2010 Apple Research Review
January 26-28, 2011
Red Lion Hotel Yakima Center
Yakima, WA

Wednesday , January 26 **APPLE COMMITTEE**

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FINAL PROJECT REPORT
WTFRC Project Number: AP-09-901AB

Project Title: A sensitive indicator of Honeycrisp fruit N status for maximal quality

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Total Project Funding: **Year 1:** $34,595 **Year 2:** $35,391

Budget 1
Organization Name: Cornell University

Item	Year 1 (2009)	Year 2 (2010)	
Salaries	11,500	11,960	
Benefits	6,095	5,856	
Supplies	6,100	6,675	
Travel	900	900	
Total	24,595	25,391	

Footnotes:

Salaries budgeted are for a 4-month postdoc working on this project at $34,500 per year in the first year and at $35,880 in the second year. The fringe benefit rate for the second year is 48.96%.

Supplies include cost of leaf and fruit nitrogen analysis, analytical columns, guard columns, standards, solvents, vials and service for the HPLC separation and quantification of amino acids.

Travel expense is budgeted for one trip per year to the experimental site in WA to set up the trial and collect samples.

Budget 2
Organization Name: Cornell University

Item	Year 1 (2009)	Year 2 (2010)	
Fruit loss compensation*	10,000	10,000	
Total	10,000	10,000	

Footnotes: * This was a contingency plan for the worst case scenario where the high N treatments (120lbs and foliar N treatments) might make some of the fruit unmarketable so that the co-operative grower gets compensated for the loss: 20 bins of unmarketable fruit x $500/bin = $10,000. We only used a small portion of the budget in 2009 and we did not use any in 2010.

Objectives

The objectives of this project are 1) to determine how asparagine and other free amino acids in Honeycrisp fruit respond to N supply and relate the levels of free amino acids to fruit N status and fruit quality and 2) establish the optimal range of fruit N status expressed as asparagine level, with the goal of developing a sensitive indicator of Honeycrisp fruit N status for maximal quality.

Significant Findings

- Over the range of N fertilization rate 0 to 120 lbs per acre, N fertilization had only limited effect on fruit yield, fruit size, quality and physiological disorders during the first two years of the trial. Although trees in the highest N treatment tended to have higher yield and bigger fruit, N fertilization beyond 30 lbs/acre didn't bring any significant benefit.

- Both leaf N and fruit N increased only slightly in response to increasing rate of N fertilization. However, fruit asparagine concentration increased significantly when N fertilization rate increased from 0 to 30 lbs N per acre. Any further increase in N fertilization rate didn't result in significant increase in fruit asparagine concentration.

- Asparagine accounted for about half of the total free amino acids in Honeycrisp fruit. As a result of the increase in asparagine concentration, the concentration of total free amino acids increased in the same way as asparagine in response to N fertilization.

- Both fruit asparagine concentration and total free amino acid concentration are more sensitive than leaf N or fruit N in response to N fertilization.

Methods

Two field trials were set up in commercial Honeycrisp orchards in WA in 2009, one at Brewster Flats and the other at Naches Heights. The trees at Brewster Flats were 6-yr-old trees on M.26 at 8 x 14 foot spacing (389 trees/acre). The trees at Naches Heights were 5-yr-old over-grafts on Delicious/MM106 at 6 x 16 foot spacing (454 trees/acre). At both sites, trees were fertilized with 0, 30, 60, 90, or 120 lbs actual N per acre in 2009, whereas in 2010 only the trial at Brewster Flats was continued. Each treatment was replicated 5 or 6 times, with 5 to 6 trees per replicate, in a randomized complete block design. For each nitrogen treatment, urea fertilizer was applied at tight cluster and 3 weeks later in an equal split. The fertilizer was spread under the tree canopy and was watered into the soil within a few days of application. The cropload of the trees at Brewster site was 5 to 7 fruit per cm^2 trunk cross-sectional area (TCA) and that at Naches site was 9 to 10 fruit per cm^2 TCA, after chemical thinning followed by hand-thinning.

In addition, a field trial was set up at Hill Top orchards in 2010 to use foliar urea applications during fruit development as a method to generate a range of fruit N status. Briefly, 7-yr-old Honeycrisp/M26 trees at 3 x 15 foot spacing (968 trees/acre) received 0, 1, 3, 5, or 7 foliar sprays of 5 lbs urea/100 gal water at weekly intervals centered around 6 weeks before expected harvest in 2010. Each treatment was replicated 5 times in a randomized complete block design with 4 trees per replicate. The cropload of the experimental trees was adjusted to 5 to 7 fruit per cm^2 trunk cross-sectional area (TCA) after chemical thinning followed by hand-thinning.

The effects of N treatments on leaf and fruit N status, levels of free amino acids, yield and fruit quality were monitored. Leaf samples were taken in mid-August for nitrogen analysis. Fruit samples were taken at harvest for analysis of N and individual free amino acids. Fruit yield and size were measured at harvest and one bushel of fruit per replicate was stored in a regular cold room. Fruit quality (color, firmness, soluble solids, and occurrence of bitterpit and other disorders) were assessed at fruit harvest and after 4 months of cold storage. Asparagine and all other free amino acids were

separated and quantified with an Agilent 1100 high performance liquid chromatography using the AccQ-Tag method (Waters Corporation).

Results and Discussions

1. Effects of N fertilization on fruit yield, fruit size and quality

At Brewster site, all the trees had relatively low cropload in 2009. Fruit number and fruit yield per tree were higher in the highest N treatment (120 lbs/acre) than in the control (Table 1). No significant difference in fruit size was detected between any N treatments and the control, but the trees fertilized with nitrogen tended to have slightly bigger fruit. N fertilization did not significantly affect fruit firmness, soluble solids, acids, or bitterpit incidence. Percentage of clean fruit (free of russet) was significantly higher in the 120 lbs N treatment than in the control. In 2010, tree cropload was medium. Compared with the control, fruit size was slightly larger, and correspondingly fruit firmness was slightly lower in the N treatments (Table 2). As N rate increased, percentage of fruit with greasiness increased. Sunburn incidence was significantly higher at 120 lbs N rate than the control. N fertilization did not significantly affect fruit soluble solids, acids, color, bitterpit incidence or russet incidence.

At the Naches site, all the trees had relatively high cropload in 2009. N fertilization did not significantly affect fruit number or fruit yield per tree (Table 3). This may be partly due to the fact that the grower had put on about 30 lbs of nitrogen on all the trees before the trial was set up. Fruit size was the largest in the highest N treatment (120 lbs/acre), but for some reason, trees in the 30 lbs N /acre treatment produced the smallest fruit. Fruit firmness was slightly affected by N fertilization. No difference in fruit soluble solids or acids was detected between any N treatments and the control. Bitterpit was highest in the control, which we don't have a clear explanation for. Perhaps the trees were relatively vigorous and fruit size was relatively big. Sunburn incidence at this site was higher than that at Brewster site in 2009, and N fertilization slightly affected sunburn incidence. Similar to that found at Brewster site, percent of clean fruit was significantly higher in the highest N treatment (120 lbs N/acre) than in the control.

No difference in fruit yield, fruit size, firmness, soluble solids, acids, or any disorder was detected between foliar N-treated and the control (Table 4).

2. Effects of N treatments on leaf N, fruit N, fruit asparagine and total free amino acids

At Brewster site, both leaf N and fruit N increased only slightly from 1.9% to 2.2% and from 0.24% to 0.31% respectively in 2009, and from 2.1% to 2.4% and from 0.20% to 0.32% respectively in 2010, as N fertilization rate increased from 0 to 120 lbs/acre (Fig 1). However, both fruit asparagine concentration and total free amino acid concentration increased significantly. In 2009, fruit asparagine concentration and total free amino acid concentration increased from 4.4 to 7.2 mg/g and from 10.4 to 13.9 mg/g, respectively in 2009 when N fertilization rate increased from 0 to 30 lbs/acre. Any further increase in N fertilization rate (beyond 30 lbs/acre) did not result in any significant increase in either fruit asparagine concentration or total free amino acid concentration. In 2010, the increase in both asparagine concentration and total free amino acid concentration in response to N fertilization was larger at the same N rate (Fig. 1).

At Naches site, leaf N was higher than at Brewster site, but it increased only slightly at the two highest N fertilization rates (Fig 1). Surprisingly, fruit N levels were slightly lower than those at Brewster site, but it increased from 0.30% to 0.37% as N fertilization rate increased from 0 to 120 lbs/acre. Both fruit asparagine concentration and total free amino acid concentration showed the same trend in response to N fertilization: they increased significantly (from 6.8 to 9.3 mg/g and from 13.2 to 16.8 mg/g) when N fertilization rate increased from 0 to 30 lbs/acre, but no additional significant increase was detected with further increase in N fertilization rate.

At the Hill Top site, fruit N did not respond significantly to foliar urea application (Fig. 2). Both fruit asparagine concentration and total free amino concentration showed an increase when

foliar urea was applied one time, but no further increase was observed when the number of foliar urea applications was increased beyond one application.

Compared with leaf N and fruit N, fruit asparagine concentration and total free amino acid concentration were more sensitive to N supply, and therefore should be better indicators of fruit N status. Since both fruit asparagine concentration and total free amino acid concentration responded more to the same soil N fertilization during the second year (2010) than during the first year (2009), and we only have one-year data on foliar urea application, both trials need to be repeated for another season in order to draw firm conclusions.

Table 1. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Brewster site, 2009)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Bitter pit (%)*	*Sunburn (%)*	*Russet % clean*
0	11.1b	61b	180	18.1ab	12.3	0.529	1	7ab	39b
30	18.1 ab	95 ab	187	18.3 a	11.8	0.485	1	8 ab	39 b
60	18.5 ab	98 ab	186	17.9 b	12.7	0.474	1	9 a	43 ab
90	14.7 ab	73 ab	191	18.2 ab	12.3	0.520	2	5 ab	49 ab
120	22.5 a	119 a	192	18.0 ab	12.4	0.532	1	1 b	68 a

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

Table 2. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Brewster site, 2010)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Grease (%)*	*Sunburn (%)*	*Russet % clean*
0	23.0	89	210 b	16.0 a	12.9	0.47	5 c	3 bc	0
30	25.7	97	228 ab	15.5 ab	13.4	0.5	21 b	1 c	0
60	25.2	95	231 a	15.6 ab	13.1	0.50	15 bc	9 ab	0
90	23.0	86	229 ab	15.5 ab	13.4	0.52	25 b	5 bc	2
120	29.0	109	223 ab	15.5 b	13.1	0.49	47 a	15 a	4

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

Table 3. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Naches site, 2009)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Bitter pit (%)*	*Sunburn (%)*	*Russet % clean*
0	43.0	216	217ab	16.9bc	12.1	0.507	11a	16ab	57b
30	43.1	237	187 b	17.2 ab	12.5	0.499	4 b	21 a	72 ab
60	42.6	229	201 ab	17.5 a	12.6	0.537	3 b	20 a	68 b
90	49.4	274	191 ab	16.8 c	12.3	0.497	2 b	10 b	59 b
120	48.2	231	222 a	16.7 c	12.6	0.540	3 b	13 ab	85 a

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

Table 4. Effects of foliar urea sprays on Honeycrisp yield, fruit size and quality (Hilltop site, 2010)

Sprays	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Grease (%)*	*Bitterpit (%)*	*Russet % clean*
0	36.8	127	285	13.3	12.1	0.45	0	7	N/A
1	36.8	123	296	13.5	12.3	0.47	0	8	N/A
3	38.2	128	305	13.3	11.8	0.47	0	15	N/A
5	43.9	151	305	13.3	12.3	0.44	0	10	N/A
7	41.8	142	282	13.4	11.8	0.41	0	2	N/A

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

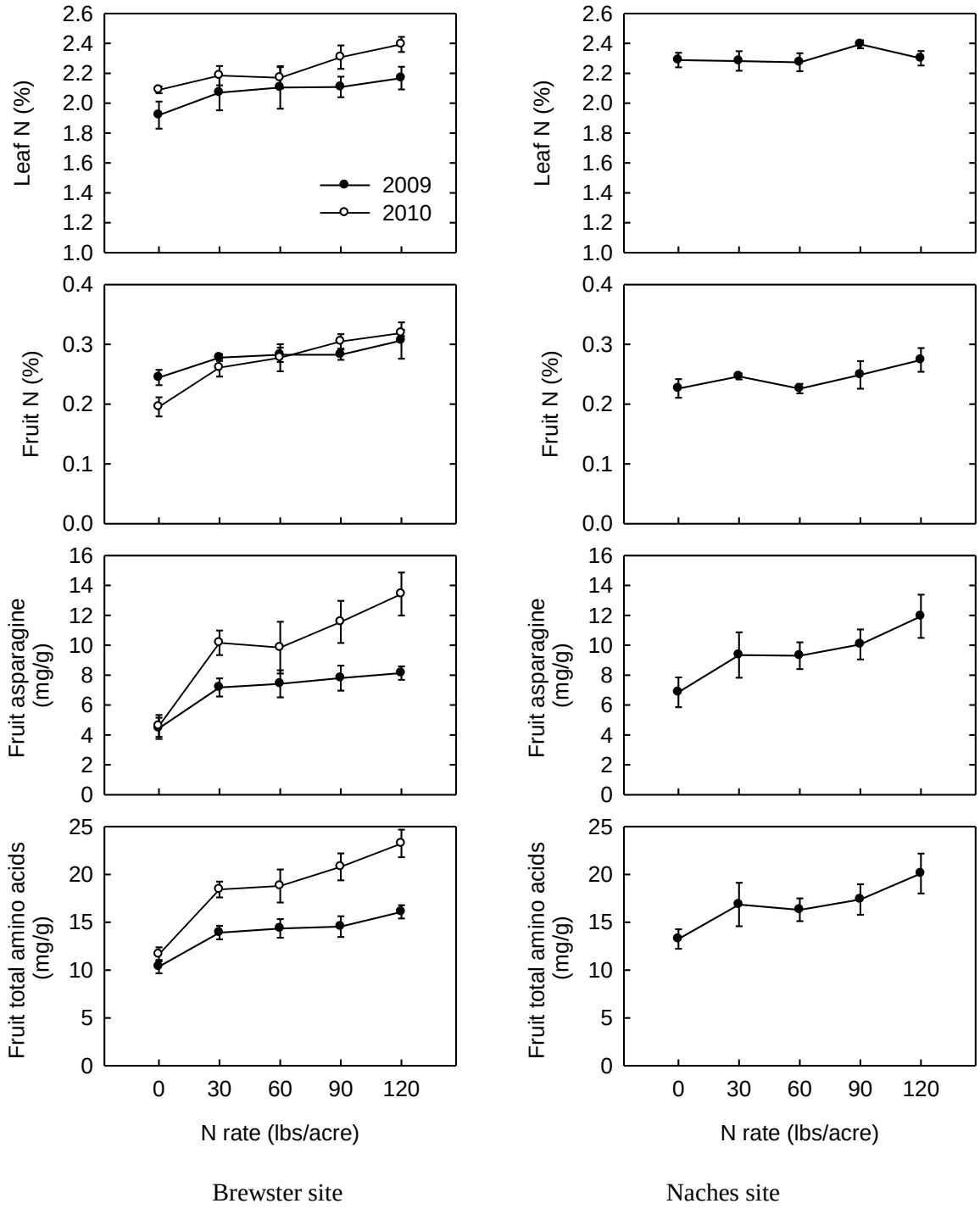

Fig 1. Nitrogen content of leaf samples taken in mid-August, and concentrations of nitrogen, asparagine and total free amino acids in Honeycrisp fruit at harvest in response to nitrogen fertilization at both Brewster site and Naches site.

