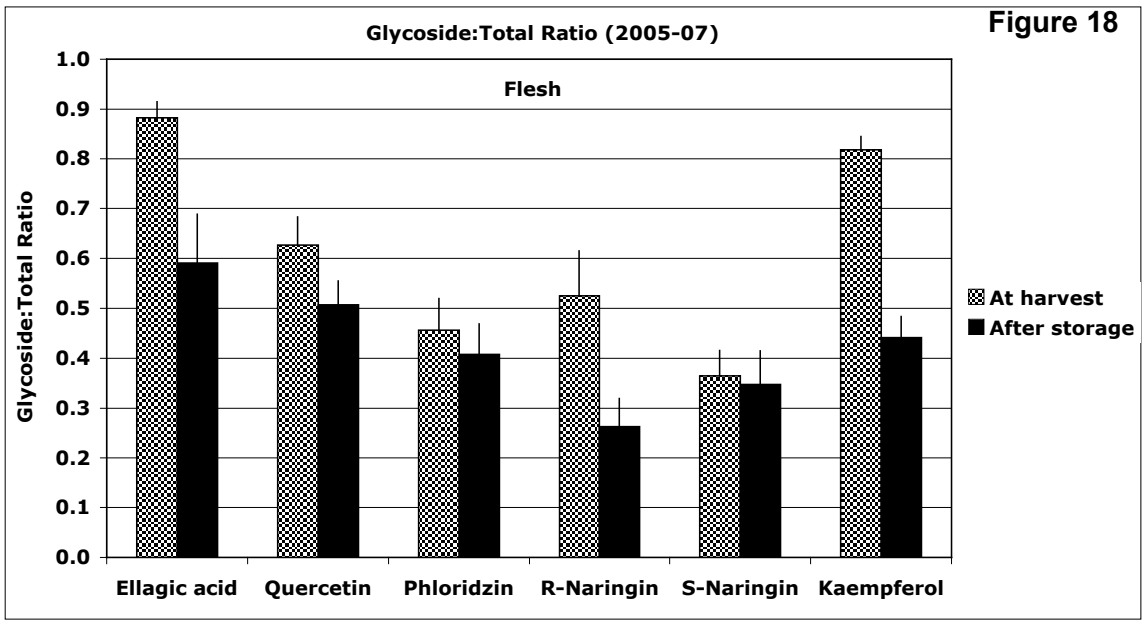

Total Quercetin (2005-07)
(410)
Figure 13
Quercetin (mg/100 g FW)
300
250
200
150
100
50
0
Flesh
Peel
Gala
Golden Delicious
Red Delicious
Granny Smith
Fuji


Total Ellagic Acid (2005-07)
Figure 14
Ellagic Acid (mg/100 g FW)
120
100
80
60
40
20
0
Flesh
Peel
Gala
Golden Delicious
Red Delicious
Granny Smith
Fuji

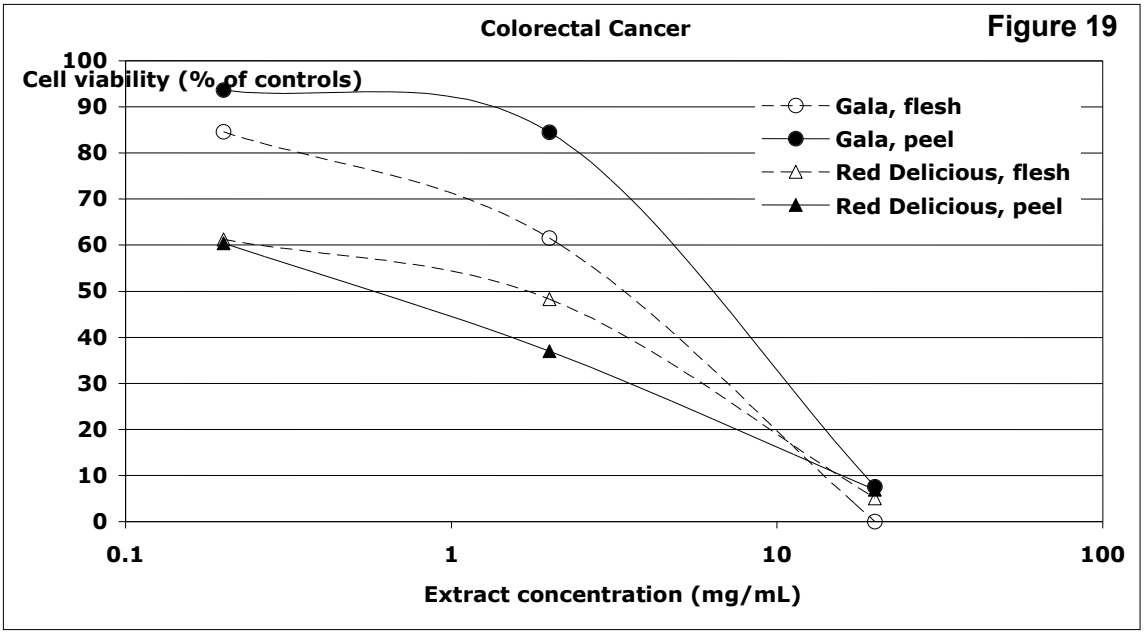

Flesh
Figure 15
Ellagic Acid (mg/100 g FW)
80
70
60
50
40
30
20
10
0
0 week
2 week
At harvest
After storage

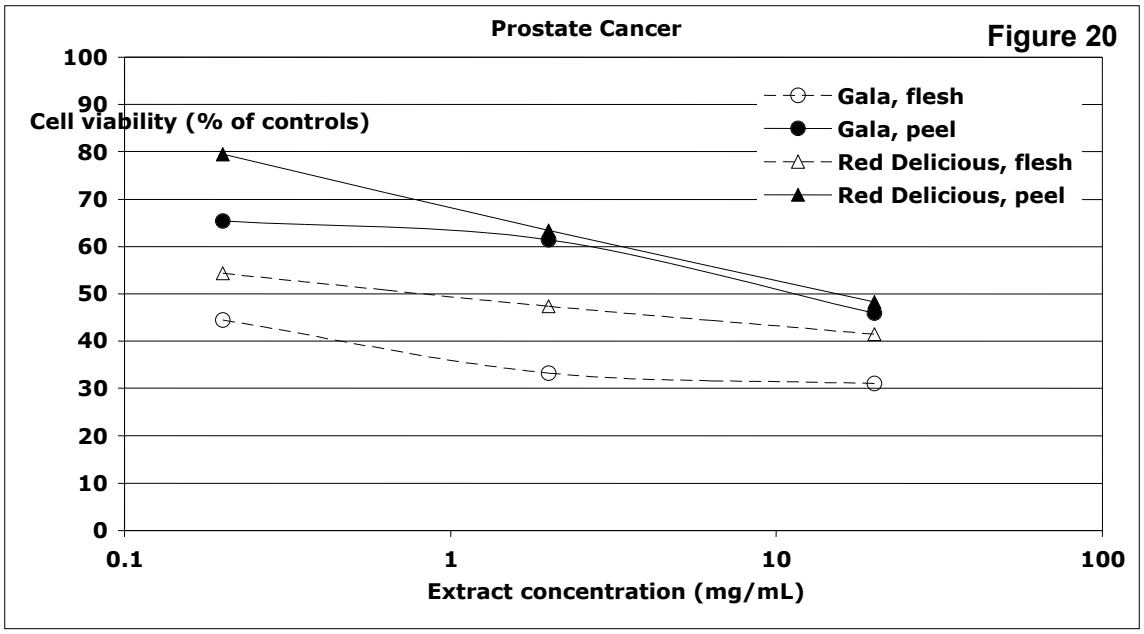

Peel
Figure 16
Ellagic Acid (mg/100 g FW)
80
70
60
50
40
30
20
10
0
0 week
2 week
At harvest
After storage