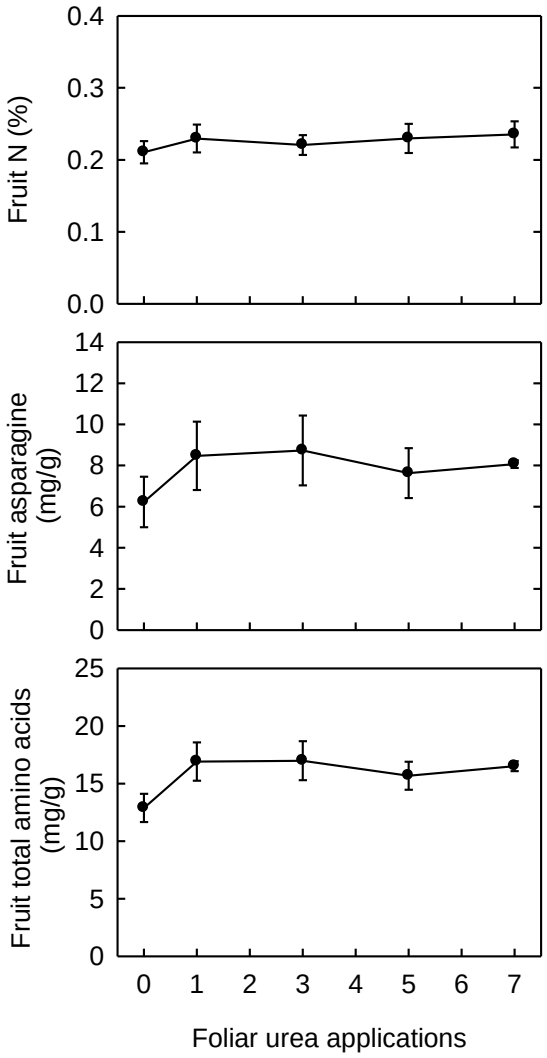

Fig. 2. Concentrations of nitrogen, asparagine and total free amino acids in Honeycrisp fruit at harvest in response to foliar urea applications

Executive Summary

The objectives of this project are 1) to determine how asparagine and other free amino acids in Honeycrisp fruit respond to N supply and relate the levels of free amino acids to fruit N status and fruit quality and 2) establish the optimal range of fruit N status expressed as asparagine level, with the goal of developing a sensitive indicator of Honeycrisp fruit N status for maximal quality. Over the last two years, we have used both soil application of nitrogen and foliar urea sprays to alter tree and fruit N status. The effects of N supply on fruit yield and quality and the concentrations of asparagine and total free amino acids in fruit were evaluated.

Over the range of N fertilization rate 0 to 120 lbs per acre, N fertilization had only limited effect on fruit yield, fruit size, quality and physiological disorders during the first two years of the trial. Although trees in the highest N treatment tended to have higher yield and bigger fruit, N fertilization beyond 30 lbs/acre didn't bring any significant benefit. Both leaf N and fruit N increased only slightly in response to increasing rate of N fertilization. However, fruit asparagine concentration increased significantly when N fertilization rate increased from 0 to 30 lbs N per acre. Any further increase in N fertilization rate didn't result in significant increase in fruit asparagine concentration. Asparagine accounted for about half of the total free amino acids in Honeycrisp fruit. As a result of the increase in asparagine concentration, the concentration of total free amino acids increased in the same way as asparagine in response to N fertilization. No significant effect on fruit yield, quality, or fruit N content was found in foliar urea treatments. However, both fruit asparagine concentration and total free amino concentration showed an increase when foliar urea was applied once, but no further increase was observed when the number of foliar urea sprays was increased further.

Compared with leaf N and fruit N, fruit asparagine concentration and total free amino acid concentration were more sensitive to N supply, and therefore should be better indicators of fruit N status. Since both fruit asparagine concentration and total free amino acid concentration responded more to the same soil N fertilization during the second year (2010) than during the first year (2009), and we only have one-year data on foliar urea application, both trials need to be repeated for another season in order to draw firm conclusions.

FINAL REPORT

Project Title: Identifying causes of variability in fruit quality

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Other funding sources

Agency Name: Emerging Research Issues, WSU-ARC
Amt. awarded: $64,756 awarded

Budget 1
Organization Name: WSU **Contract Administrator: Mary Lou Bricker**
Telephone: **Email address: mdesros@wsu.edu**

Item	2009	2010	
Salaries	26,952	28,030	
Benefits	1,955	2,033	
Wages	7,500	7,500	
Benefits	728	728	
Equipment			
Supplies	2,000	2,000	
Travel	10,000	10,000	
Miscellaneous			
Total	49,135	50,291	

OBJECTIVES

1. Document role of fruit developmental history at key stages (i.e., flowering, thinning, fruit maturation) and management interventions in fruit quality
2. Map fruit quality and position within high resolution 3D digital canopies
3. Develop practical strategies for reducing variability and maximizing genetic potential.

SIGNIFICANT FINDINGS

- Quality can vary considerably among fruit in high density fruiting wall architectures
- Variability in quality is not related to fruit position in high density fruiting wall architectures
- In general, the later opening blooms yielded lesser quality fruit
- Timing of flowering did not account for a great amount of the variability in fruit quality
- Fruit quality was negatively related with fruit number per tree underscoring the importance of crop load management
- Tree architecture and fruit quality 'maps' can be recreated in 3D using data points captured by laser-scanning
- In Gala there was no difference in fruit quality potential between king and side blooms

RESULTS AND DISCUSSION

Experiments were conducted in 2009 and 2010 at C & M Orchards, Prosser, WA on 11th and 12th leaf 'Buckeye Gala', and 3rd and 4th leaf 'Aztec Fuji'. Both were trained to super spindle architecture. Initially, before bud break, we gathered a geospatial electronic spectrum and limb diameters of three trees per variety. At harvest, we removed the leaves, collected geospatial data on fruit position and woody structure, measured limb diameters, and picked every fruit. During bloom, each flower was tagged using 3M ScotchCode™ numbered wire marker tape on the day it opened (i.e., was accessible to pollinators).

To evaluate the role of fruit position on quality, we used a Topcon total station to identify the position of every fruit and the woody structure of the tree at 2-inch increments. The canopy and fruits' positions were then recreated digitally using Matlab software. We assessed positional relationships with quality by analyzing quality parameters in three 'directions' – east to west, north to south, and vertically in the tree. There were no clear effects of a fruit's position on its quality irrespective of the attribute assessed (Fig. 1). Virtual 3D maps of fruit quality further illustrate a lack of relationship between fruit position and quality (Fig. 2). This suggests that thinning was effective and that light distribution was good – not surprising for 3rd leaf trees that had not yet filled the space.

In 2009, the first bloom for Gala (Fig. 3) and Fuji (Fig. 3) was on 22nd and 23rd of April, respectively. The final blooms for Gala and Fuji were tagged on 12th and 8th of May, respectively. Gala had a total of 2094 tagged flowers with trees 1, 2, and 3 having 669, 643, and 782 flowers, respectively. Progression of bloom is being modeled with growing degree hours currently (data not shown). By 10 days from the first bloom, 60% of the floral buds were open and these flowers accounted for 92% of the fruit retained for harvest. At harvest on 26 August, there were 189 Gala fruit with tree 1, 2, and 3 yielding 64, 61, and 64 fruit, respectively. The fruit to bloom ratio was 9% for Gala in 2009. In Fuji for 2009, we counted a total of 524 tagged blooms with tree 1, 2, and 3 having 124, 128, and 271 flowers, respectively. The fewer flowers compared to Gala were due to incomplete canopies of the 3rd leaf trees. At harvest on 7 October, there were 99 Fuji fruit with tree 1,

2, and 3 yielding 49, 21, and 29 fruit, respectively. The fruit to bloom ratio overall for Fuji was 6% in 2009 – slightly lower than expected due to frost damage.

The bloom count/tagging for Gala in 2010 started 13 April and finished 3 May with 2022 total blooms tagged with tree 1, 2, and 3 having 572, 732, and 718 flowers, respectively. We followed the same three trees in both years and can draw no cause-effect relationship between fruit number per tree in 2009 and return bloom in 2010. Interestingly, tree 1 had about 28% fewer flowers than tree 2 despite the trees having similar fruit numbers in the previous year, and being pruned similarly. At harvest on 30 August, there were a total of 221 fruit with tree 1, 2, and 3 yielding 78, 71, and 72 fruit, respectively. Fruit set (meaning here the number of fruit harvested per number of flowers) was therefore about 14%, 10%, and 10%, respectively. For Fuji, bloom tagging started on 15 April and finished on 3 May totaling 1329 with tree 1, 2, and 3 having 531, 314, and 494 flowers, respectively. At harvest on 5 October, there were a total of 174 Fuji fruit with tree 1, 2, and 3 yielding 73, 35, and 66 fruit, respectively, or, a final fruit set of about 14%, 11%, and 13%. The similar final 'fruit set' percentage between Fuji and Gala (despite significantly different flower numbers per tree) reflect the effectiveness of the thinning crew and the managers' knowledge of optimizing fruit load per tree.

Crop load management was accomplished in both years and for both Gala and Fuji with 'standard' chemical bloom and post-bloom programs (lime sulphur and carbaryl) followed by hand thinning. Lime sulfur was applied in both years on both cultivars at 50% bloom with following applications at 70% bloom. Further, Fuji trees received applications of carbaryl and ethephon to reduce crop load attempting to reduce the effects of biennial bearing. Prior to hand thinning, whether the result of chemical thinning or natural drop, the Gala trees had a reduction in flower/fruitlets of 74% and 71% in 2009 and 2010, respectively. Hand thinning fruit accounted for reductions of crop load of only 8% and 2% for 2009 and 2010, respectively. The Fuji trees had a reduction in flower/fruitlets of 67% and 81% in 2009 and 2010, respectively. Hand thinning accounted for 20% and 4% of thinning in 2009 and 2010, respectively. We continue to analyze cluster composition in relation to thinning applications to judge efficacy. The efficacy of the two thinning treatments (bloom and post-bloom) is questionable since we did not record notable fruit drop in the weeks following application (data not shown).

The orchard managers' goal in 2010 was to increase the crop load and this meant there was less hand-thinned fruit (trees in 2010 bore an additional 7 to 14 fruit compared to 2009). The increased crop load in 2010 did not have a significant effect on Gala, whereas Fuji by contrast, had reduced fruit quality in 2010 compared to 2009. In Gala, the average mass difference was only 4% with average fruit weighing only 9 g more in 2009. The other quality parameters assessed were similarly variable, suggesting that the increase in fruit number in 2010 was nearer the target crop load. Indeed, the increase of about 20 fruit per tree did not reduce the average harvested fruit size below size 88. However, Fuji fruit quality varied considerably between 2009 and 2010 seasons. In 2009, weight was 21% greater, diameter was 16% greater, and color was 28% greater. In addition, starch was higher in 2009 by 48% and conversely firmness was lower by 18%. Though variability appears large between the years, the average fruit quality for packout did not fall below 88's.

The variability within the individual trees in 2010 cannot be attributed to a single aspect of crop load per limb, position in canopy, or multiple fruits per spur. We found, for example, that a 53 g fruit was at the top of the canopy and that a 272 g fruit was harvested from the same limb (i.e., ostensibly a similar environment). Further, at approximately a height of 2 m (6 feet), in the same tree, four fruit were harvested, weighing 248 g, 181 g, 261 g, and 278 g, a difference of greater than 50%, again from fruit in a similar environment. As another example in the same year in Fuji, at 1.5 m (about 5 feet), a single limb carried 5 fruit that weighed 284 g, 244 g, 229 g, 289 g, and 293g. These

preceding examples may demonstrate variable capacity for some limbs to partition photoassimilates, or a pre-determined limit to fruit size. When work is finished on the geo-spectral mapping, we will be able to gather a clearer understanding of limb structure and position of the limb's capacity for crop load. Multiple fruit per spur, left to mature, have varying quality. Generally, in our study multiple fruit per spur was uncommon. In 2010, there were only 6 spurs with more than singles after hand thinning in Gala. One spur held 3 fruit and the others had 2. On the spur with the 3 fruit, the largest apple weighed 204 g followed by 198 g and 145 g. Of the 13 fruit observed, only 5 fruit weighed greater than 215 g or graded larger than 88. We continue to evaluate dynamic cluster composition data to determine the role of fruit-fruit competition on final fruit quality. Our current analyses are evaluating cluster composition of 1) the poor quality fruit (10^{th} percentile) vs. high quality (90^{th} percentile) and, 2) fruit from flowers that opened on the same day yet varied in quality significantly at harvest.

Comparing bloom time and fruit quality reveals a subtle relationship that does not account for the significant variability among fruit in their quality. Overall, the fruit from earlier bloom tended to be heavier and larger than fruit from the late-opening flowers (Figs. 5 & 6). Conversely, the earlier blooms yield fruit that have slightly lower starch and are less firm. Over the 20 days of Gala flowering in 2009, mean fruit weight dropped by 42%, diameter decreased by 17% (Fig. 5), starched decreased by 37% and firmness increased by 11%. In 2010, bloom was complete in 20 days with weight decreasing by 46%, diameter reduced by 18% (Fig. 3), starch rating was 83% lower, and firmness was 9% greater. For Fuji over an 11-day bloom period, mean fruit weight decreased by 50%, diameter was reduced by 19% (Fig. 4), starch decreased by 17%, and a reduction of 22% was recorded in firmness. In 2010, over a 13-day bloom period, Fuji fruit lost 45% weight, 16% diameter (Fig. 4), 15% starch, and firmness increased by 18%. While the variability among fruit from flowers opening on a given day remains substantial (Figs. 4 & 5), crop load management strategies should focus on removing late-opening flowers/fruit.

King and Side Bloom Comparison. Gala floral buds/blooms/fruitlets were pinched back to either one king bloom or side bloom to assess fruit quality at different stages in floral development. Statistical comparisons of king, side, and control (fruit from the timing of flowering experiment above) show that quality of fruit from king blooms is not any better than fruit from side blooms considering fruit quality characteristics such as weight, diameter, and height (Table 1). Trees in this study that had similar crop loads (kg/cm^2) to the floral timing trees, fruit quality was not significantly different. However, trees with crop loads above 6 fruit/cm^2 began to show greater variability within the treatment (Table 2). Our trees in this study were not hand-thinned to adjust crop load. To truly identify fruit superiority the whole crop load adjustment regime should have been applied through the treatments. However, our data underscore the importance of crop load management for growing superior quality fruit. It is not sufficient in many orchard systems to limit crop load to a single fruit per cluster – cluster thinning to a target fruit number per tree is necessary to achieve high quality.

Table 1. Comparisons of fruit quality of 'Buckeye' Gala trees thinned to one flower per cluster (king, side) with control (hand thinned) by least significant difference ($P \leq 0.05$)

Treatment	% Red (Closest 10%)		Weight g		Diameter mm		Height mm		Firmness lb		Water Core Rating 1-5		Starch Rating 1-6		Brix	
Control	86.057	a	229.86	a	78.13	a	78.37	a	17.47	a	1	a	3.77	a	11.96	a
King	54.701	c	203.42	b	74.13	b	74.96	c	17.28	a	1	a	2.92	c	10.68	b
Side	62.704	b	229.39	a	77.92	a	76.44	b	17.47	a	1	a	3.23	b	10.62	b
lsd	3.105		7.67		1.16		1.23		0.39		0.02		0.22		0.17	
r x r	0.35		0.08		0.08		0.04		0.002		0.002		0.07		0.28	

Table 2. Comparisons of mean fruit yield and quality from trees thinned to only king or side bloom at various timings. All comparisons are calculated as least significant difference ($P \leq 0.05$).

Treatment	# of Fruit		Yield (kg)		Fruit Weight (g)		TCSA (cm*cm)		Fruit/cm2
4/17/ King	107	ab	23.3	a	216	a-c	16.4	a-d	6.5
4/17/ Side	87	bc	18.5	b-c	211.2	bc	13.5	cd	6.4
4/21/ King	93	a-c	18.6	a-d	200.8	bc	15.5	b-d	6.0
4/21/ Side	87	bc	18.6	a-d	214.1	a-c	11.9	d	7.3
4/27/ King	97	a-c	19.7	a-c	204.13	bc	16.3	a-d	6.0
4/27/ Side	60	d	14.8	de	246.6	a	22.9	ab	2.6
5/5/ King	91	a-c	20.1	a-c	226.5	ab	21.5	a-c	4.2
5/5/ Side	52	d	12.5	e	244.6	a	18.3	a-d	2.8
5/13/ King	109	ab	21.4	a-c	196.7	bc	17.6	a-d	6.2
5/13/ Side	97	a-c	21.7	ab	223.3	ab	23.9	a	4.1
5/26/ King	110	a	20.2	a-c	183.5	c	12.3	d	8.9
5/26/ Side	84	c	16.8	c-e	199.1	bc	20.9	a-c	4.0
lsd	21.7		4.6		33.3		8.13		
r x r	0.73		0.62		0.56		0.49		

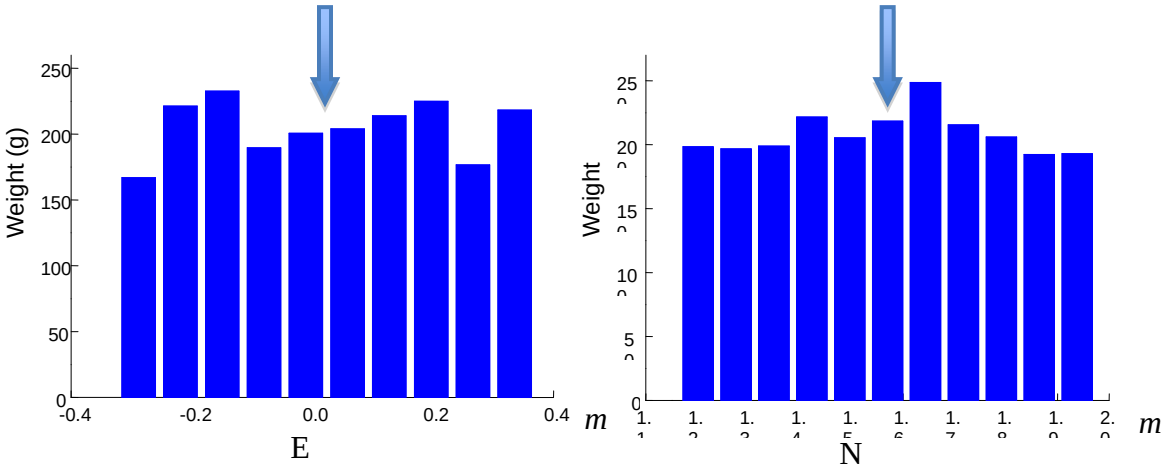

Figure 1. Distribution of mean apple weight (g) in E-W (left) and N-S (right) directions within 3rd leaf 'Aztec Fuji' canopies. The canopy central axis is at the middle along the x-axis (indicated by arrow). Row orientation is N-S.

Figure 2. Model of 3 consecutive 3rd leaf 'Fuji' trees illustrating fruit position, size and color. Size of model fruit is proportional to actual size and color is indicative of actual fruit color. Data points were collected with Topcon laser total station; 3D virtual trees and fruit were constructed using Matlab software.

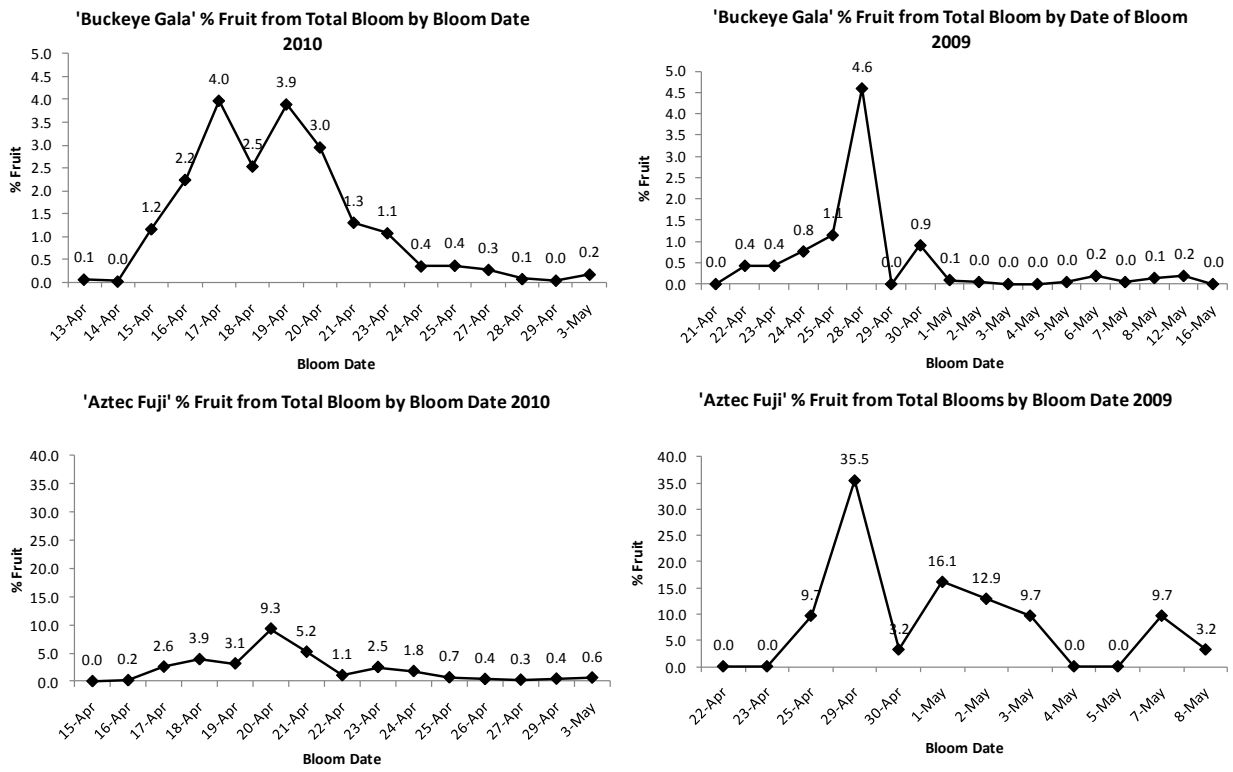

Figure 3. The fruit harvested as a percentage of total blooms open throughout the bloom period for 3 consecutive trees of 'Buckeye Gala' or 'Aztec Fuji'. 'Buckeye Gala' had 2094 blooms with 189 fruit harvested in 2009 and 2022 blooms with 221 fruit harvested in 2010. 'Aztec Fuji' had 524 blooms with 99 fruit harvested in 2009 and 1329 blooms with 174 fruit harvested in 2010.

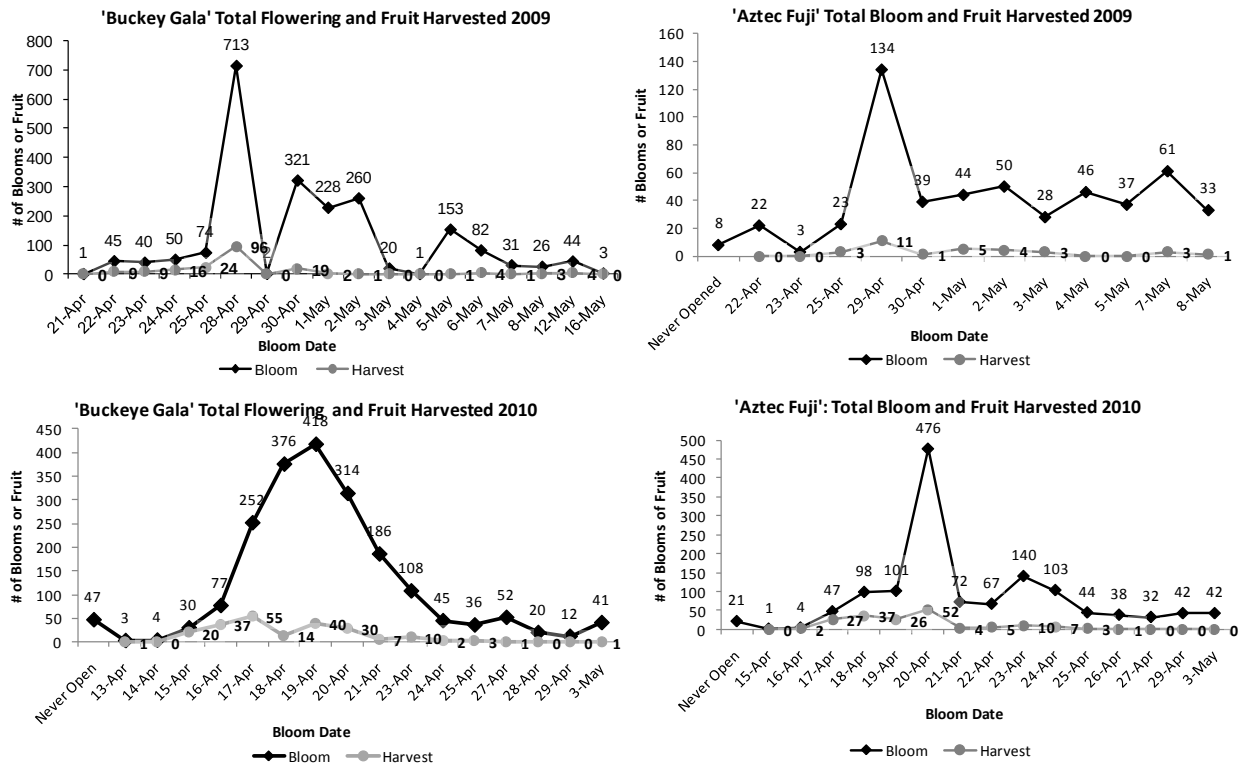

Figure 4. Total flowers opening by date (black line), and total fruit harvested from flowers open on that date (grey line) for 'Buckeye Gala' and 'Aztec Fuji' in 2009 and 2010.

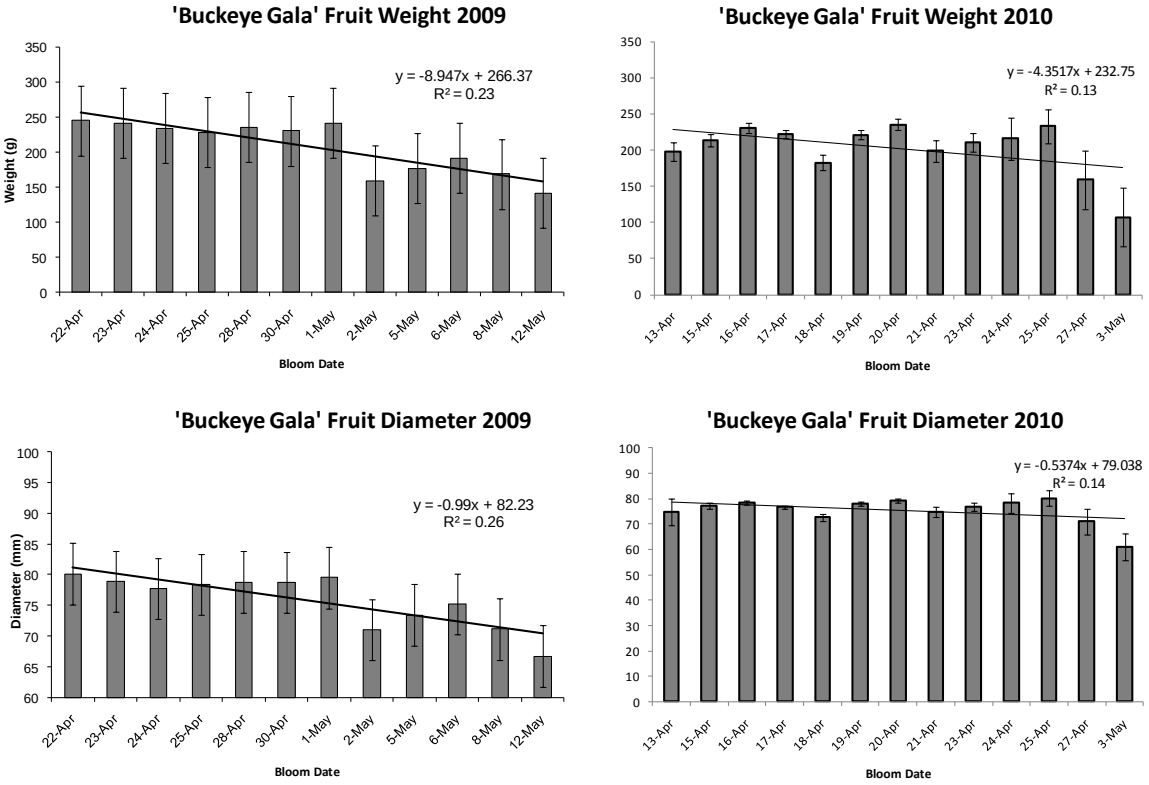

Figure 5. Fruit weight (g) and diameter (mm) of 'Buckeye Gala' by the date of the flower opening in 2009 and 2010, $P < 0.05$.