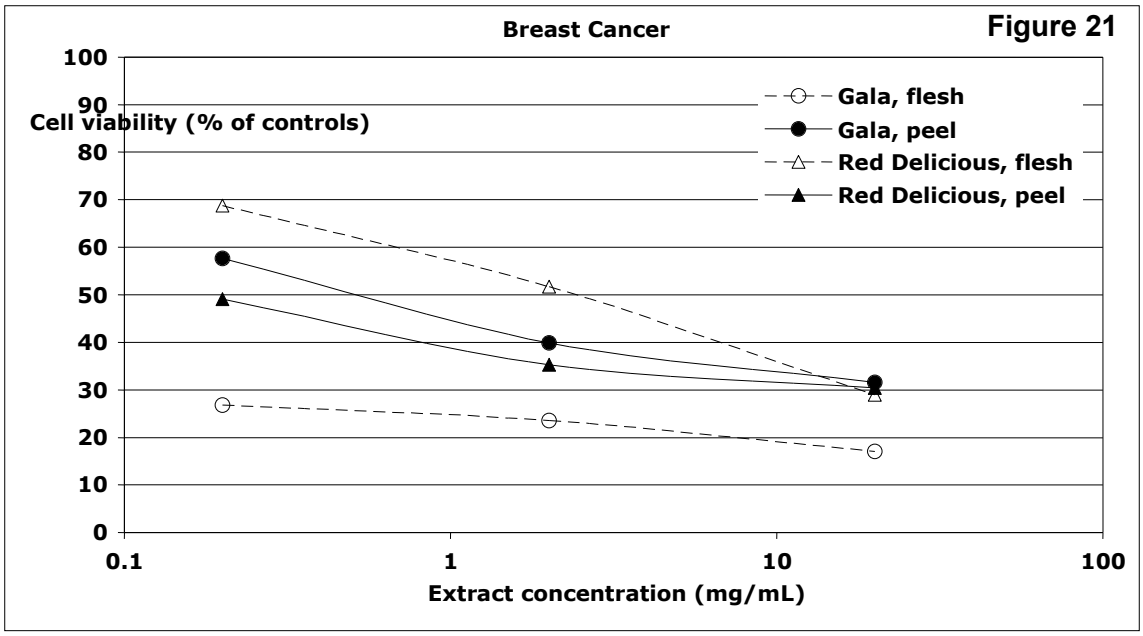




Anti-cancer activity. Extracts of flesh and peel tissues of Gala and Red Delicious apples were active, in a dose-dependent manner against colorectal (HCT-116), breast (MDA-MB-231), and prostate (PC-3) adenocarcinoma cancer cell lines using an *in vitro* Alamar Blue (resazurin) fluorescent dye assay to determine cytotoxicity. Both peel and flesh extracts of Gala and Red Delicious reduced colorectal cancer cell viability, especially when sugars were cleaved from the flavonoids by enzymatic hydrolysis, although Red Delicious was more active than Gala (Fig. 19). Against prostate cancer cells, after cleavage of sugars from the flavonoids, the Gala flesh extract was most inhibitory followed by Red Delicious flesh extract (Fig. 20). Again, the aglycone Gala flesh extract was most inhibitory against breast cancer cells, followed by the peel extracts of both Red Delicious and Gala apples (Fig. 21).


Colorectal Cancer
Figure 19
Cell viability (% of controls)
100
90
80
70
60
50
40
30
20
10
0
0.1
1
10
100
Extract concentration (mg/mL)
Gala, flesh
Gala, peel
Red Delicious, flesh
Red Delicious, peel


Prostate Cancer
Figure 20
Cell viability (% of controls)
100
90
80
70
60
50
40
30
20
10
0
0.1
1
10
100
Extract concentration (mg/mL)
Gala, flesh
Gala, peel
Red Delicious, flesh
Red Delicious, peel



Anti-inflammatory activity. Inflammatory bowel activity was measured using an *in vitro* colitis model in colorectal adenocarcinoma (HT-29) cancer cells after inflammatory insult with tumor necrosis alpha (TNF-α) and measuring prostaglandin E_2 (PGE_2) levels. Quantification of the anti-inflammatory activity of flesh and peel tissues of Gala and Red Delicious apples is still underway, and so, the results of this disease model will be presented at the research review meeting.

Anti-adipogenic activity. This method replaced the proposed anti-hyperlipidemia disease model, because of its direct relevance to obesity. Pre-adipocyte (3T3-L1) cells were chemically induced to accumulate triglycerides, and then challenged with apple extracts from Gala and Red Delicious flesh and peel tissues. Adipogenesis was evaluated using a commercially available adipogenesis assay kit that stains triglycerides in the adipocytes. Lipid droplets are visualized microscopically and quantified by measuring absorbance at 492 nm. Quantification is still underway, and so, the results of this disease model will be presented at the research review meeting.

Significance and impact. The presence in five major Washington apple varieties of both known and novel phytochemicals that are active in various disease models is a very positive finding of this research. These phytochemicals, which represent different classes of flavonoids, appear to be durable to both extensive refrigerated CA storage and shelf-life periods at room temperature. These flavonoids have powerful anti-oxidant properties, which may contribute in large measure to the long-lasting anti-oxidant activities of Washington apples.

Two of the major apple varieties grown in Washington, Gala and Red Delicious, exhibited pronounced anti-cancer activities against three major cancers – colorectal, breast, and prostate. The expected anti-inflammatory and anti-adipogenic properties of these apple varieties will also provide strong evidence for the health benefits of consuming Washington apples. It is known that inflammation plays a role in gastrointestinal and other disease states. Increased consumption of apples, as well as other fruits and vegetables, could help counter various inflammatory disease states and the growing U.S. obesity epidemic (14).

This research provides a strong basis for claims regarding the health benefits of consuming Washington apples, which could be utilized by Washington apple growers to enhance their marketing efforts. However, more detail studies should be undertaken to identify other beneficial phytochemicals in Washington apples, to determine how the environment and crop management practices influence their contents in apples, and the role that these phytochemicals in apples play in preventing various diseases. These efforts would provide additional evidence for the presumed health benefits of apple consumption.

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FINAL PROJECT REPORT
WTFRC Project Number: 2005-09 and AP-06-606

Project Title: Estimating Apple firmness using Tensile Mechanical Properties

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Cooperators:

Other funding Sources: None

Agency Name:
Amount awarded:
Notes:

Total Project Funding: $60,073

Budget History:

Item	**Year 1:** 7/2005 – 6/2006	**Year 2:** 7/2006 – 1/2007
Salaries	6 478	11 622
Benefits	2 656	4 251
Wages	2 720	0
Benefits	272	0
Equipment	800	0
Supplies	573	400
Travel	1 500	800
Sensory Panel	15 000	13 000
Miscellaneous	0	0
Total	30 000	30 073

Introduction and Summary

This report covers the activities performed in Fall 2005 and Spring and Fall of 2006 comparing the tensile and compressive mechanical properties of apples and pears to human sensory intensity ratings of texture, and the Guss Penetrometer. The project was funded by WSTFRC grants awarded in August 2005 and February 2006. Data was collected in Fall 2005, Spring 2006 and Fall 2006.

Following Spring 2005 (year 1), we refined existing protocols for future data collections. These revisions were based on results from the Spring 2005 tests which indicated a low correlations between the mechanical properties and sensory evaluations. Revisions for Year 2 of the study (Fall 2006) included selection of apple and pear varieties, sample size and loading tests to measure the mechanical properties. Specifically, in Fall 2006, we compared the mechanical properties of apples and pears to sensory evaluations of texture, Sinclair (nondestructive) and the Guss (destructive) Penetrometer. Analysis of the Fall 2006 data indicate a relatively strong correlation between one sensory evaluation measurement (crispness) and the tensile material properties of apples and pears.

Objectives:

2005

1. Determine if human perceived apple firmness is related to the tensile material properties (elastic modulus, failure stress) of tissues from cultivars of apples and pears commercially grown in Washington State.
2. Determine if there is a relationship between the tensile material properties of the apple and pear varieties and the firmness pressure test originally developed by Magness and Taylor and refined over the years.

2006

1. Confirm 2005 results.
2. Develop design tools for use in designing and evaluating destructive and nondestructive firmness sensors.

Significant Findings:

Fall 2005

- Developed procedure (sample size, loading rate, photographic settings) to measure tensile forces in apple and pear tissue
- Weak correlations between tensile mechanical properties (elastic modulus, failure stress and failure strain) and compressive material properties (elastic modulus, failure stress and failure strain) in both apples and pears.
- Poor correlations between tensile mechanical properties and Guss Penetrometer measurement of firmness in both apples and pears
- Good correlations between compressive mechanical properties and Guss Penetrometer
- As the apple or pear matures, the tensile elastic modulus decreased more rapidly the compressive elastic modulus

Spring 2006

- Good correlations between compressive material properties and sensory evaluations
- Good correlations between Guss Penetrometer and sensory evaluations
- Good sensory correlations between Guss Penetrometer and compressive material properties
- Poor correlations between tensile material properties (measured in an orientation perpendicular to the core line) in apples and sensory evaluations (crispness, hardness, juiciness , chewiness and fracturability) lead to a redesign of the experimental techniques used in Fall 2006

Fall 2006

- Good correlations between tensile material properties (measured in an orientation parallel to the core line) in apples and pears and at least one sensory evaluation (crispness)

- Good correlations between compressive material properties and some sensory evaluations (hardness, fracturability).
- Good correlations between compressive material properties and Guss Penetrometer
- Good correlations between Guss Penetrometer and sensory texture attributes (hardness and fracturability)
- Computer models of typical apples and Anjou pears were constructed and verified.