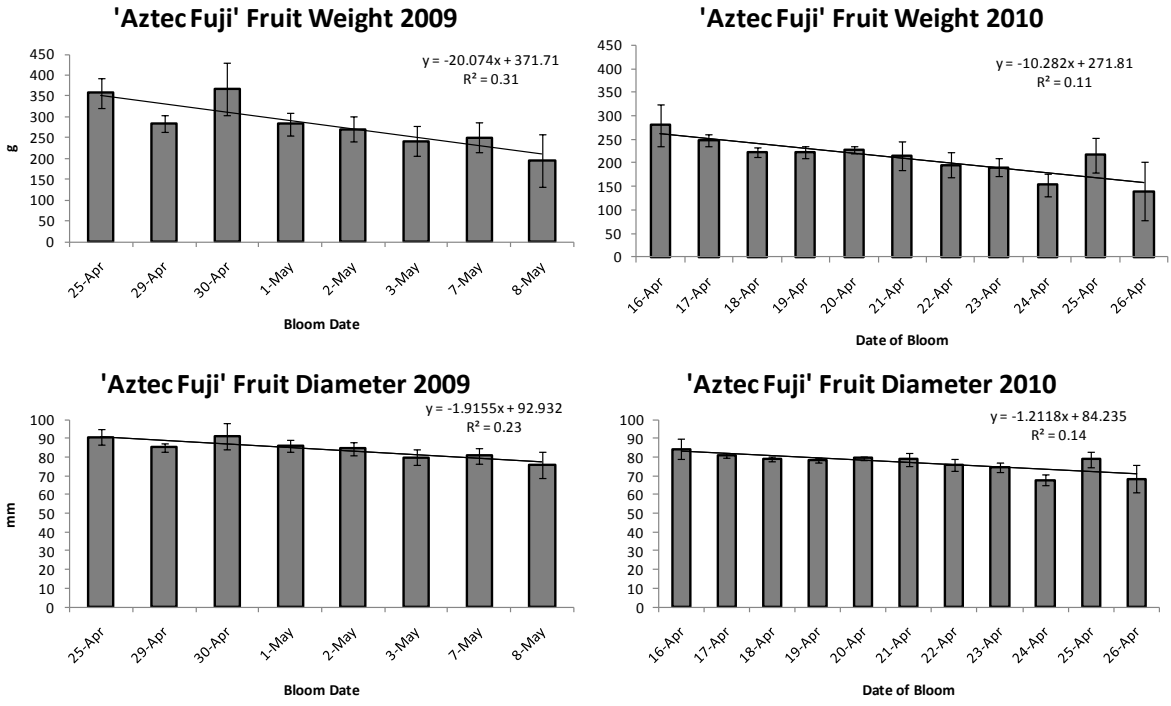

Figure 6. Fruit weight (g) and diameter (mm) of 'Aztec Fuji' by the date of the flower opening in 2009 and 2010, $P < 0.05$.

EXECUTIVE SUMMARY

Our research team set out better understand the role of fruit position, timing of flowering, floral hierarchy (i.e., king vs. side bloom), and fruit developmental context (i.e., a single vs. triple-fruited spur prior to thinning, etc.) on fruit quality in apple. Our previous data had identified tremendous variability in fruit quality at harvest despite being grown with high management inputs and in compact fruiting wall architectures. We hypothesized that much of this variability is due to differences in floral timing, fruit position, and/or fruit developmental context. We evaluated these elements for 'Buckeye Gala' and 'Aztec Fuji' over two years in commercial orchards north of Prosser, Washington. To test the hypotheses, we labeled each flower when it had opened sufficiently for a pollinator to access. Starting May 19 2009 and 2010, we counted and recorded position of the number of fruit/fruitlets remaining and measured them for diameter weekly until harvest. At harvest, the fruit quality was analyzed by measuring weight, diameter, height, color (as % red), firmness, water core, starch, soluble solids and recorded any blemish affecting quality (e.g. sunburn, mechanical, insect and/or animal damage). In addition, at harvest we made three-dimensional structural maps of the harvested trees and each fruit's position using data acquired by a Topcon total station laser scanner and Matlab software.

In the high-density fruiting wall orchards studied, we found no role of fruit position on fruit quality – there were high and poor quality fruit in every canopy position, though, overall, fruit quality was very high. We created high resolution 3D virtual canopies that demonstrate this point dramatically. In trees of the 'standard' system – central leader, moderate density (e.g., 7 x 13), we documented a significant role of position on quality with the highest quality (size and color) fruit positioned around the periphery of the canopy and the poor quality fruit situated in the lower canopy tiers and to the interior (data not shown). Our studies now focus on the poor quality fruit and understanding what made them so. It appears that timing of flowering is not entirely responsible for variability in fruit quality, though we did document a slight relationship between final fruit quality and timing of flowering, with fruit from the later-opening flowers being poorer quality than those from the early- and mid-opening flowers. The significant variability that exists among fruit that were from flowers opened on the same day becomes the next concern. This can be as much as 100 g – the difference between several box sizes. Our current efforts are to reconcile these differences with fruit developmental context, reviewing what cluster composition was and when thinning occurred within the individual cluster. Future studies should also investigate the possibility of there being differences in fruit quality potential among flowers (i.e., already established by the time of flowering) that would have been established in the previous season, post initiation.

FINAL PROJECT REPORT

Project Title: Integration of storage technologies for fruit quality management

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Other funding sources

1. Agency Name: USDA-NIFA (competitive grant)
(Federal + non-Federal): $2.4 million (total) over 4 years.
Notes: D. Rudell is Project Director, J. Mattheis is a Co-PI. The Standard Research and Extension Project, "A diagnostic toolbox for integrated management of postharvest apple necrotic disorders" was submitted (01/12/10) for the current funding cycle. WTFRC and AgroFresh, Inc. are co-sponsors.

2. Agency Name: AgroFresh, Inc.
Amount awarded: $19,000
Notes: Funding supports 'Honeycrisp' storage research.

Total Project Funding: $83,613

Budget History:

Item	2008	2009	2010
Salaries	$19,605	$20,198	$20,808
Benefits	$6,470	$6,665	$6,867
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	$1000	$1000	$1000
Travel	0	0	0
Miscellaneous	0	0	0
Total	$27,075	$27,863	$28,675

RECAP ORIGINAL OBJECTIVES:

The project had 4 objectives, 2 for 'Honeycrisp' storage and disorders, 2 for postharvest use of chlorophyll fluorescence monitoring technology. The objectives reflected perceived knowledge gaps in information available to the industry generated using fruit produced in Washington. Increasing 'Honeycrisp' volume suggests long term storage protocols are needed, the basis for objective 1. The use of 'Honeycrisp' as a breeding parent and the heritability of disorder (soft scald, soggy breakdown) susceptibility in light of a near complete lack of knowledge pertaining to disorder biochemistry and genetics was the basis for objective 2. While commercial technology for fruit chlorophyll fluorescence monitoring is used in a number of warehouses, questions existed related to longevity of superficial scald prevention following low O_2 storage based on fluorescence monitoring (objective 3) and the utility of this technology for assessing CO_2 injury risk.

SIGNIFICANT FINDINGS:

Objective 1. Identify postharvest protocols to maximize storage life of 'Honeycrisp' and other softscald-sensitive cultivars. Substantially met for 'Honeycrisp', not met for other cultivars.

1. 'Honeycrisp' storage regimes that included 7 days at 50 ^{o}F after harvest then 36-39 ^{o}F largely controlled development of soft scald and soggy breakdown.
2. Late harvest 'Honeycrisp' apples (starch 6.0, yellow ground color, slight greasiness) are at high risk of soft scald, soggy breakdown, internal radial browning, senescent browning, peel dimpling and greasiness within 2 months after harvest.
3. Risk of 'Honeycrisp' internal radial browning increased with decreased O_2 concentration in CA; and decreased with use of SmartFresh®.
4. Harvista™-treated 'Honeycrisp' had less softscald and soggy breakdown compared to non-treated or SmartFresh®-treated fruit.
5. CA (1.5% O_2/1% CO_2) storage of 'Honeycrisp' reduced greasiness and acid loss regardless of temperature (33, 36, 39 ^{o}F) compared to fruit stored in air at 39 ^{o}F.
6. Differences in 'Honeycrisp' volatile production were evident between controls and SmartFresh® - treated fruit stored in air or CA.
7. Final storage temperature of 36-39 ^{o}F reduced soft scald and soggy breakdown risk compared to 33 ^{o}F.
8. Internal CO_2 injury developed in 'Honeycrisp' fruit stored in 3 or 5% CO_2 but not 1% CO_2, all with 2% O_2.
9. Based on all experiments, 'Honeycrisp' harvested with starch score 4-5 on a scale to 6, breaking ground color, held 7 days at 50 ^{o}F then 36-39 in CA (2% O_2, 1% or less CO_2) are likely to perform well during long-term storage. SmartFresh® use can contribute to enhanced titratable acidity and lack of greasiness after storage in air or CA.

Objective 2. Characterize physiological events that result in softscald symptom development. Partially met.

1. Changes in 'Honeycrisp' metabolism were detected prior to symptom development.
2. Differences exist in volatile and non-volatile compounds between fruit that did and did not develop softscald.

Objective 3. Identify minimum storage duration required to control superficial scald by use of oxygen setpoints below 1%. Partially met.

1. 'Granny Smith' apples stored at 0.2% O_2 above the low O_2 fluorescence deflection point + 1% CO_2 did not develop superficial scald through 7 months in CA +21 days RA + 21 days at 68 ^oF, or 7 months CA +10 days RA + 28 days at 68 ^oF.

Objective 4. Identify what if any limits for CO_2 exist during apple storage in oxygen below 1%. Partially met.

1. 'Granny Smith' apples stored in up to 3% CO_2 with 0.2% O_2 did not develop CO_2 injury symptoms through 9 months in storage plus 7 days at 70 ^oF.
2. No change in chlorophyll fluorescence of 'Fuji' apples was detected in CO_2 concentrations up to 10%. 'Fuji' apples from the same lot stored in 1% O_2 with 3 or 5% CO_2 developed internal browning consistent with CO_2 injury.

RESULTS & DISCUSSION
The utility of 7 days at 50 ^oF followed by lower storage temperature for 'Honeycrisp' apples to avoid soft scald and soggy breakdown was standard practice for most 'Honeycrisp' experiments in this project (Table 1). While some lots will not develop these chilling disorders if cooled directly to 33-36 ^oF after harvest, no methods are currently known to predict lot to lot susceptibility to chilling injury. Bitter pit development was not significant in any of the experiments we have conducted during the 2008-2010 period. A 7 day period at 50 ^oF to condition fruit to lower temperatures, first reported by Chris Watkins of Cornell University, in our experiments usually has prevented chilling injury. Some instances where the 50 ^oF conditioning has not completely prevented the disorders have been relatable to the final storage temperature, where disorder incidence increased as temperature decreased (Table 2). Harvest maturity is also a factor influencing chilling injury risk, with injury susceptibility increasing with later harvest. Horticultural practices that favor red color development and initiation of degreening (nitrogen and crop load management) may allow earlier maturity and harvest. In the absence of red color development, the lack of softening during maturation and storage may lead to harvest (late) and storage duration (long) decisions that compromise other fruit quality attributes including disorder susceptibility.

	2008	2009	2010
Soft Scald 7d @ 50 ^oF	0,13,0,2,0,0	1,0,7,5,0,9,3	4,1
Bitter pit	0, 4,0,1,0,0	0,0,0,0,0,0,0	0,0
Soft scald 33 ^oF continuously	80	45	1

Table 1. Incidence (%) of soft scald and bitter pit in 'Honeycrisp' apples during multiple ARS experiments in 2008-2010. Fruit were held in air at 50 ^oF for 7 days then at 33, 36, or 39 ^oF, or continuously at 33 ^oF (one experiment per year only).

Harvest Date	Storage ^oF	% Soft Scald	% Soggy Breakdown
Sep 21	33	12a	12a
	36	0b	0b
	39	0b	0b
Oct 3	33	49a	26a
	36	10b	3b
	39	1b	1b

Table 2. 'Honeycrisp' cumulative (n=72) chilling disorders during 7 months cold storage in air. All fruit held 7 days at 50 ^oF after harvest. Disorder incidence recorded after 1,3,5 ,and 7 months. Means (n=18) followed by different letters are significantly different, Tukey's HSD, $p<0.05$.

Consequences of higher storage temperature on fruit quality are minimized within the 33-39 ^oF range by storing near 36 ^oF (Tables 3,4). After 1-7 months in air plus 7 days at 70 ^oF, firmness and acidity

were either enhanced or similar for fruit stored at 33 or 36 ^{o}F compared to 39 ^{o}F. Soluble solids content (SSC) is typically not responsive to cold storage conditions, and although statistically significant differences were observed related to temperature in various experiments, real differences in eating quality relatable to the magnitude of SSC variation among treatments is suspect.

Controlled atmosphere storage at 2% O_2 and 1% CO_2 slows 'Honeycrisp' ripening as measured by titratable acidity and peel greasiness. No chilling disorders or bitter pit developed on fruit in this experiment. In general, lots used in the 3 years of this project were not susceptible to bitter pit, and where the disorder was present, incidence was low and no treatment differences in bitter pit incidence were observed.

Month	Storage ^{o}F	Lbs	% TA	% SSC
Harvest	--	15.6	0.506	13.4
1	33	15.6ab	0.477ab	13.3b
	36	16.4a	0.498a	13.8a
	39	15.0b	0.436b	13.2b
3	33	16.2a	0.420a	13.2a
	36	16.0a	0.424a	13.0ab
	39	14.1b	0.363b	12.6b
5	33	15.1	0.379a	13.3a
	36	15.2	0.371a	13.1a
	39	14.5	0.342b	12.6

Table 3. 'Honeycrisp' fruit firmness, titratable acidity (TA) and soluble solids content (SSC) after air storage. All fruit held 7 days at 50 ^{o}F after harvest, and for 7 days at 70 ^{o}F after storage. Means (n=18) followed by different letters are significantly different, Tukey's HSD, $p<0.05$.

months	atmosphere	^{o}F	% titratable acid	% soluble solids	% greasy	lbs
harvest	--	--	0.494	13.2	0	13.7
4	Air	33	0.339cd	12.7ab	4b	13.9
		36	0.358bc	12.9a	13b	14.9
		39	0.313d	12.2b	40a	14.0
	2% O_2 1% CO_2	33	0.414a	13.0a	4c	14.0
		36	0.365bc	12.6ab	4c	13.8
		39	0.383b	12.6ab	0c	14.4
7	Air	33	0.290bc	12.3bc	6bc	13.8
		36	0.276c	12.1c	14b	13.8
		39	0.276c	12.8ab	83a	14.0
	2/1 O_2/CO_2	33	0.372a	12.7abc	0c	13.4
		36	0.382a	13.4a	3c	13.9
		39	0.321b	12.3bc	0c	13.3

Table 4. 'Honeycrisp' fruit quality after air and CA storage. All fruit held 7 days at 50 ^{o}F after harvest, and for 7 days at 70 ^{o}F after storage. Means (n=18) followed by different letters are significantly different, Tukey's HSD, $p<0.05$.

'Honeycrisp' demonstrated sensitivity to low O_2 and high CO_2 during this project. Low O_2 injury was manifested as interior radial (Table 5) or diffuse and calyx end browning (Table 6). Internal and calyx end browning occurred with fruit for which the O_2 concentration was set based on chlorophyll fluorescence monitoring (HarvestWatch^{TM}) indicating metabolic events leading to low O_2 injury may not be detectable by monitoring fluorescence. As lowering O_2 increased risk of radial browning, without substantial enhancement of fruit quality, results to date do not support CA protocols with O_2 less than 1.0%.

months	treatment	% titratable acidity	ground color 1-5	soft scald %	% radial browning	lbs
4	air	0.314c	5a	0	0	15.0
	O_2: 0.3	0.346bc	5a	0	3	14.1
	0.5	0.346bc	4b	3	3	14.7
	0.8	0.413a	5a	0	11	15.5
	1.5	0.358b	5a	3	6	14.9
6	air	0.249d	4.9a	0	0b	13.8
	O_2: 0.3	0.387a	4.3b	6	31a	14.9
	0.5	0.380ab	4.4ab	8	33a	14.8
	0.8	0.350bc	4.3b	0	19a	14.4
	1.5	0.325c	4.2b	0	6b	14.9

Table 5. 'Honeycrisp' fruit quality after storage, 2008 harvest. All CA treatments contained 0.5% CO_2. All fruit held 7 days at 50 ^oF after harvest, final storage temperature 33 ^oF. Fruit held 7 days at 70 ^oF after storage. Change in fruit chlorophyll fluorescence detected at 0.3% O_2. Means (n=18) followed by different letters are significantly different, Tukey's HSD test, $p<0.05$.

Month	% O_2	trt	Peel color 1-5	% titratable acidity	% internal browning	% calyx browning	lbs
Harvest			2.9	0.589	--	--	15.1
4	Air	Ck	4.7*	0.391	0	0	14.3
		SF	3.6	0.472*	0	3	14.4
	2	Ck	3.4	0.427	3	0	14.6
		SF	3.8	0.446	0	0	13.9
	1	Ck	3.6	0.441	3	0	14.6
		SF	3.7	0.467	22*	3	14.6
	0.5	Ck	3.3	0.449	14	9	14.6
		SF	3.7	0.490*	36*	19	14.2
7	Air	Ck	5*	0.360	0	0	14.7
		SF	4	0.461*	0	0	14.6
	2	Ck	3.4	0.419	8	0	14.4
		SF	3.1	0.423	3	0	14.2
	1	Ck	3.5	0.411	9	0	13.7
		SF	3.7	0.439*	10	17*	14.3
	0.5	Ck	3.3	0.426	17	22	14.5
		SF	3.5	0.460*	37	21	14.1

Table 6. 'Honeycrisp' fruit quality after storage, 2009 harvest. Ck: check, SF: SmartFresh®. Starch at harvest = 5.7 (1-6). CO_2 =0.5% all CA treatments. All fruit held 7 days at 50 ^oF after harvest, storage temperature 36 ^oF. SmartFresh® applied the day of harvest. Fruit held 7 days at 70 ^oF after storage. Change in fruit chlorophyll fluorescence detected at 0.3% O_2. *: means (n=18) are significantly different, Bonferroni t-test, $p<0.05$.

Incidence of internal browning and cavities increased with storage CO_2 concentration during 'Honeycrisp storage (Table 7). Browning symptoms from CO_2 exposure (diffuse, light brown color) were distinguishable from chilling-related soggy breakdown (solid patches, saturated tan color). Likelihood that injury is induced by CO_2 is supported by DPA use in a 2010 experiment where injury has occurred in untreated fruit but not DPA-treated (1000 ppm) fruit stored in 5% CO_2 (not shown).

Month	% CO_2	% internal browning	% cavities
4	1	0*	0
	3	14	0
	5	25	0
7	1	0*	0
	3	15	15
	5	36	10

Table 7. 'Honeycrisp' disorders after high CO_2 CA storage. O_2 =2% all CA treatments. All fruit held 7 days at 50 ^oF after harvest, storage temperature 36 ^oF, fruit held 7 days at 70 ^oF after storage. *:significant linear trend, r^2=0.94.

Use of SmartFresh® with 'Honeycrisp' slows fruit ripening resulting in higher acid retention, slower ground color change (in air storage), reduced greasiness and radial browning (Table 8, Figure 1). While the field 1-MCP formulation Harvista™ reduced chilling disorders (Figure 1), SmartFresh® has not consistently impacted development of soft scald or soggy breakdown in our experiments. Production of ripening/senescence-related ethyl volatiles (Figure 2) is delayed by SmartFresh® treatment, an impact that may prolong the optimal eating period. Ethyl ester production increases in many cultivars including 'Honeycrisp' as ripening and senescence progress. The lack of softening in 'Honeycrisp' can be misleading as an indicator of overall fruit quality when compared with flavor and taste (i.e. good firmness and texture, overripe flavor and taste).

	Air	SF air	1.5% O_2/1% CO_2	SF 1.5% O_2/1% CO_2
Ground color	5.0a	5.0a	4.2b	4.3b
% titratable acid	0.258d	0.287c	0.320b	0.336a
% radial browning	0b	0b	28a	6b

Table 8. 'Honeycrisp' CA 1.5 O_2 1 CO_2. final temperature 33 ^oF. SF: SmartFresh® applied the day of harvest. Means (n=18) followed by different letters are significantly different, Tukey's HSD, p<0.05.

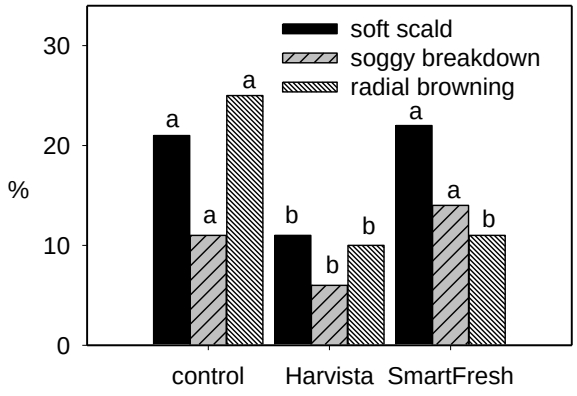

Figure 1. Cumulative soft scald, soggy breakdown, and radial browning incidence for 'Honeycrisp' fruit stored up to 6 months in air. Means followed by different letters are significantly different, Tukey's HSD test, p<0.05.

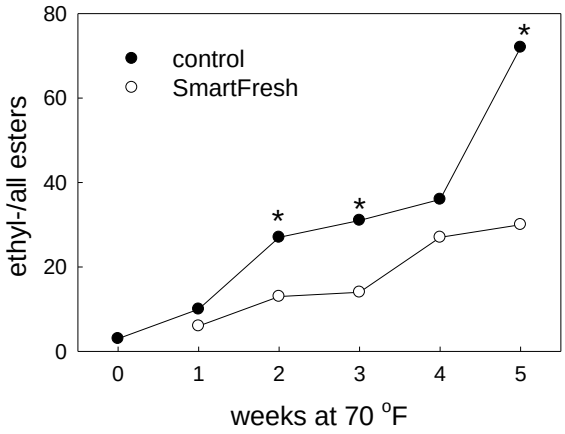

Figure 2. Ratio of ethyl esters to all esters during 'Honeycrisp' ripening at 70 ^oF. SmartFresh® applied the day of harvest. *: means significantly different, Bonferroni t-test, p<0.05.

Objective 2. Characterize physiological events that result in softscald symptom development. In an experiment during which 80% of 'Honeycrisp' apples held continuously at 33 ^oF developed soft scald while fruit from the same lot stored 7 days at 50 ^oF then 33 ^oF did not develop the disorder, several

differences in fruit chemistry were detected. Volatile compounds indicative of overripe/senescent apples including acetaldehyde, ethanol, and many ethyl esters were produced in much higher amounts compared to fruit without symptoms. Two unique volatiles, ethyl butenoate and ethyl 2-methylbutenoate, were produced only by fruit that developed soft scald. A group of non-volatile compounds, acylated sterol glycosides, were also detected only in injured fruit. Volatiles typical of ripening 'Honeycrisp' including acetate, butyrate and propionate esters were higher in sound fruit. A number of the compounds typical of fruit that developed soft scald were detected prior to symptom development indicating a potential use as biomarkers for soft scald prediction. The butenoate esters and acylated sterol glycosides unique to damaged fruit have been found in other cultivars with other disorders (superficial scald, CO_2 injury) indicating a relationship with fruit stress response.

Objective 3. Identify minimum storage duration required to control superficial scald by use of oxygen setpoints below 1%. 'Granny Smith' apples stored in air, 1.5 or 0.5% O_2 with 1% CO_2 were stored at 33 ^oF for up to 9 months. The low O_2 setpoint was determined by monitoring fruit chlorophyll fluorescence using the HarvestWatch^{TM} system. Fruit were moved from CA after 3, 5, 7, or 9 months to air and held 10 or 21 days, then moved to a warm room (70 ^oF) and held up to 28 days. Scald symptoms were assessed weekly during the 28 day warm room period. Scald developed only on air-stored fruit after 3 months plus 7 days at 70 ^oF, and on air- or 1.5% O_2-stored fruit after 5 months plus 7 days at 70 ^oF. After 7 months, scald was present at removal from cold storage on air-stored fruit, and on fruit stored in 1.5% O_2 after 7 days at 70 ^oF following 10 days in air at 33 ^oF (Table 9). Fruit stored in 0.5% O_2 then 10 days at 33 ^oF in air did not scald in 28 days at 70 ^oF, but did scald when held 21 days at 33 ^oF in air after CA plus 28 days at 70 ^oF. Fruit were not marketable at that point due to low quality (soft, yellow, greasy). After 9 months, fruit stored in either O_2 regime developed scald when moved to air storage at 33 ^oF followed by 7 days at 70 ^oF, and all fruit was marketable at that point. This example demonstrates the low O_2 benefit (at 0.5 compared to 1.5% O_2) for scald control is detectable through 7 but not 9 months CA.

Month	Treatment	Days in RA at 33 ^oF after CA	Days at 70 ^oF after RA until superficial scald symptoms	Scald Incidence %
7	Air	10	0	67
	O_2: 1.5%	10	7	100
	0.5	21	28	22
9	Air	10	0	72
	O_2: 1.5%	10	7	100
	0.5	10	7	25

Table 9. 'Granny Smith' scald incidence following storage. Fruit moved from CA to 33 ^oF in air, then to 70 ^oF after 7 or 9 months.

A similar study conducted with 'Delicious' apples was unsuccessful as no fruit developed scald.

Objective 4. Identify what if any limits for CO_2 exist during apple storage in oxygen below 1%.

Experiments were conducted with 'Granny Smith' and 'Fuji' apples. Although normally at low risk of CO_2 injury under CA, 'Granny Smith' was used due to the utility of CA in O_2 less than 1% for scald control. 'Fuji' was used due to its high CO_2 injury susceptibility.
'Granny Smith' apples were stored in air or CA with 0.3 (determined with HarvestWatch^{TM}) or 1.5% O_2 combined with 1,3, or 5% CO_2. No change in chlorophyll fluorescence was detected related to CA CO_2 concentration. No symptoms of CO_2 injury were observed on fruit evaluated after 3,6, or 9 months plus 7 days at 70 ^oF (Table 10). Fruit stored at 0.5% CO_2 were greener compared to fruit stored in 1.5 or 3% CO_2. Fruit stored in CA developed less core- and senescent browning compared to air-stored fruit (except for fruit stored in 1.5% O_2, 0.5% CO_2).

% O_2	% CO_2	Peel color	% CB	% SB	% scald	lbs
air	air	1.9a	13a	92a	100a	11.4b
0.3	0.5	1.0c	4b	0b	0b	18.0a
	1.5	1.9a	0b	0b	0b	17.3a
	3.0	1.5b	0b	0b	0b	17.4a
1.5	0.5	1.0c	13a	0b	83a	17.8a
	1.5	1.8a	0b	0b	79a	16.6a
	3.0	1.7a	0b	0b	0b	17.4a

Table 10. 'Granny Smith' color, disorders, and firmness after 9 months storage at 33 ^{o}F. Low O_2 setpoint determined by monitoring fruit chlorophyll fluorescence. Fruit held 7 days at 70 ^{o}F after removal from storage. Means (n=18) followed by different letters are significantly different, Tukey's HSD, $p<0.05$.

'Fuji' fruit harvested with slight-moderate watercore, starch score 4.2 (18 fruit average) were stored at 33 ^{o}F in 1% O_2 with 0.5, 1.5, or 5% CO_2. In 1% O_2, no change in fruit chlorophyll fluorescence was detected at CO_2 concentrations up to 10%. Symptoms of CO_2 injury (core browning, cavitation) were observed after 1 month storage for fruit stored at 5% CO_2 (Table 11). As with 'Honeycrisp' (Table 7), risk and development of CO_2 injury was not indicated by chlorophyll fluorescence assessment.

% CO_2	% core browning	% cavitation
0.5	0	0
1.5	0	0
5.0	27	11

Table 11. 'Fuji' apple internal disorders after 1 month storage. Fruit were held 7 days at 33 ^{o}F in air, then in 1% O_2 with 0.5, 1.5, or 5% CO_2. Fruit held 7 days at 70 ^{o}F after removal from storage.