Methods

The three testing sessions (Fall 2005, Spring 2006 and Fall 2006) used similar methods to select fruit, conduct the sensory evaluation, and measure the mechanical properties. This common methodology is described below.

Fruit Selection

Spring 2006

Gala, Granny Smith, Braeburn, Red Delicious, Golden Delicious apples were removed from air storage in late February 2006 and transported to WSU Pullman. the apples selected had a range of firmness values when evaluated by the sensory panel – a difficult prediction task. To increase the likelihood of having apples with a range of firmness values, we identified apples from historically strong and weak lots of apples. Fruit was screened twice, once nondestructively with Sinclair and samples tested destructively in Wenatchee. In February 2006, apples from these lots were pressure tested with the Slinclair nondestructive firmness to ensure a wide range of firmness.

Fall 2006

Apples were selected in Wenatchee as follows:
Golden Delicious - soft overmature fruit provided from an orchard, stored in air storage. Selected as being less than 13.5 lbf and less than 34 on the Sinclair.
Gala - soft fruit provided from an orchard stored in air. Less than 14.0 lbf and less than 37 on the Sinclair.
Red Delicious - medium firmness provided from an orchard stored in air. 15-16 lbf, not correlated with the Sinclair.
Braeburn - firm fruit again from an orchard stored in air. 19-20 lbf and above 49 on the Sinclair
Granny Smith - firm fruit from a commercial packer. Stored in air and commercially sorted. 18-20 lbf and above 38 on Sinclair.

Pears were selected in Wenatchee as follows:
Anjou pears - from an orchard stored in air.
Bosc and Bartlett pears purchased from a commercial packer stored in air.

Classification of apples for sensory evaluation

Apples from regular cold storage (1-3°C) were brought up to room temperature 24 hours before analysis. Prior to evaluation by the sensory panels, the fruit were characterized using instrumental measures of hardness using the Guss Penetrometer and the Sinclair iQ. These measurements were performed by the Kupferman group in Wenatchee and apples arrived in Pullman, characterized by their hardness level. On the day of the sensory evaluation panels, the measurements using the Guss Penetrometer and the Sinclair iQ were verified as some time had elapsed between the original measurements.

Trained sensory evaluation panel

A sensory panel of 10 panelists (2005) and 17 panelists (2006) was recruited using advertising in the WSU/Pullman community. Panelists were screened for any known allergies and anosmias. Panelists will be trained to recognize the apple texture attributes of hardness, juiciness, crispness and fracturability as defined in Table 1. In 2005, the panelists were also trained to recognize chewiness; however, this attribute was excluded from evaluations in 2006 as it was not found to yield significant

results. The texture attributes were selected based upon previous literature. For training, published texture scales were used for the different texture attributes and panelists were trained to both recognize the attribute and assign it an intensity rating. Fruit of varying texture intensities and different varietals were used for the training process.

Table 1: Texture attributes of apples that were evaluated during the 2005 and 2006 trained sensory panels.

Texture:	
Hardness	Force required to bite completely through sample placed between molars
Crispiness	Amount of pitch of sound generated when the sample is first bittern with the front teeth
Juiciness	Amount of juice released on mastication in the first three chews
Fracturability	Force with which sample ruptures when placing sample between molars and biting down completely at a fast rate

During apple evaluation, panelists were presented with 6 sections of apple per evaluation session and these sessions were replicated. Following apple classification by hardness level, apples from the low, medium and hardness groupings were split in half. Half of the sample was used for tensile property measurement and half of the apple was used for sensory testing. The apple was labeled such that the sensory data and the tensile data for that apple could be compiled. The half that was used in the sensory testing was split in half. Thus, each panelist was presented with ¼ of a washed apple for evaluation and a knife to peel his/her own fruit.

Evaluations took place in individual sensory booths equipped with lap top computers for recording data. The apple sections were randomly presented to the panelists at room temperature. Apple selections were identified using three-digit codes and presented one at a time to panelists. Each panelist was provided with water to rinse between samples as well as a cuspidor for sample expectoration. The samples were scored for intensity of each texture attribute using a 15-cm unstructured line scale, with the left end of the scale corresponding to the lowest intensity (0 mm=absent) and the right end corresponding to the highest intensity (150 mm=extreme). Results were collected and analyzed using Compusense 6.0 software (Guelph, ON) and sensory data was quantified by measuring the distance of the mark along the line.

Mechanical Properties

Compressive elastic modulus, failure stress and failure strain

Cylindrical tissue samples 15 mm in length and 9.22 mm in diameter were excised from the fruit. In the Fall 2005 and Spring 2006 tests, these the centerline of these samples was perpendicular to the core line of the fruit. Based on the redesign of the experimental techniques following the Spring 2006 test, in the Fall 2006 test the samples' center line was parallel to the core line of the fruit. The cylinders were compressed to failure between the parallel plates of a universal testing machine (Fall 2005, Instron Model 1350, Spring and Fall 2006, Texture Analyzer TAXT2 by SMS). Force and deformation data was collected at intervals of 10 milliseconds. Stress values were computed from the recorded force data and sample diameter; strain values were computed from recorded deformation data and the original length of the sample. From the stress and strain data the compressive elastic modulus (slope of the stress vs. strain data), failure stress and failure strain values were computed.

Tensile elastic modulus

Measuring the tensile material properties of fruit tissue is problematic due to the difficulty of forming and gripping a suitable test specimen. In our tests, the failure mechanical properties were computed

using a bending apparatus and image analysis. Central to this analysis is the determination of the location of the neutral axis – the plane about which the sample deforms in response to a bending load. In this project, the neutral axis of the fruit tissue samples were determined using digital image analysis.

A rectangular block of tissue, 8.16 mm wide, 26.76 mm in length, and 8.16 mm in height was removed from the fruit. The sample was excised from the fruit so that the length dimension of the sample was parallel to the core line, and the height dimension of the sample was perpendicular to the core line.

The sample was placed in a 3 point bending jig (Image 1) and slowly deformed to failure. A digital video record was made of the deformation. Two digital images were extracted from the video; one prior to deformation of the sample (Image 1), and a second image at a point where the sample had been deformed to a point near failure (Image 2). Image 2 was then subtracted (on a pixel-by-pixel basis) from Image 1, resulting in a difference image (Image 3). Of particular interest are the dark and light triangular regions on the sides of Image 3. The dark region is where the side of the sample rotated toward the center of the sample due to the bending load. The light region is where the side rotated away from the center of the sample due to the bending load. The point where the two regions meet was the pivot of the side's rotation. This pivot point is on the

Image 1 Image 2 Image 3

neutral axis of the sample. The square of the ratio of the distance between the bottom of the sample and the neutral axis to the distance between the neural axis and the top of the sample is equal to the ratio of the compressive elastic modulus to the tensile elastic modulus. Using the compressive elastic modulus computed from the compressive test and the square of the ration of distances to the neutral axis, the tensile elastic modulus was computed.

RESULTS AND DISCUSSION

Sensory attributes ANOVA results for apple firmness level characterization (soft, intermediate and hard as determined by instrumentals measures), panelists and interaction between apples (sample) and panelists are shown in Table 2. Differences between panelists and interaction between apples and panelists were not significant. This reveals consistency between panelists and that the level of error was small. Significant differences between the apple samples were observed at $p \leq 0.05$ (Tukey's HSD test) for all the sensory parameters (crispness, hardness, fracturability, juiciness and chewiness) in the 2006 cultivars. Apple results from 2006 showed that the panelists were able to differentiate apples based on firmness level. However, in 2005 there were not significant differences found between intermediate and hard apples for all texture sensory parameters.

Table 2. Interaction between Apple Sensory Parameters

2005	Crispness	Hardness	Fracturability	Juiciness	Chewiness
Sample (S)	241.67*	2676.63*	285.91*	837.42*	172.99*
Panelist (P)	2.04	132.90	11.64	48.73	29.19
Interaction (S x P)	0.42	13.52	2.13	12.07	2.25
2006					
Sample (S)	130.00*	416.00	240.47*	271.49*	N/A
Panelist (P)	0.42	10.61	1.27	3.74	N/A
Interaction (S x P)	0.18	1.09	0.63	2.25	N/A

F value and significant levels from a two-way ANOVA
*** Significant at P < 0.05**

A logarithmic relationship between the physical properties of fruit and associated sensory response can be observed in our 2005 data. When fruit is soft, the consumers might be expected to be more sensitive to texture differences than any instrument is capable of measuring. When fruits are hard, the ability of consumers to sense texture differences may become saturated, and thus instrumental measurement is better than the consumer at discriminating between hard and very hard fruit.