EXECUTIVE SUMMARY

Project results indicate 'Honeycrisp' postharvest quality, usually not limited by firmness/texture, is best managed by optimizing harvest maturity, avoiding chilling injury, and recognizing low O_2 or high CO_2 can induce damage in CA. Late maturity and harvest increases postharvest disorder risk, therefore horticultural practices that enable maturation and red color development to occur relatively early will contribute to successful postharvest management. How best to achieve early maturation remains to be defined, however, experience with other cultivars indicates nitrogen and crop load management are important factors regulating fruit development. 'Honeycrisp' also appears sensitive to pre-harvest stress, particularly temperature, that may contribute to diffuse browning we have observed in some trials and in samples brought to the lab by fieldmen. This tendency has been noted in other North American production areas as well. Situations where harvest is delayed due to lack of red color development can increase risk of postharvest disorders.

'Honeycrisp' can also be sensitive to post-conditioning storage temperature, and CA environments with very low O_2 or high CO_2. Low temperature and low O_2 sensitivity do not appear to be quality management issues due to 'Honeycrisp's slow softening characteristic, and utility of 36 ^oF and 2 % O_2 for reduced loss of acidity, greasiness delay, and slower ground color change. Ripening-related volatile production is also slowed at moderate O_2 concentrations, a factor we feel is important for long-term storage quality due to delay in production of ethyl volatiles that can contribute to over-ripe flavor and taste. 'Honeycrisp' high CO_2 sensitivity to be less than that of 'Braeburn' and 'Fuji', and while controllable by DPA, reasonable CO_2 storage management is likely to be sufficient to control CO_2 induced disorders.

Use of 1-MCP as SmartFresh® can slow 'Honeycrisp' ripening similar to CA alone, and some SmartFresh® CA combination benefits are evident. SmartFresh® does not appear to enhance chilling disorder susceptibility, however, as with other CO_2 sensitive cultivars, CO_2 management of SmartFresh® treated 'Honeycrisp' fruit should receive close attention.

'Honeycrisp' temperature management, particularly during the conditioning period after harvest, is an area where further research may be productive. Are fewer days at temperatures higher than 50 ^oF needed to acclimate fruit to avoid chilling injury? Higher temperatures typically cause more fruit acid to be consumed and less acid is a factor limiting edibility of fruit from long-term storage.

Characterization of metabolic events preceding and following chilling injury development are providing insight into the disorder development process. Further work is needed to determine if monitoring of specific compounds has utility as predictive or diagnostic tools. Information to date raises many questions about the disorder process, particularly the role of acylated sterol glycosides in stress response and injury. Information from this project was used in the successful SCRI grant proposal, and further work in this area is continuing with SCRI funds.

Monitoring fruit chlorophyll fluorescence as a function of storage O_2 concentration is increasingly used by industry for CA storage management. Our results indicated the longevity of 'Granny Smith' scald control from low O_2 storage is enhanced as room O_2 content decreases through 7 but not 9 months. While our results do not indicate fruit stress from high CO_2 is detectable using the HarvestWatch™ system, further work is needed to determine safe CO_2 concentrations in rooms where O_2 is managed using chlorophyll fluorescence based information for O_2 content. While its has long been known that apple fruit CO_2 sensitivity increases as O_2 content decreases, specific safe O_2/CO_2 combinations for individual cultivars remain to be determined.

FINAL PROJECT REPORT

Project Title: Finding scald control tools using apple peel chemistry

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Other funding sources

Agency Name: NIFA, USDA (Grant no. 2010-51181-21446**)**
Amt. awarded: $1,483,438 (federal total over 4 years)
Notes: Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle. David Rudell (Project Director) will manage and participate in the project and James Mattheis (Co-PI) and Yanmin Zhu (Co-PI) will also participate. This is a multi-state, multi-national project. The final year's funding of this WTFRC project provided cash support for ARS-Wenatchee's activities in the first year of this SCRI project.

Agency Name: AgroFresh, Inc.
Amt. awarded: $232,235 (for Rudell and Mattheis role in SCRI project over 4 years)
Notes: Cash donation to support activities and objectives outlined in the Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle (see above).

Total Project Funding: **Year 1:** $53,305 **Year 2:** $55,784

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	$37,927	$39,065	
Benefits	$11,378	$11,719	
Wages			
Benefits			
Equipment			
Supplies	$4000	$5000	
Travel			
Miscellaneous			
Total	$53,305	$55,784	

Objectives:

1. Find peel apple peel chemicals that link scald to cultivar, harvest maturity, and other factors.
2. Identify additional apple peel chemicals that are important to fruit quality, ripening, and scald.
3. Identify apple peel chemicals or genes useful as early scald prediction or breeding selection tools.

Significant Findings:

Objectives 1 and 3

1. A total of 202 peel chemicals were selected as candidate biomarkers for assessing scald risk based on differences among storage duration, DPA treatment, and 1-MCP treatment.
2. Other peel chemicals associated with scald symptoms may provide a diagnostic "scald fingerprint", distinguishing scald from other similar looking disorders.
3. 53 candidates that reflected final scald incidence and severity as affected by harvest maturity were selected as *scald risk assessment biomarkers* (SRABs).
4. 27 SRABs were selected and may be useful for monitoring (monthly) and managing scald risk during CA storage after further lot by lot validation.
5. Delayed warming treatments of 1 week (68 ^{o}F) following 1-4 weeks of storage significantly reduced scald development. Peel content of related SRABs, acylated steryl glycosides (ASGs), reflected changes in scald development provoked by intermittent warming treatment.
6. ASG levels rose prior to a variety of peel disorders including scald indicating these SRABs may be more general biomarkers of risk for other peel disorders.
7. Candidate biomarkers that could potentially assess at-harvest risk were identified.

Objective 2

8. Between additional 'Honeycrisp' and 'Granny Smith' peel chemistry experiments, an additional 100+ apple peel chemicals were characterized.
9. Important SRABs including ASGs and CTs (conjugated trienols) were either identified or tentatively identified.

Results and Discussion

Categorizing candidate biomarkers. Candidate biomarkers were selected and categorized from the first experiment in collaboration with Dr. Hertog into groups that may predict or diagnose scald development. An in-depth evaluation of data from the first year of the study provided a more focused picture of the role(s) different chemicals may play in a biomarker-based superficial scald storage management system. Peel chemicals with different levels between control and DPA-treated fruit during the pre-symptomatic, predictive period were segregated from compounds reflecting scald symptom presence or absence. Candidate biomarker quality was ranked and overlap between candidates that predict and those associated with superficial scald symptoms were revealed. Candidate biomarkers associated with DPA- treated or control fruit during each period (Table 1) were evaluated in subsequent validation experiments. Results indicate candidate biomarkers can predict and may have uses beyond predicting scald for storage management. Other uses include accurate diagnosis and/or distinguishing superficial scald from other peel defects with similar visual symptoms to troubleshooting storage, packing-line, and other supply chain issues.

Relationships among peel chemistry, superficial scald incidence, and post-storage ripening after different storage durations and final selection of candidate biomarkers. Another goal was to evaluate how post-storage ripening may influence peel chemistry and possibly illuminate new candidate biomarkers or potential testing protocols. 'Granny Smith' apples were picked 1 month prior to commercial harvest and treated with 0 or 2000 ppm DPA, or 1 ppm 1-MCP then stored for 0, 1, 2, 4, or 6 months in 33 ^{o}F air. Scald incidence and peel chemistry was evaluated 0, 7, and 14 days after removal from storage. The storage stress leading to scald, as in previous years, principally transpired within the first month which was 3 months before symptoms were apparent. Peel chemical levels following 0, 7, and 14 days of ripening demonstrated that peel chemistry was different in control and DPA-treated fruit as early as 1 month after harvest confirming our results from the previous year. Results also show that levels of many candidate biomarkers change over the 2 week post-storage ripening period, either accentuating or reducing differences between control and DPA-treated fruit. Levels of a specific group of aroma volatiles associated with scald during the previous year's experiments only after scald symptoms appeared, the methanol based esters, were elevated in control peel after only 1 month storage plus 7 or 14 days. This indicates that post-storage ripening after shorter storage periods could provide earlier information related to scald status and storage stress.

Re-examination of predictive candidate biomarkers found in the previous year indicated over 60% of the candidates distinguished fruit with and without scald during symptom development. This experiment also identified an additional 137 candidate biomarkers. Candidate selection was based on the most important factors that reduce scald: storage duration, DPA treatment, and 1-MCP treatment (Fig. 1). Candidate biomarkers were screened in subsequent validation experiments to find only the most useful biomarkers. Further harvest maturity, CA, and intermittent warming experiments sought to validate this list of candidate markers.

Selection of scald risk assessment biomarkers (SRABs) based on harvest maturity. 'Granny Smith' apples were harvested 4 (H1) or 2 weeks before (H2) commercial maturity, at commercial maturity (H3), or 2 (H4) or 4 weeks (H5) following commercial maturity. Control and DPA (2000 ppm) treated fruit were then stored in air for up to 26 weeks (6 months). Levels of candidate scald biomarkers were screened at 0, 2, 4, 8, 16, and 26 weeks. Scald was evaluated monthly from 8-26 weeks and 26 weeks + 7 days at 68 ^{o}F. Scald symptoms developed first (at 16 weeks) in H1-4 and were more severe at 26 weeks + 7 days than H5 (Fig. 2).

The experiments identified SRABs useful regardless of harvest maturity and reflect the severity of scald that developed by the end of the test. A total of 53 SRABs met these criteria (Fig. 1). In most cases SRAB levels reflected not only the magnitude of scald developed at the end of the study but also the timing (first appearing at 16 weeks as opposed to 26 weeks) (Fig. 3). These SRABs were further tested to determine which can be used as tools to assess scald risk.

Partial validation of SRABs under CA conditions. 'Granny Smith' apples were harvested 1 month prior to commercial maturity, treated with DPA (0 or 2000 ppm) and placed immediately into 33 ^{o}F air, or CA (0.5%, 1.0%, or 5% O_2; all 1% CO_2). Fruit were stored for up to 9 months and SRABs (validated by the harvest maturity experiment) were screened at 0, 1, 2, 6, and 9 months. Scald incidence and severity and incidence of other peel injuries were rated after 0 and 7 days at 68 ^{o}F.

Fruit stored in 0.5% O_2 developed a dark anomalous peel injury after 9 months storage. However, superficial scald developed only on air-stored apples not treated with DPA starting at 6 months, and at 9 months on air-stored, DPA treated fruit. As scald did not occur on the untreated, air-stored fruit until 6 months in this study, it was not surprising that none was observed on any of the CA-stored fruit by 9 months. However, it is possible that scald would have developed later than 9 months, especially in 5% O_2 which would be expected to have the least scald control potential. Using this assumption, we set up selection criteria to find which SRABs can be employed as risk assessment tools.

Line plots of levels of all 53 SRABs during storage in every CA regime were visually evaluated and categorized. SRABs were further ranked within each category by a factor representing the % change in level during storage and the % difference between peel from DPA treated and untreated fruit. Highly ranked biomarkers changed the most during storage and had the greatest difference between control and DPA treated peel. Of the 53 SRABs, 15 were selected as scald risk assessment tools for CA (Fig. 1) with another 6 that require further validation. Still another 6 SRABs may actually indicate risk for multiple types of peel injury as they were linked with scald and the anomalous browning injury occurring on fruit stored 9 months in 0.5% O_2 (Fig. 4).

Impacts of intermittent warming on scald incidence and peel chemistry. 'Granny Smith' apples harvested 1 month prior to commercial maturity were placed in 33 ^{o}F air storage immediately or following 1 week at 68 ^{o}F. Of the fruit placed in storage immediately, additional subsets were removed after 1, 2, 4, and 8 weeks for 1 week at 68 ^{o}F after which they were placed back into cold storage. Apple peel chemistry was evaluated at harvest, before and after warming treatment, and after 26 weeks (6 months) storage. Scald severity was evaluated following 13, 17, 21, and 26 weeks. No scald developed after 26 weeks on fruit held 1 week at 68 ^{o}F beginning 1 or 2 weeks after harvest, and warming beginning at 4 and 8 weeks significantly reduce scald development. Levels of one SRAB group, the acylated steryl glycosides (ASG) were permanently lowered by intermittent warming and reflected scald development. Evaluation of other SRABs is ongoing for this experiment.

Expanded apple peel chemical library and identification of key scald-associated chemicals. The peel chemical analysis was expanded to include over 100 additional chemicals in 'Granny Smith' and 'Honeycrisp' experiments. In collaboration with Dr. Bruce Whitaker, ARS-Beltsville, we identified a key "family" of peel chemicals called ASGs and their chemical building blocks, the steryl glycosides (SGs) in 'Granny Smith' peel. ASGs modify important plant cell components in response to low temperatures. While there is no change in concentrations of the ASG "building blocks" (SG and phytosterols), ASG levels are elevated in control peel both prior to and during scald symptom development. ASG levels decrease in peel from both control and DPA-treated fruit after removal from storage. The same relationship between scald-risk and elevated ASG levels was confirmed and validated in subsequent experiments including differences associated with harvest maturity and CA conditions. Results indicate increasing ASG levels also precede soft-scald in 'Honeycrisp' and anomalous peel browning in 'Granny Smith'. Elevated ASG levels may indicate chilling stress leading to many of these disorders has occurred, and ASG metabolism may also serve as a potential genetic target to modify susceptibility to chilling related peel injuries. Expression of genes that possibly control this pathway was evaluated during storage with and without DPA with inconclusive results. It is possible that control of this little-known pathway is complex and requires a much more comprehensive gene expression analysis to discover the correct candidate biomarkers associated with this pathway.

Other compounds, collectively called conjugated trienols (CT), were identified. These natural peel chemicals have been associated with oxidative stress and scald in this and many other studies over the last 40 years. Results indicate that these may be useful SRABs. In addition to identified CTs, this study revealed other peel chemicals with some CT-like features. These unidentified SRABs were among the highest ranked for identifying scald risk.

Finding candidate biomarkers for at-harvest scald risk assessment. Five harvests of 'Granny Smith' [see above "Selection of scald risk assessment biomarkers (SRAB) based on harvest maturity"] were stored in air for 26 weeks (6 months) and scald evaluated at 26 weeks + 7 days at 68 ^{o}F. Apple peel chemistry (+700 natural peel chemicals) was evaluated at harvest. Statistical comparison of the apple peel chemistry at harvest with scald incidence was used to select candidate biomarkers for at-harvest scald risk assessment. Most highly-ranked candidates were associated with later harvests suggesting higher levels may indicate less likelihood of scald development (Fig. 5). A few compounds were

associated with fruit that did develop severe scald. It is important to note that this very preliminary evidence may also indicate these candidate biomarkers are associated with only harvest maturity which is, in turn, associated with scald incidence. Considerably more experimentation is necessary to establish which, if any, of these candidates would be useful at-harvest tools.