Table 3 shows the two-way ANOVA sensory attributes F values for pear parameters (soft, intermediate and hard groupings) panelists and interaction between apples and panelists. Significant differences were found between pear samples at $p < 0.05$ (Tukey's HSD test) for all attributes with the exception of juiciness indicating that the training received was adequate and that panelists were able to differentiate pears with varied firmness levels. There were not significant differences between the panelists demonstrating consistency within the group. Also, not significant interactions were found between pear samples and panelists for all attributes.

Table 3. Interaction between Pear Sensory Parameters

	Crispness	Hardness	Fracturability	Juiciness
Sample (S)	330.29*	258.55*	1771.90*	204.84*
Panelist (P)	11.97	8.05	50.65	63.17
Interaction (S x P)	3.61	2.26	19.87	21.54

F value and significant levels from a two-way ANOVA
*** Significant at P < 0.05**

Table 4 showed the one-way ANOVA results of instrumental determinations (Guss, Sinclair, elastic modulus by compression and tension) and their relationship with apple groups. The one-way ANOVA results of instrumental determinations (Guss, Sinclair, elastic modulus by compression and tension) and pear groups are shown in Table 5. Significant differences at $p < 005$ (Tukey's HSD test) between apple and pear groups were observed, indicating that all instrumental measurements were able to differentiate between different groups of apples and pears. Instrumental measures were originally used to characterize the apples and pears and these results support these initial groupings.

Table 4. One Way ANOV for Instrumental Analysis of Apples

2005	Guss	Sinclair	AEMC	AEMT
Sample (S)	765.39*	420.93*	255.45*	27.01*
2006				
Sample (S)	1468.33*	1283.47*	195.76*	98.63*

F value and significant levels from a One-way ANOVA
*** Significant at P < 0.05**

Table 5, One Way ANOV for Instrumental Analysis of Pears

	Guss	Sinclair	AEMC	AEMT
Sample (S)	891.78*	138.25*	79.87*	110.12*

F value and significant levels from a One-way ANOVA
*** Significant at P < 0.05**

Correlation matrices for sensory texture attributes of apples are presented in Table 6. Strong correlations were observed between crispiness, hardness, fracturability and juiciness in 2005. In 2005, chewiness showed weaker correlations with the other sensory attributes, indicating that this term was not a good predictor of apple firmness. Thus chewiness was removed from the apple texture profiling in 2006. In the 2006 harvest year, correlations between sensory attributes were slightly lower, especially juiciness which was not as highly correlated to crispness, hardness, and fracturability as previously demonstrated in the 2005.

Correlation matrices for sensory attributes for pears are presented in Table 7.
In pears, strong correlations were observed between crispness, hardness, and fracturability. However, juiciness was weakly correlated to the other sensory texture attributes. These findings demonstrate that the mechanism for releasing juice in the mouth is not the same between apples and pears, with the release of cell fluids depending upon the biology of the fruit.

Table 6. Correlation Matrix of Sensory Attributes in Apples

Year 1	Crispness	Hardness	Fracturability	Juiciness	Chewiness
Crispiness	1.00	**0.88**	**0.91**	**0.82**	**0.62**
Hardness	**0.88**	1.00	**0.92**	**0.80**	**0.64**
Fracturability	**0.91**	**0.92**	1.00	**0.85**	**0.61**
Juiciness	**0.82**	**0.80**	**0.85**	1.00	**0.58**
Chewiness	**0.62**	**0.64**	**0.61**	**0.58**	1.00
Year 2	**Crispness**	**Hardness**	**Fracturability**	**Juiciness**	**Chewiness**
Crispiness	1.00	**0.82**	**0.79**	**0.73**	**N/A**
Hardness	**0.82**	1.00	**0.85**	**0.65**	**N/A**
Fracturability	**0.79**	**0.85**	1.00	**0.67**	**N/A**
Juiciness	**0.73**	**0.65**	**0.67**	1.00	**N/A**

Table 7. Correlation Matrix of Sensory Attributes for Pears

Year 1	Crispness	Hardness	Fracturability	Juiciness
Crispiness	**1.00**	**0.86**	**0.87**	**-0.25**
Hardness	**0.86**	**1.00**	**0.90**	**-0.32**
Fracturability	**0.87**	**0.90**	**1.00**	**-0.28**
Juiciness	**-0.25**	**-0.32**	**-0.28**	**1.00**

In firm apples, tissue fracture is associated with breakage of individual cells and results in the release of all fluids. In soft apples, fracture occurs as a result of cell to cell debonding. Individual cells do not always break open and release their contents, and these results in a mealy apple.

Pears appeared to behave differently from apples, in that increased firmness resulted in a low amount of juice released in the fruit as evaluated by the sensory panel. This relationship between firmness and juice release was attributed to cell to cell debonding and little juice release. Soft pears are associated with breakage of individual cells, resulting in the release of juice often associated with a

juicy pear. Differences between apples and pears in the way juice contents are released may be attributed to fruit physiology and how the starch hydrolyses during ripening.

In apples, correlation analysis of the degree of association between instrumental and sensory measurements is provided in Table 8. Large positive or negative values indicated a strong association. In 2005, strong to moderate correlations were observed between the Guss, Sinclair and compressive elastic modulus, and the sensory attributes of crispness, hardness, fracturability and juiciness. Weaker correlations were observed between the tensile elastic modulus and all sensory texture attributes. In 2006, strong correlations were found between the Guss, Sinclair, compressive elastic modulus, tensile elastic modulus, and the sensory attributes of crispness, hardness and fracturability. Guss, Sinclair, and compressive elastic modulus provided measurements that did not significantly differ ($p < 0.05$) in their relationship to sensory attributes for both harvest years. However, tensile elastic modulus measurements differed significantly ($p < 0.05$) between apples from 2005 and 2006.

Table 8. Correlation Matrix of Apples Sensory Attributes and Instrumental Measurements

Year 1	Crispness	Hardness	Fracturability	Juiciness	Chewiness
Guss	0.72	0.78	0.74	0.66	0.64
Sinclair	0.81	0.82	0.83	0.76	0.65
Compressive EM*	0.76	0.78	0.78	0.70	0.64
Tensile EM*	0.57	0.62	0.63	0.53	1
Year 2	**Crispness**	**Hardness**	**Fracturability**	**Juiciness**	**Chewiness**
Guss	0.78	0.83	0.76	0.66	N/A
Sinclair	0.75	0.79	0.74	0.63	N/A
Compressive EM*	0.68	0.73	0.67	0.57	N/A
Tensile EM*	0.88	0.78	0.74	0.69	N/A

***EM: Elastic Modulus**

An increased predictability of apples crispness, hardness, and fracturability was observed in 2006. Also, some small variability in correlations between instrumental and sensory measurements was observed in apples between both harvest years. In 2005, correlations between the Sinclair and the Guss measurements and sensory attributes were higher than the 2006 correlations. This variability may be associated with the structural differences in different varieties of apples and the differences where the fruit was taken when sampling. The possible reasons for the range of correlations obtained over different harvest years include the different range of firmness of fruit presented to different panelists, the difference between the texture of apples at the point of instrumental measurement and region eaten by each panelist, and the range of sensory acuities and cognitive abilities of individual panelists. The mechanical and texture characteristics of apples and pears are influenced by the structural features of the flesh and are affected by storage conditions that cause a high structural variability.

Correlation analysis of the degree of association between instrumental and sensory measurements for pears is provided in Table 9. Strong correlations were observed between the Guss, tensile elastic modulus and the texture sensory attributes of crispness, hardness and fracturability. Measurements made using the Sinclair and the average elastic modulus by compression showed poor correlations at predicting sensory texture attributes in pears. The term juiciness was negatively and poorly correlated to all instrumental measurements.

Table 9. Correlation Matrix of Pear Sensory Attributes and Instrumental Measurements

Year 1	Crispness	Hardness	Fracturability	Juiciness
Guss	0.79	0.83	0.81	-0.41
Sinclair	0.68	0.71	0.71	-0.25
Compressive EM*	0.59	0.61	0.59	-0.21
Tensile EM*	0.85	0.79	0.81	-0.31

*** EM = Elastic Modulus**

Tensile elastic modulus differed significantly between apples from the first and second harvest years. Differences of tensile measurements between harvest years may be attributed to the difference in how the measurement was made between the two years. In 2005, the tensile elastic modulus and failure modulus were measured in a direction parallel to the core line. However in 2006, the measurements were made perpendicular to the core line due to the redesign of the experimental technique. Sensory evaluation techniques and training did not differ between years.

Tensile material properties have been found to be highly orthotropic in that the properties change with orientation of the tissue sample with respect to the core line of the fruit. Strong correlations of tensile measurements and crispness for apples and pears were observed when samples were taken perpendicular to the core line as opposed parallel to the core line. These observations were associated with the fact that tissue failure from biting with the front teeth was crack-related. Tensile material properties played a dominant role when a crack propagates and the length of the crack propagation. In the current study, fracturability and hardness were measured with the molars where compressive material properties dominated. The finding showed that tensile material properties were correlated to compressive properties, and compressive properties were related to fracturability and hardness.

Fruit firmness or strength is a function of the mechanical properties of the cell wall, cell turgor, and bonding between cells. Another factor that impacts fruit firmness is the contents of the cell. Cell strength is a hydrostatic phenomenon that is diminished in the absence of cell contents. Studies using pressure probes as a measure of compressive forces showed that the cell wall elastic modulus increased with increased turgor pressure in the cell. The results of the tensile material properties studies were attributed to the dependence on the strength of the pectin bonds between cells and the cell wall strength. The compressive material properties were attributed to a high dependence on the turgor pressure in the cell, and to a lesser extent on the pectin bonds and cell wall strength. Under certain storage environments, the fruit could mature without noticeably changing cellular turgor pressure.

An advantage of tensile tests is that they provide the opportunity to determine the mechanism of tissue failure through the examination of the fracture surface in fruit. There are three forms of tissue failure: cell fracture, cell rupture, and cell-to-cell debonding.

There is a difference of mechanical properties of a population of cells versus individual cells. In puncture tests of whole fruits, the compression and shear properties of the cell population is evaluated, while during tensile testing, the strength of thin layers of individual cells is determined. In the tensile measurements, the strength of the weakest cell may define the strength of the entire sample. Generally, failure in uniaxial compression is associated with an increase in turgor pressure which involves a change of volume in the cells and rupturing of cell walls. Failure during tension involves tearing of the cell walls and/or cell to cell debonding. Compressive tests may be relevant to understanding factors affecting the development of bruises while tensile measurements may be closely related to biting and chewing of food.

The analysis on the Fall 2005 fruit indicate that the tensile material properties decline at a faster rate than compressive material properties as the fruit matures. One explanation of this observation could be that the tensile material properties are highly dependent on the strength of the pectin bonds between cells and the cell wall strength, while the compressive material properties are highly dependent on the tugor pressure in the cell, and to a lesser extent on the pectin bonds and cell wall strength. Under certain storage environments, the fruit could mature without noticeably changing cellular tugor pressure.

The tensile material properties are highly orthotropic (the properties change with orientation of the tissue sample with respect to the core line of the fruit. The sensory evaluations and tensile properties measured in the Spring 2006 test were orientated perpendicular to each other, and showed little correlation, while in the Fall 2006 test the tensile material properties and sensory evaluations were both taken parallel to the core line, and there was a high correlation between the sensory and tensile measurements. Although we did not measure tensile material properties and sensory evaluations perpendicular to fruit core lines, we suspect that the correlations between sensory and tensile measurements would also be high in this orientation.

One very clear outcome from this project is that the orientation of the load applied by a firmness sensor must be specified. The correlation between material properties parallel and perpendicular to the core line is low, and comparing firmness measurements taken without specifying the orientation will vary widely.

FINAL PROJECT REPORT
WTFRC Project Number: AP-07-704

Project Title: Sensory profiles and consumer acceptance of apple breeding selections

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Other funding Sources

Agency Name:
Amount awarded:
Notes:

Total Project Funding:

Budget History:

Item	Year 1: $35,000	Year 2:	Year 3:
Salaries	17000		
Benefits	5780		
Wages			
Benefits			
Equipment			
Supplies	10720		
Travel	1500		
Miscellaneous			
Total	**35000**		

SIGNIFICANT FINDINGS

- The work will commence January 2008.
- The trained panel has been recruited and we have 12 panelists interested in participating.
- Training is scheduled to start the week of January 14th, with 3 sessions per week.
- We have received the list of apple selection to be evaluated by the trained panel.
- We plan to drive over to Wenatchee early January to pick up the selections as well as apples to use during training.
- Final trained panel evaluations scheduled for February 4th and 6th 2008
- Consumer panels are planned for February 5th and 7th 2008
- Both panels are scheduled to be completed by February 7th 2008

RESULTS AND DISCUSSION

A list of the apple selections was provided by Bruce Barritt. This list will be shortened to no more than 10 selections as described in the original proposal.

Table 1. Apple selections to be evaluated by the trained and consumer panels.

Selection Number	Harvest Date	Original Seedling Number	Parentage
Allen 2	10/8/07	T19-17-3-9427	Splendour open
Fuller 5	9/25/07	T19-10-5-9435	Splendour x Coop 15
Fuller 7	9/25/07	T19-46-95-9434	NJ90 x Goldrush
Fuller 10	9/18/07	C16-17-4-6-9737	Honeycrisp x Cripps Pink
Fuller 17	10/9/07	C12-10A-9-9623	Honeycrisp x Chinook
Fuller 18	10/9/07	C16-5-1-14-9735	Honeycrisp x Enterprise
Fuller 20	9/25/07	T18-5-26-9515	Coop 25 x Goldrush
Fuller 24	10/23/07	T21-18-25-9523	Gala x Cripps Pink
Allen 26	10/8/07	T21-20-49-9519	Gala x Fuji
Fuller 29	10/23/07	T21-25-13-9530	Fuji x BC 85-27-2
Fuller 30	10/9/07	T21-25-19-9530	Fuji x BC 85-27-2
Fuller 34	10/16/07	C12-7B-34-9624	Honeycrisp x Delicious
Fuller 36	10/9/07	C12-10A-16-9623	Honeycrisp x Chinook
Fuller 37	10/2/07	C12-16A-35-9724	Gala x Delblush

Sensory Attributes

Based on input from Bruce Barritt and on our previous experience with sensory evaluation of texture in apples, we have short-listed the sensory attributes to the following:

<u>Trained panel attributes to evaluate:</u>

- Flavour and taste: sweetness, sourness, overall apple flavor intensity, astringency
- Texture: firmness/hardness, juiciness, mealiness
- Appearance: colour intensity, size, presence of lenticels

<u>Consumer panel attributes to evaluate:</u>

- Flavour and taste: sweetness, sourness, apple flavour intensity, astringency, overall rating
- Texture: firmness/hardness, juiciness, mealiness, overall rating
- Appearance: overall rating
- Final overall rating of the apple

Reasons for expected success

- We successfully completed a similar study with cherries in June/July 2007 and obtained successful results. The same people are involved in this study (Ross, Chauvin, Weller and Plotka) as were involved in the cherry sensory evaluation study.

- We have also conducted apple texture studies for the past two years. This work composed Andrea Chauvin's Ph.D. dissertation topic. She has now completed her Ph.D. and is looking forward to starting on her post-doctoral work in this area.

Significance to Industry

- Draw relationships between sensory profiles obtained from a trained panel and the evaluations of a consumer panel.
- Assist the WSU apple breeding program regarding commercialization decisions of appropriate apple selections using sensory data from trained and untrained (consumer) sensory panels.
- More broadly, this research will give insight into the apple consumer, the attributes that he/she is seeking in an apple and the influence of different sensory attributes of the apple acceptance.

FINAL PROJECT REPORT
WTFRC Project Number: AP-06-601

Project Title: Flower bud development in apple

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Item	Year 1 (2006)	Year 2 (2007)	Total
Salaries[1]	3380	3481	6861
Benefits	1159	1194	2353
Wages	4893	4893	9786
Benefits	495	495	990
Supplies	200	200	400
Travel[2]	700	700	1400
Total	**10827**	**10963**	**21792**

[1] 5% of Hirst time spent on this project

[2] Travel to Washington state to set up the field study and establish the treatments.

Objectives:

The objective of this project was to further understand the root causes of biennial bearing by tracking the development of potential flower buds from early in the season through to dormancy. Bourse buds were selected with different histories:

- vegetative
- flowering but not fruiting
- fruiting

Our goal was to determine whether buds on vegetative spurs differed from those that flowered but did not set fruit, and whether in turn these differed from spurs that carried fruit. We were interested to learn not only whether spur history affected whether a flower developed, but how well developed the flower was by the end of the season. More highly differentiated buds are likely to result in larger fruit the following year.

Significant findings:

- Floral/fruiting status of spurs had no effect on whether they would form flower clusters for the following year
- Floral/fruiting status of spurs had little effect on the quality of flowers formed for the following year
- Overall tree crop load may be as important or more important than localized fruiting effects in determining biennial bearing
- Flower formation may start earlier in the season in Fuji than in Gala.