Adapting peel chemical measurement platforms for biomarker based scald-management tools. Some preliminary work has been conducted towards development of non-laboratory based platforms to estimate changes in apple peel chemistry. Dr. Renfu Lu (ARS, East Lansing) and Dr. Mike McCarthy (UC-Davis) have collaborated by performing preliminary evaluations of two different on-line devices for superficial scald prediction. Dr. Lu used near infra-red spectroscopy and fluorescent scattered light imaging to attempt to differentiate apples at 0, 1, 2, and 4 months. It was not possible to segregate untreated or DPA treated apples using either technique under these experimental conditions.

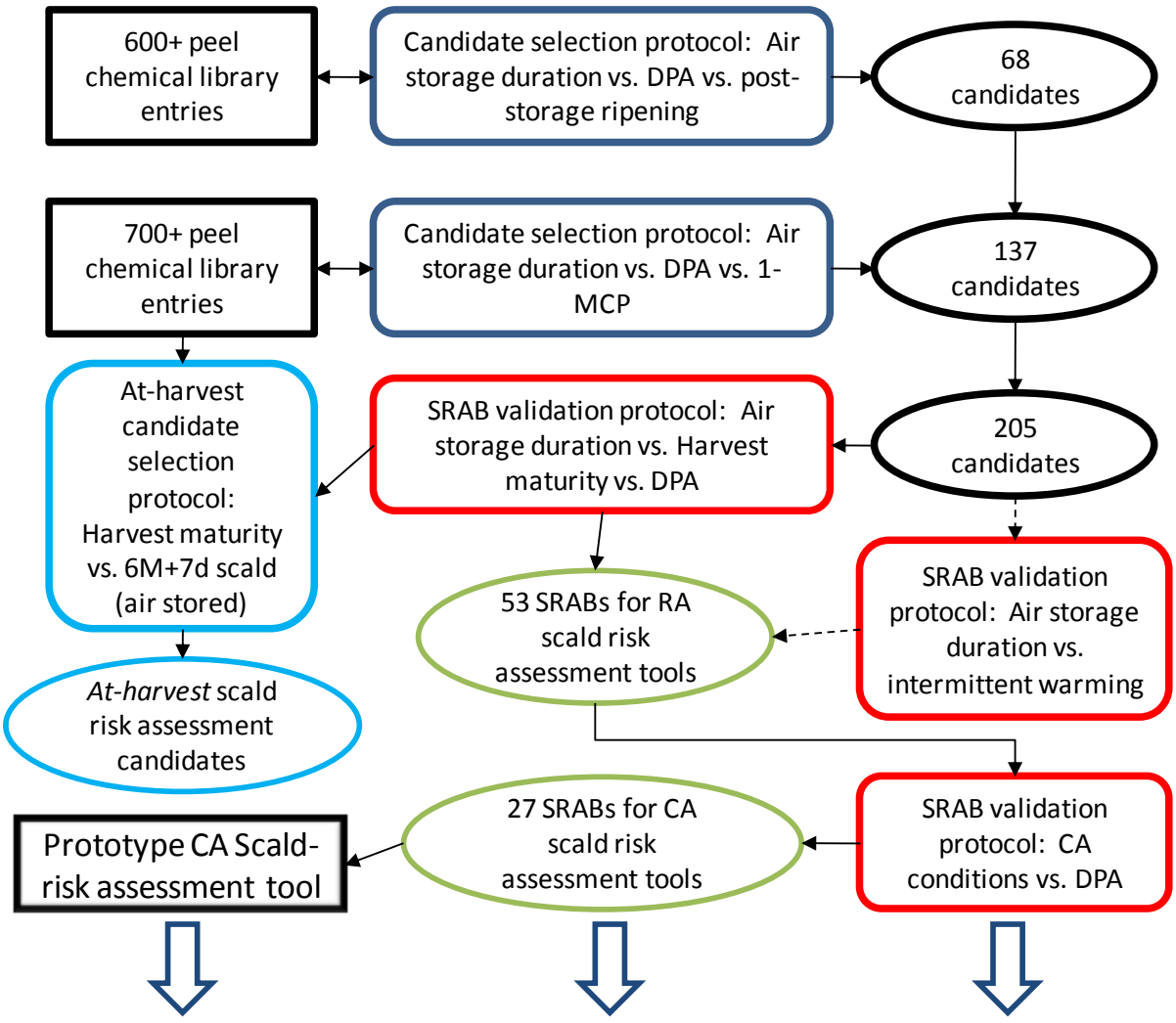
In an ongoing study, Dr. McCarthy is evaluating the possibility of using a small magnetic resonance imaging platform to differentiate untreated and DPA treated apples at 0, 1, 2, and 4 months. Both collaborators generously performed this work using our apples without direct funding.

Assessment of natural apple peel chemicals as scald-reducing crop protectants
Evaluation of natural apple peel chemicals selected in this study as pre-harvest treatments is ongoing. Preliminary results indicate a possible relationship between scald protection and one peel compound. An additional evaluation of this natural peel chemical and related metabolites is in progress.

Conclusions and future directions
Results indicate that measuring various scald-risk assessment biomarkers (SRABs) may be a useful apple storage management tool by indicating when and which fruit are at higher risk to develop scald. By selecting SRABs using storage conditions typically required for scald to develop (storage stress and ripeness) and then validation under storage conditions similar to commercial practice, risk assessment tools are expected to widely applicable. Furthermore, ASGs may have an added value of indicating risk of other peel browning disorders in addition to superficial scald. Candidate biomarkers potentially indicating scald risk *at-harvest* were also selected and require validation.

SRABs selected for scald risk assessment tools need to be validated using multiple lots in commercial settings. Measurement protocols should also be refined under these settings. Platforms used to measure SRABs must be adapted or developed for both service laboratory and field use. A similar protocol should be employed to reveal useful at-harvest scald risk assessment biomarkers. Improved protocols to find gene expression SRABs should be employed.

Figure 1. Overview of project activity coordination and accomplishments.



	High 50	VIP>0.8
Pre-scald (7d-2m model)		
total candidates	33	68
Greater in control	23	32
Greater in DPA	10	36
Scalding (2m-6m model)		
total candidates	38	232
Greater in control	14	82
Greater in DPA	24	150

Table 1. Ranked candidate biomarkers from initial selection categorized by superficial scald development period and treatment association. This demonstrates peel chemicals associated with unstressed, "healthy" peel can also be useful biomarkers indicative of storage stress levels.

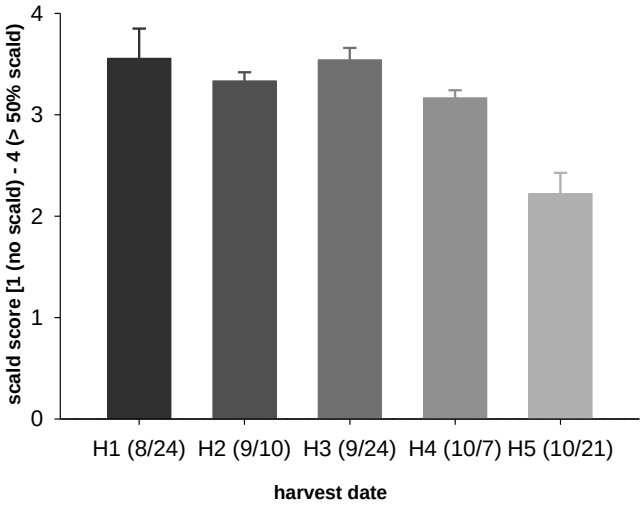


Figure 2. Scald severity after 26 weeks (6 months) 31 °F air storage (+7 days at 68 °F) of 'Granny Smith' apples harvested 4 or 2 weeks prior to commercial harvest, commercial harvest, and 2 or 4 weeks following commercial harvest. Scald developed less on H5 apples. This data was used to compare effects among harvest maturity, scald severity and candidate biomarker. This comparison provided validation to select Scald Risk Assessment Biomarkers (SRAB).

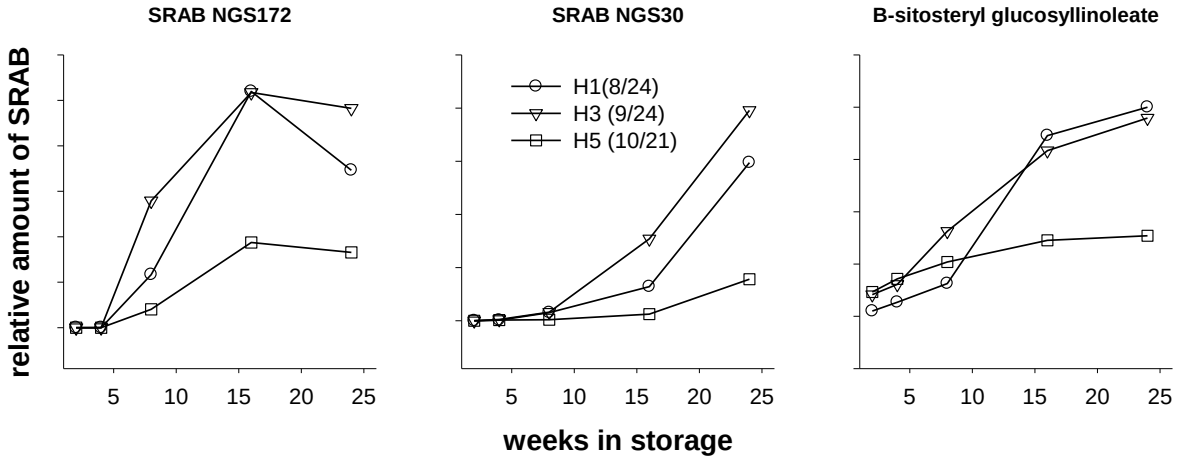


Figure 3. Levels of select Scald Risk Assessment Biomarkers (SRAB) in 'Granny Smith' peel of apples harvested 4 weeks prior to commercial harvest, commercial harvest, and 4 weeks following commercial harvest. Results demonstrate that SRAB levels reflect the earlier onset and greater severity of scald after 26 weeks (6 months) 31 °F air storage (+7 days at 68 °F) in the earlier harvests.

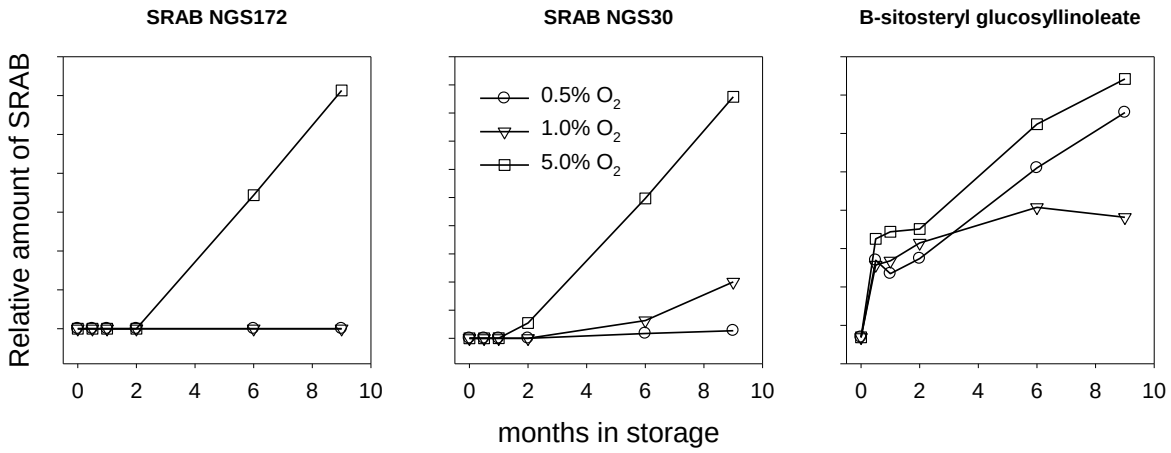


Figure 4. Levels of selected Scald Risk Assessment Biomarkers (SRAB) in 'Granny Smith' peel of apples harvested 1 month prior to commercial harvest and stored for up to 9 months in 0.5, 1.0, or 5.0% O_2 at 31 ^{o}F. Although no scald developed on any of the CA stored fruit within 9 months (it took 6 months to appear on air stored controls), SRAB levels were elevated in the 5.0% treatment, which would be most likely to develop scald. ASG levels (far right, for example) also increased prior to the development of an anomalous peel browning disorder that began to appear in 0.5% O_2. With further validation, it is expected that monthly monitoring of these and other CA-specific SRABs may be a useful storage management tool for scald.

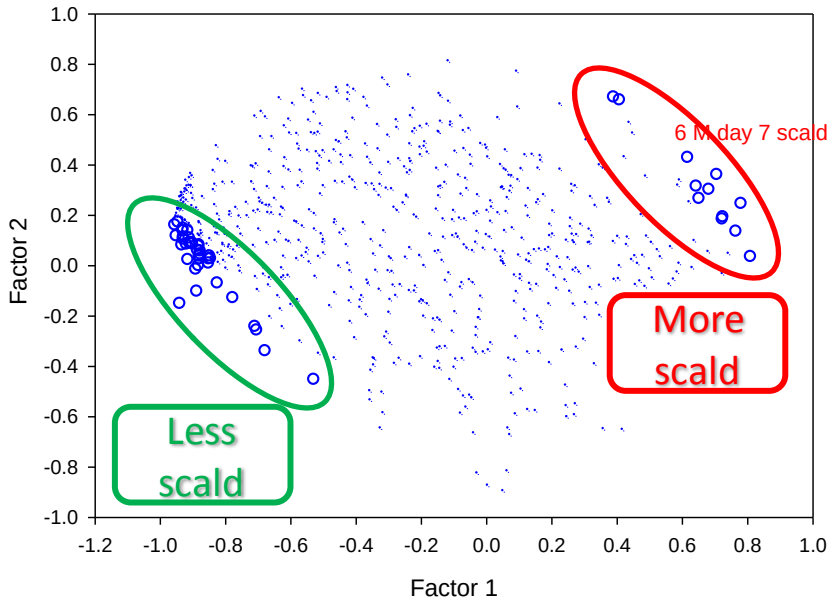


Figure 5. Selection of candidate *at-harvest* scald risk assessment biomarkers in 'Granny Smith' peel of apples harvested 4 or 2 weeks prior to commercial harvest, commercial harvest, and 2 or 4 weeks following commercial harvest. Scald was evaluated after 26 weeks (6 months) 31 ^{o}F air storage (+7 days at 68 ^{o}F). Total at-harvest apple peel chemistry was compared to final scald severity to highlight candidates associated with harvests that developed more or less scald. As this is only an evaluation of one lot, it is suggested that this experiment be repeated with multiple lots and multiple seasons to establish a final list of candidates.