Methods:

On 10 mature trees each of Gala (regular bearing, small fruit size) and Fuji (biennial, larger fruit size), 20 buds from each of the following categories were selected at flowering.

- vegetative (Gala trees flowered very heavily in 2006 and insufficient vegetative buds could be found)
- flowering but not fruiting (flowers removed at full bloom)
- fruiting (thinned to the king flower at full bloom)

Buds were selected and tagged at full bloom and sampled throughout the season. After tagging buds for later sampling, the trees were hand-thinned to a light-moderate commercial crop load. On each sampling date, 2 buds per tree were sampled and stored in a fixative solution until later dissection.

During dissection, the number of bud scales, transition leaves, true leaves and bracts were counted. The degree of floral differentiation was measured using a 1-5 rating scale in 2006 where 1=vegetative and 5=highly differentiated floral bud (sepals clearly differentiated on king and lateral flowers. This scale was expanded slightly in 2007 so that 0=vegetative and 5=highly differentiated floral bud. We also measured the diameter of the king flower within the bud.

From these data, we determined:

- the degree to which the presence of a flower or a fruit on a spur inhibits floral bud formation for the following years crop.
- the degree to which the presence of a flower or a fruit on a spur affects the complexity of flower buds (and therefore fruit size potential).

Results and Discussion

Because temperature plays such a central role in tree development, we looked at the course of growing degree-day (GDD) accumulation over each growing season. During the period of our sampling, the accumulation of GDD was essentially linear. Therefore graphs of bud development appeared almost identical, whether they were plotted against days after full bloom or GDD. Since days after full bloom is more easily interpreted, plots presented in this report will use that basis.

As buds develop, they form (in order from the outside) bud scales, transition leaves (appearing as a cross between bud scales and true leaves), true leaves, then bracts. The true leaves represent the very small leaves that will first emerge from the buds the following spring. If the meristem of the bud appears flattened, this indicates that there is no visual sign that the bud has formed a flower therefore such buds are classified as vegetative. In floral buds however, the meristen becomes domed, then forms first the king flower then lateral flowers (see Fig. 1).

Fig. 1. Developing apple buds showing a flattened meristem indicating a vegetative status (left) and a highly developed flower cluster (right).

As buds develop over the course of the season, the number of appendages (bud scales, transition leaves, true leaves and bracts) increased markedly (Figs. 2-3), although the floral/fruit status of the bud had no effect in either Gala or Fuji in either year. In both years, Gala buds had approximately 27 appendages by the end of the season, compared with 17-21 for comparable buds of Fuji. Most of this difference was due to the presence of more bracts in Gala buds. The function of bracts in buds is unknown.

In 2006, the first visible signs of flower formation in Gala buds appeared just after 90 DAFB (Fig. 4). which coincides with the timing of floral differentiation we have found in our previous work with Red Delicious buds in Ohio and Gala buds in New Zealand. Buds of Fuji however formed flowers earlier giving a wider window during which flowers could form. Flowers formed earlier (40-80 DAFB) in 2007, and very rapidly (Fig. 5). The timing of flower formation was similar in Gala and Fuji, with almost all buds of both cultivars forming flowers by 80 DAFB. From our earlier work with Red Delicious in Ohio, flowers formed during the period 90-120 days after full bloom, and after this period essentially no more flowers formed. This is the earliest we have seen flowers form in any of our many studies examining flower formation.

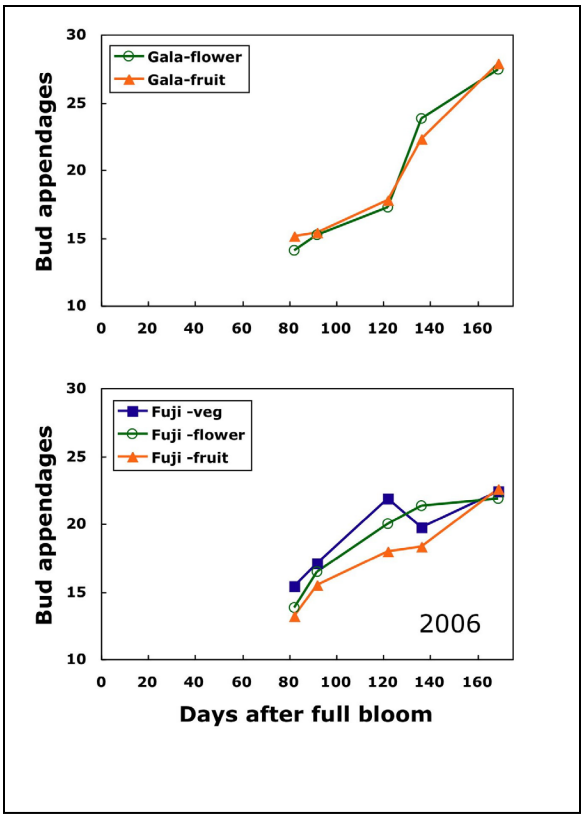

Fig. 2. Total number of appendages (bud scales + transition leaves + true leaves + bracts) in buds of Gala and Fuji in 2006. Full bloom was April 30, 2006.

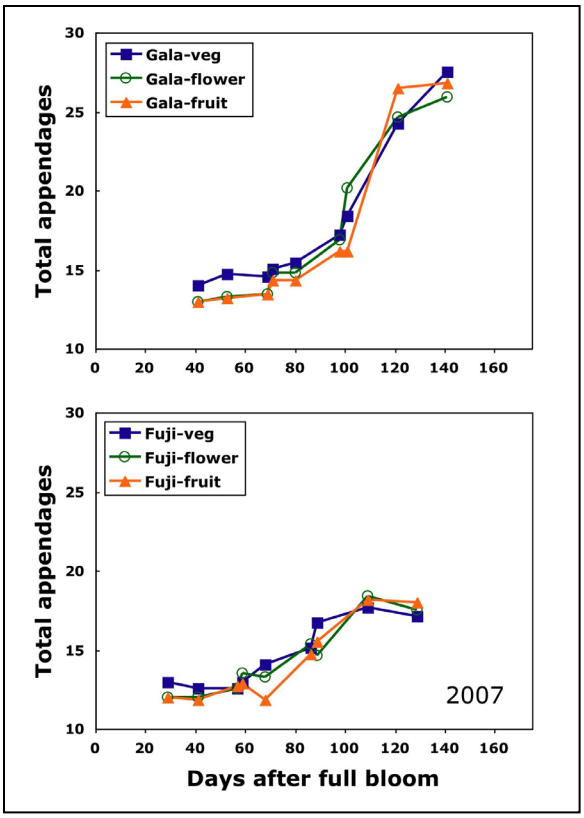

Fig. 3. Total number of appendages (bud scales + transition leaves + true leaves + bracts) in buds of Gala and Fuji in 2007. Full bloom was May 1 (Gala) or May 13 (Fuji).

Interestingly, at least 80% of all buds sampled eventually formed flowers, including those from fruitful spurs. This challenges the simple text-book idea that the presence of a fruit on a spur inhibits flower initiation in the bourse bud of that spur, especially in cultivars with a propensity for biennial bearing such as Fuji. This did not happen in either year in this study. The trees used for this study were hand-thinned to a reasonable commercial crop load, so it appears that the overall crop load of the trees may have been more important in determining flower formation that localized fruiting effects on particular spurs.

The level of complexity a bud attains before flowers are formed can be determined by linear discriminant analysis and is called the critical appendage number. Basically this analysis determines the threshold level in terms of number of bud appendages that must be reached before a flower is formed. This analysis predicts the critical appendage number, then gives a measure of what proportion of all buds would have had their floral status predicted correctly using this model.

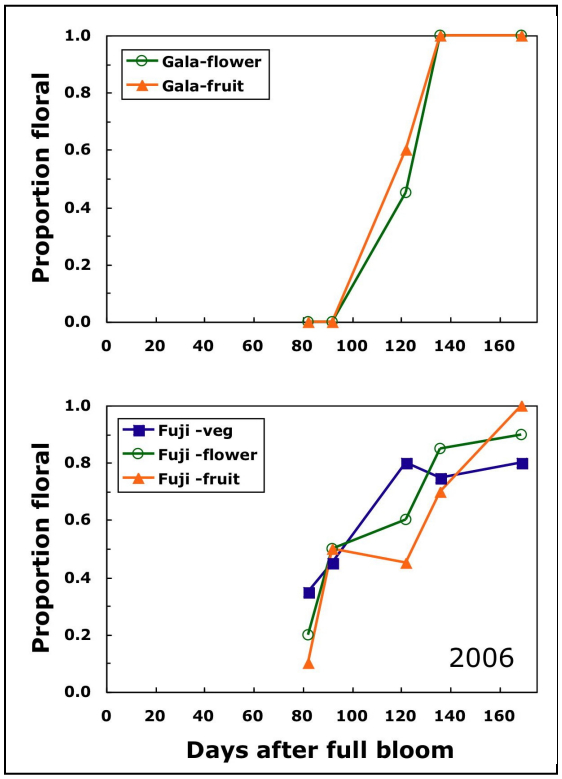

Fig. 4. The proportion of buds in which the commitment to flowering (doming of the meristem) was visible in 2006.

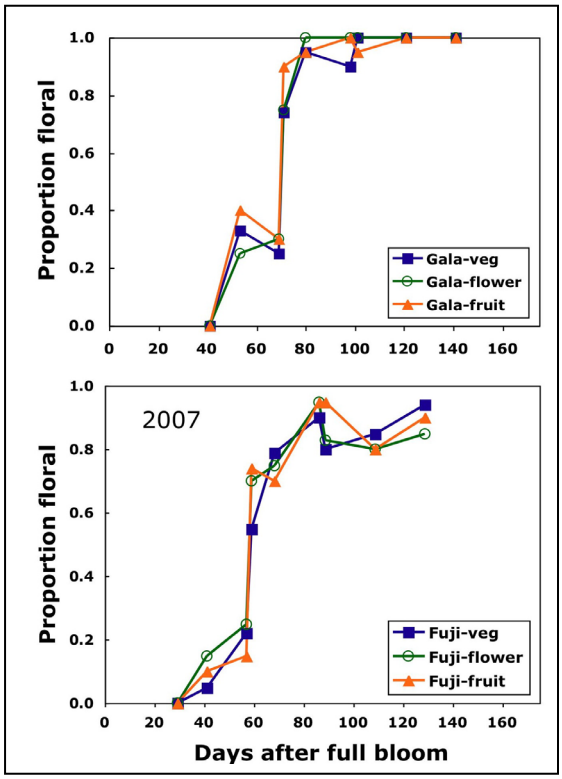

Fig. 5. The proportion of buds in which the commitment to flowering (doming of the meristem) was visible in 2007.

The models used to predict the floral status of buds performed well in 2006, in all cases classifying over 80% of buds correctly (Table 1). However in 2007 results were not as clear, with descriminant models only predicting the floral status of 60-70% of buds correctly based on their number of appendages (Table 2). With Gala (2007) and Fuji (2006 and 2007) there were slight trends suggesting that buds from fruiting spurs formed flowers at a lower level of complexity than vegetative spurs. Although there is some suggestion here that the floral/fruiting status of a bud may have had a slight influence on the complexity at which the switch from vegetative to floral was made, caution should be used in in interpreting this result since only about 65% of buds were classified correctly by these models in 2007. Furthermore, the floral/fruiting status of buds did not affect the timing of bud differentiation (Figs. 4-5) or the rating of meristem development (Figs. 6-7).

Table 1. The critical appendage number prior to flower formation and proportion of buds correctly classified as vegetative or floral using linear discriminant models in 2006.

Cultivar/bud type	Critical app. No.	% correct
Gala		
Flowering	18.5	88.8
Fruiting	18.2	87.5
All Gala	18.3	88.1
Fuji		
Vegetative	18.0	81.3
Flowering	17.7	81.3
Fruiting	16.6	88.8
All Fuji	17.4	83.3

Table 2. The critical appendage number prior to flower formation and proportion of buds correctly classified as vegetative or floral using linear discriminant models in 2007.

Cultivar/bud type	Critical app. No.	% correct
Gala		
Vegetative	17.0	61.0
Flowering	16.1	67.6
Fruiting	15.9	65.2
All Gala	16.3	63.7
Fuji		
Vegetative	14.5	65.7
Flowering	14.0	65.0
Fruiting	14.0	69.8
All Fuji	14.2	65.9

Obviously not only the number or proportion of buds that flower is important, but also the "quality" of those flowers since this is likely to affect fruit size the following year.

Although Gala flowers started differentiating later than Fuji in 2006, they developed rapidly and were highly differentiated by the end of the season (Fig. 6). Fuji buds on the other hand, showed a much more gradual development, but nonetheless were well developed by the end of the season. The floral/fruiting status of spurs did not influence bud development of either Gala or Fuji in 2006 or 2007 (Figs. 6-7). In 2007, flowers started becoming apparent in buds much earlier than in 2006 or in previous studies. Gala buds however, appear to have made the first step to becoming floral (doming of the meristem) but did not undergo further differentiation until about 50 days later. Data for Fuji buds was more variable (Fig. 7) , but it is clear that they did not differentiate to the same extent as Gala buds. It is possible that the Fuji buds may differentiate further before budbreak in the spring, and we will attempt to sample more buds at that time to check for further differentiation of floral buds.

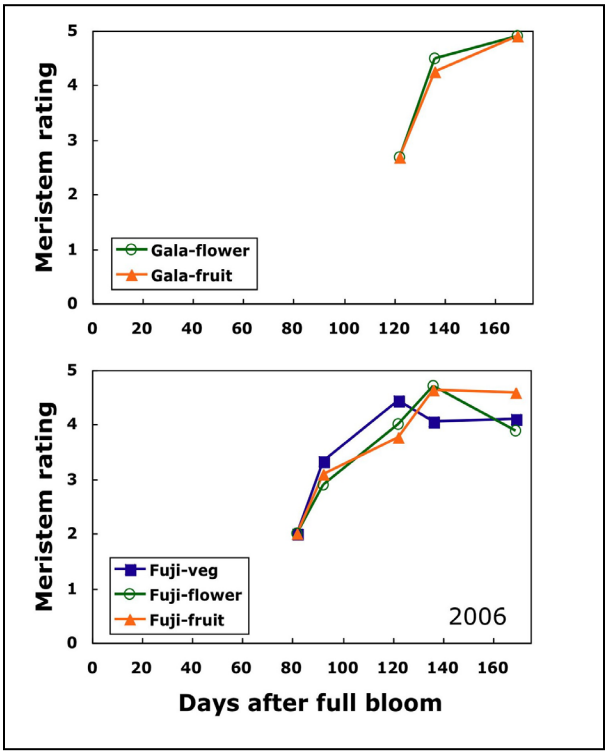

Fig. 6. The complexity of floral meristems in 2006, where 1=vegetative and 5=highly differentiated.

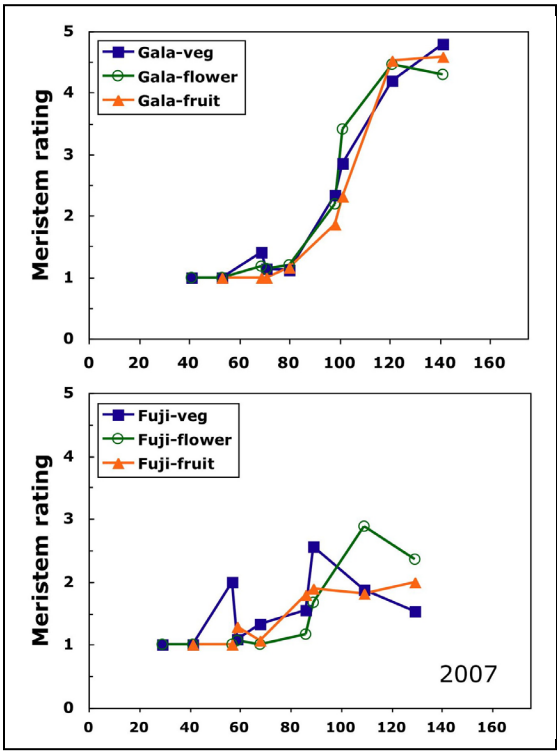

Fig. 7. The complexity of floral meristems in 2007, where 0=vegetative and 5=highly differentiated.

Another measure of the extent of floral bud differentiation is king flower diameter, which was measured in 2007. Obviously such data can only be collected after buds have become floral and developed to a point where the king flower is obvious. Again, the floral/fruiting status of a spur did not affect flower bud quality, as measured by king flower diameter (Fig. 8). King flowers in well developed Gala and Fuji buds were approximately 0.4-0.5 mm by the end of the season. Although the Fuji data presented in Fig. 7 may seem to contradict those in Fig. 8, in must be borne in mind that Fig. 7 includes all buds that have made the first visible step towards a floral status, whereas Fig. 8 only includes well-developed buds on which the king flower was obvious and could be measured. While the diameter of king flowers within buds doubled in diameter as they developed (0.25-0.5 mm), the external diameter of sampled buds did not change during the course of the season (Fig. 9). Generally, sampled buds of both cultivars ranged from 3-4 mm in diameter. For buds sampled at the end of the 2007 season, there was no relationship between the external dimensions of a bud and the diameter of the king flower contained within the bud (Fig. 10). This is not to say that large buds do not have larger flowers and higher fruit size potential than smaller buds, but within the narrow range of buds selected for this study, there was no relationship between bud size and flower quality.

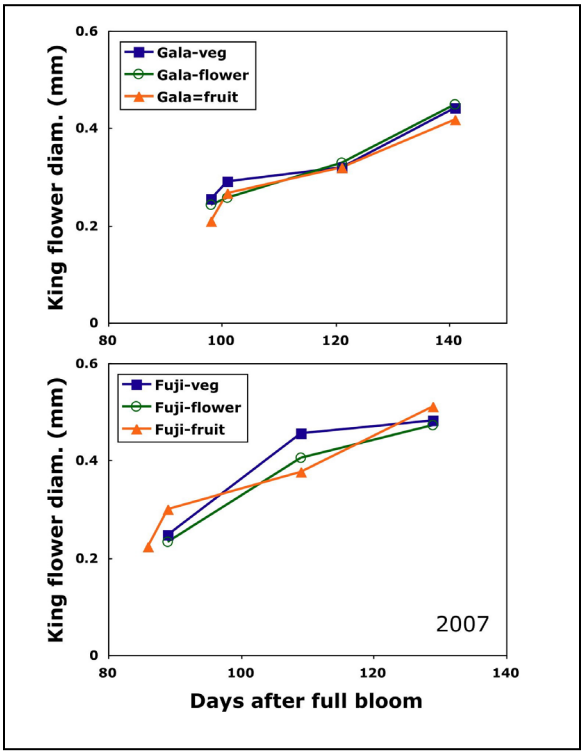

Fig. 8. The diameter of king flowers in buds during 2007.

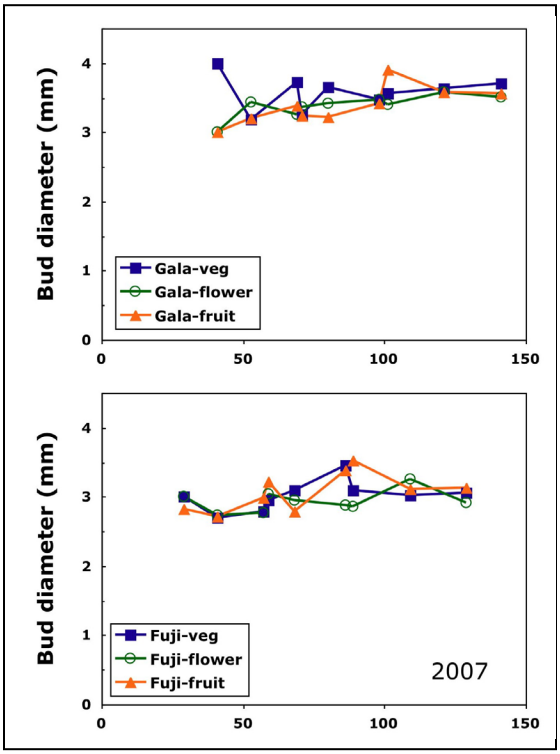

Fig. 9. The external diameter of buds sampled during 2007.

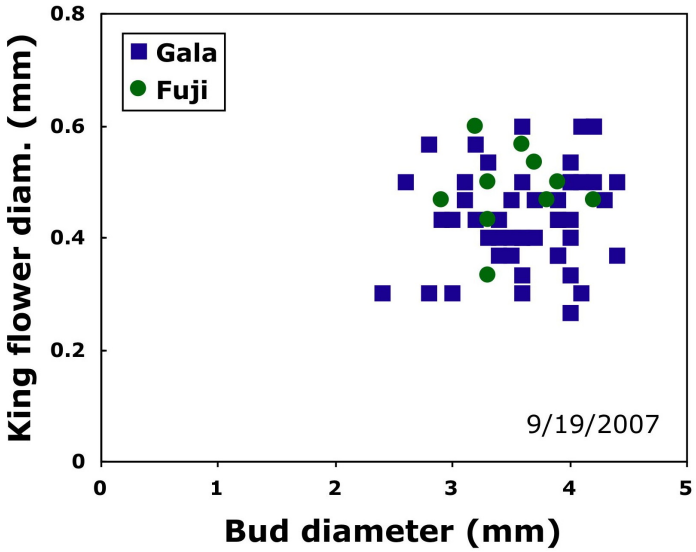

Fig. 10. Graph showing no relationship between bud external diameter and king flower diameter in 2007.

FINAL PROJECT REPORT

Project Title:	Mechanism of apple fruit growth under Washington conditions

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No report submitted.

FINAL PROJECT REPORT

Project Title: Adapting available genomics tools to enhance WA breeding

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No report submitted.

FINAL PROJECT REPORT
WTFRC Project Number: AH-05-511

Project Title: Consulting for the Washington Apple Breeding Project
PI: Fredrick A. Bliss
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City: Davis
State/Province/Zip CA 95616

Cooperators: Bruce Barritt, Yanmin Zhu, Jim McFerson.

Budget History:

Item	Year 1: 2008	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel	$ 5,280		
Miscellaneous	$ 7,680		
Total	**$12,960**		

Significant Activities and Findings:

Presented ideas and plans for integrating molecular marker opportunities into the apple breeding program.

- Developed a decision tree showing where molecular markers can be used effectively in an apple breeding program.
- Led assembly of a comprehensive list of apple traits for setting genetic and breeding priorities.
- Developed the document - Apple Trait Decision Tree for Implementing Marker Assisted Selection to guide decisions about potential for marker-assisted selection in apple.
- Coordinated monthly teleconferences at which important traits were discussed to determine feasibility of MAS. Traits discussed were: fruit acidity, fruit firmness, fruit crispness, fruit juiciness, fruit sweetness, mildew reaction, tree juvenility, lenticel breakdown, and skin overcolor.

Conducted literature reviews and prepared reports for the consulting work.

- Reviewed literature related to apple breeding and genetics for use by Bruce Barritt, Yanmin Zhu and Jim McFerson.

Traveled to meeting in Washington to evaluated project and participate in program activities.

- January 18 – 20, 2006. Traveled to Pasco, WA to participate in the Apple Research Meeting. Met with Bruce Barritt, Jim McFerson and industry members from Washington to discuss activities and progress in the apple breeding program. Reviewed research proposals and reports to become more familiar with activities related to apple improvement.

Submitted invoices for expenditures on a quarterly basis.

- Quarter one (Oct.1, 2005 – Dec. 31, 2005): $ 720.00
- Quarter two ((Jan. 1, 2006 –Mar. 31, 2006): $3,096.70
- Quarter three (Apr. 1, 2006 – June 30, 2006): $ 440.00
- Quarter four (July 1, 2006 – Sept.30, 2006): $ 240.00
    - Total $4,496.70

Results and Discussion:

A priority recommendation from the review of the WTFRC Apple Breeding Program conducted Oct. 10-12, 2004 was, “Incorporation of molecular tools – Tools for practical DNA-marker aided selection are nearly ready for routine incorporation in apple breeding programs. They may be useful for selection of certain traits. … It will be important to identify a researcher with the interest and capability to add this dimension to the program. This capability could be developed at WSU or contracted at another institution or the private sector” (From the Committee Report to the Board).

During the course of the year I initiated a series of monthly discussions among Bruce Barritt, Jim McFerson, Yanmin Zhu and myself to identify apple traits most amenable to enhanced improvement using MAS. I developed the document - Apple Trait Decision Tree for Implementing Marker Assisted Selection to guide decisions about potential for marker-assisted selection in apple. This decision tree includes scientific and economic factors that are evaluated for each trait in order to determine

feasibility and commercial potential for improving these traits leading to new cultivars and whether MAS can be employed cost effectively to improve efficiency

The traits discussed were: fruit acidity, fruit firmness, fruit crispness, fruit juiciness, fruit sweetness, mildew reaction, tree juvenility, lenticel breakdown, and skin overcolor. This discussion and ranking provided guidance for Bruce and Yanmin to begin developing screens for priority traits. Also, the critique and ranking of the traits provide a basis of the newly hired scientists, Amit Dhingra and Cameron Peace to develop research proposals relevant to the needs of the apple industry.

Addition of these new scientists and their enthusiasm to work with apple improvement addresses the priority recommendation of the Committee Report of 2004.

I conducted literature reviews that identified new research findings and resources relevant to the breeding program. These were provided to the collaborators during the monthly conference calls and via email correspondence.