Executive Summary

Project outcomes:

1. *Scald risk assessment biomarkers* (SRABs) for storage management tools.
2. Prototype SRAB-based tool for monitoring scald risk during CA storage.
3. List of candidate biomarkers to assess at-harvest risk.
4. Continued federal funding to search for additional gene expression and chemical biomarkers for superficial scald and other significant postharvest disorders.
5. An apple fruit mass spectral phytochemical library containing descriptions of 700+ individual phytochemicals.
6. Identification of an important class of SRABs, the ASGs, which may be broadly applicable for monitoring general storage stress that leads to multiple disorders.
7. Improved understanding of the peel chemistry behind superficial scald, storability, and quality to direct and expedite future research.

Significant Findings:

1. A total of 202 peel chemicals were selected as candidate biomarkers for assessing scald risk based on differences among storage duration, DPA treatment, and 1-MCP treatment.
2. Other peel chemicals associated with scald symptoms may provide a diagnostic "scald fingerprint", distinguishing scald from other similar looking disorders.
3. 53 candidates that reflected final scald incidence and severity as affected by harvest maturity were selected as *scald risk assessment biomarkers* (SRABs).
4. 27 SRABs were selected and may be useful for monitoring (monthly) and managing scald risk during CA storage after further lot by lot validation.
5. Delayed warming treatments of 1 week (68 ^{o}F) following 1-4 weeks of storage significantly reduced scald development. Peel content of related SRABs, acylated steryl glycosides (ASGs), reflected changes in scald development provoked by intermittent warming treatment.
6. ASG levels rose prior to a variety of peel disorders including scald indicating these SRABs may be more general biomarkers of risk for other peel disorders.
7. Candidate biomarkers that could potentially assess at-harvest risk were identified.
8. Between additional 'Honeycrisp' and 'Granny Smith' peel chemistry experiments, an additional 100+ apple peel chemicals were characterized.
9. Important SRABs including ASGs and CTs (conjugated trienols) were either identified or tentatively identified.

Future directions:

1. Validate CA prototype tools under commercial conditions in multiple lots.
2. Explore and develop SRAB measurement platforms that can be easily adopted in multiple areas of the apple production and service industry.
3. Continue to find gene expression biomarkers for potentially more sensitive and accurate disorder risk assessment and management.
4. Continue to identify natural apple fruit chemicals that are important to fruit maturation, ripening, superficial scald, and other postharvest disorders.
5. Screen and validate candidate biomarkers that assess *at-harvest* scald risk in multiple commercial lots.
6. Use apple peel chemistry to increase the understanding of scald and related disorders.

FINAL PROJECT REPORT

Project Title: Apple *ACS3* genotypes and fruit ripening

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Budget:

Organization: USDA, ARS		**Contract Administrator:** Charles Myers, Extramural Agreements Specialist
Telephone:(510) 559-6019		**Email:**cwmyers@pw.ars.usda.gov
Item	**Year 1: 2009-2010**	**Year 2: 2010-2011**
Salaries*	$25,000	26,000
Benefits	9,000	9,000
Wages		
Benefits		
Equipment		
Supplies	11,000	11,000
Travel	1,500	1,500
Miscellaneous	1,500	1,500
Total	48,000	49,000

*0.5 FTE GS9 Postdoctoral Research Associate
Supplies include common molecular biology reagents and fruit from commercial orchards.

Other funding sources

Agency Name: AgroFresh
Amt. awarded: $35,000 for the second year study
Notes: Funds cover 0.5 FTE GS9 Postdoctoral Research Associate

OBJECTIVES

1. Characterize apple fruit ripening characteristics including ethylene evolution for 6-10 cultivars at defined developmental stages.
2. Investigate cultivar-specific fruit softening rate and ethylene regeneration in fruits treated with 1-MCP.
3. Examine expression patterns of *MdACS3* and other ethylene biosynthesis and perception related genes during fruit ripening and in response to 1-MCP treatments (including Harvista on-tree spraying).
4. Explore the potential polymorphism at the *MdACS3* locus for potential functional molecular marker generation, based on gene expression results.

SIGNIFICANT FINDINGS

1. During the 8-week period of apple fruit maturation (from -6 to +2 weeks with physiological maturity as 0 stage), considerable variation in the expression levels of *MdACS3* were observed among 14 elite apple cultivars and breeding parents.

2. Two expression patterns of *MdACS3* were identified in apple fruit peel tissues: pattern A showed higher expression level with progressively-increased patterns, and pattern B exhibited lower expression level with a transient peak.

3. Higher expression of *MdACS3* usually correlated with early ripening cultivars; and lower expression of *MdACS3* usually correlated with late ripening cultivars.

4. The two *MdACS3* expression patterns were also correlated with "on-tree fruit firmness retention" during last 6 weeks before harvest, i.e. the early-ripening cultivars showed larger decrease and late-ripening cultivars had smaller decease in fruit firmness.

5. Postharvest 1-MCP treatment did not suppress *MdACS3* expression level, instead a slight increase was observed for most cultivars; the suppression on *MdACS1* expression was apparent for most cultivars for at least three months after 1-MCP treatment and stored in cold room.

6. Correlation between cultivar-specific *MdACS3* expression patterns and 1-MCP treatment efficacy was weak. However, early-ripening cultivars showed a greater average "fruit firmness retention" (difference in firmness between 1-MCP treated fruit and non-treated controls) than that of late-ripening cultivars.

7. The effect of pre-harvest application of spraying-formula of 1-MCP, i.e. Harvista® on 'Golden Delicious' showed increased firmness and suppressed ethylene production, compared to non-sprayed control. But the effect is still less effective than postharvest 1-MCP treatment.

8. In addition to stimulation of *MdACS3* and suppression of *MdACS1* gene expression (as mentioned above), the suppressed expression of all major ethylene receptor genes by Harvista® was also observed during cold storage.

METHODS

1. Physiological characterization of apple fruit maturation/ripening:

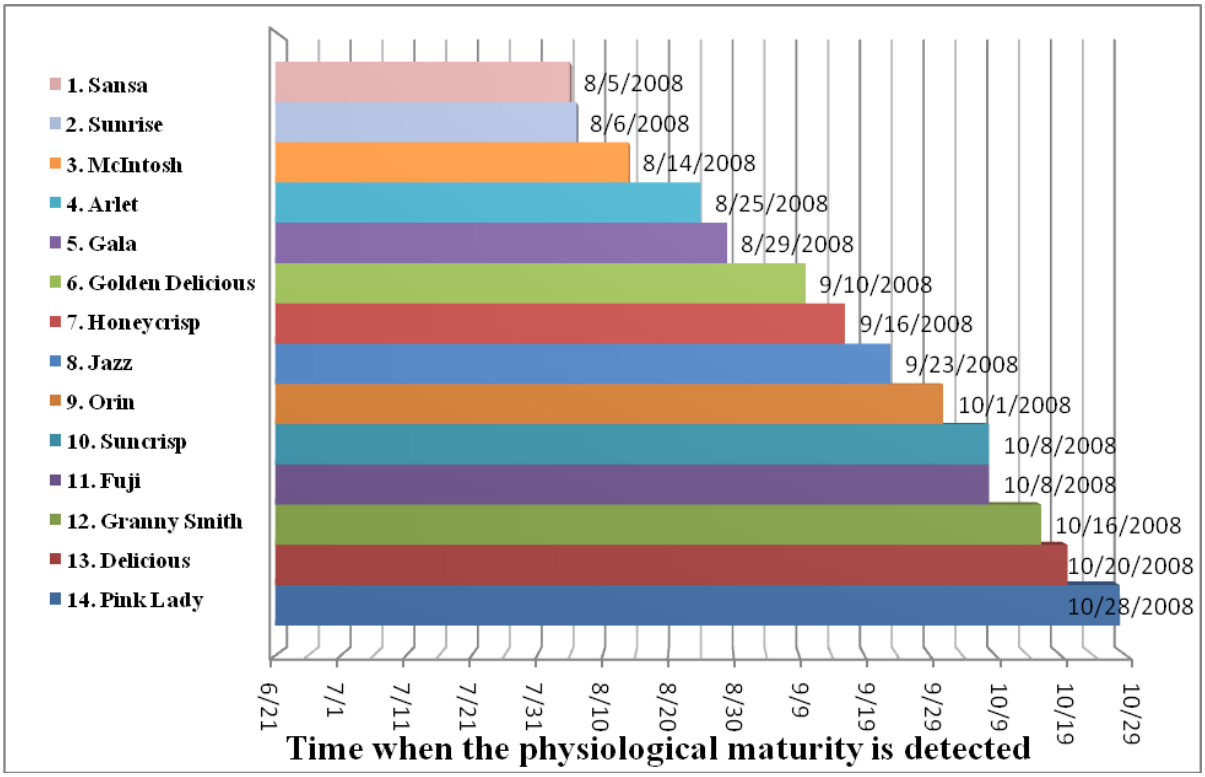
Fruit from 14 apple (*Malus* × *domestica* Borkh.) cultivars, from commercial orchards or experimental orchards in central Washington State, were subjected to systematic characterization of fruit maturation and ripening processes, starting 2 months before projected physiological maturity. Physiological maturity (stage 0) for each cultivar was retrospectively assigned based on the sample with starch staining index close to 3.5 based on 1-6 scale. Fruits harvested at stage 0 will be treated with 1-MCP then stored in air at 33° F.

2. Gene expression analysis for MdACS3 and other genes encoding ethylene receptor and signaling pathway:

Peel tissue were collected and used for total RNA isolation, followed by DNase I digestion, RNA cleanup and cDNA Synthesis. Quantitative Real-Time PCR using SYBR Green I dye were employed for gene expression analysis, including –RT (no reverse transcriptase), no template control (no cDNA) and an actin gene as internal reference genes. Quantitative PCR reaction will be repeated twice with two independent cDNAs. Target gene expression will be normalized to that of the reference actin gene and analyzed by $2^{-\Delta\Delta CT}$ method.

RESULTS AND DISCUSSION

1. A total of 14 cultivars with various ripening date were included in the study. The date at the end of the bar indicates the time physiological maturity is detected for this cultivar. The ripening dates for these cultivars span from early August to late October.



2. Two observed exxpressionpatterns of MdACS3 among 14 cultivar investigated.

Figure 2A. Cultivars with the pattern of high expression level and progressively-increased, including ‘Golden Delicious’, ‘Sunrise’ ‘Honeycrisp’, ‘Jazz’, ‘Gala’, and ‘Arlet’.

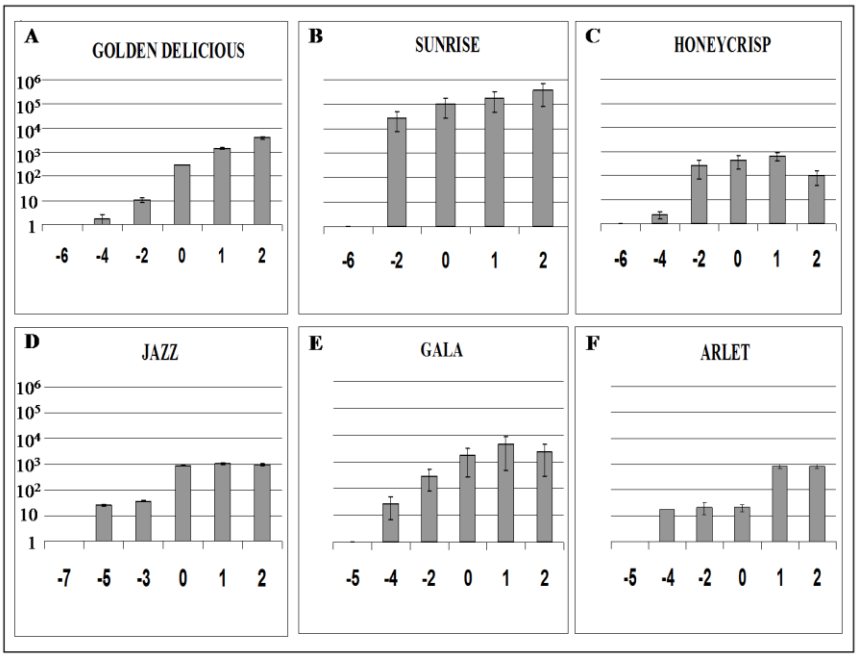
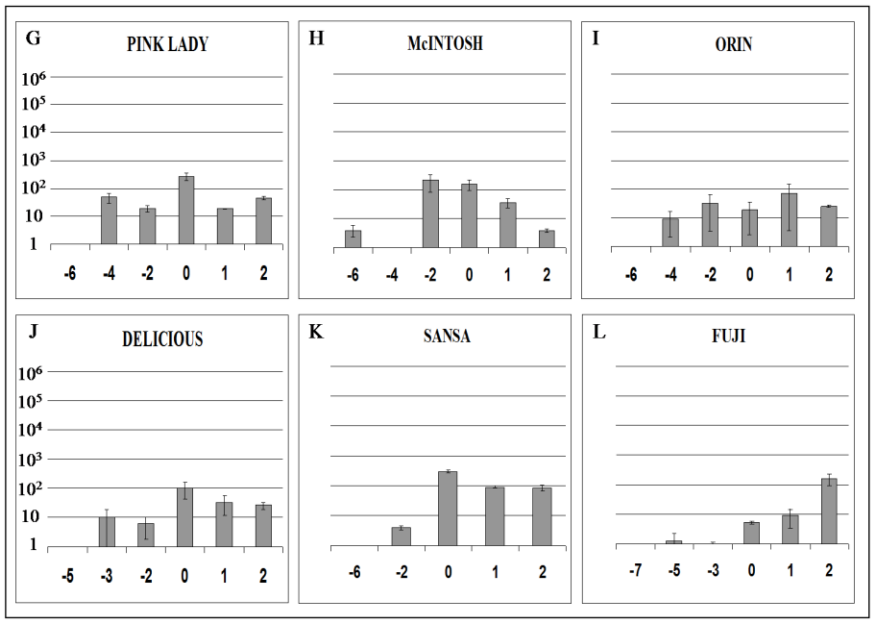


Figure 2B. Cultivars with the pattern of low expression level and transient peak, including ‘Fuji’, ‘Pink Lady’, ‘Orin’, ‘Sansa’, ‘Delicious’ and ‘McIntosh’



3. Figure 3A. The expression level of *MdACS3* was attenuated in fruit 4 weeks after harvest, 1-MCP treatment stimulated *MdACS3* expression.

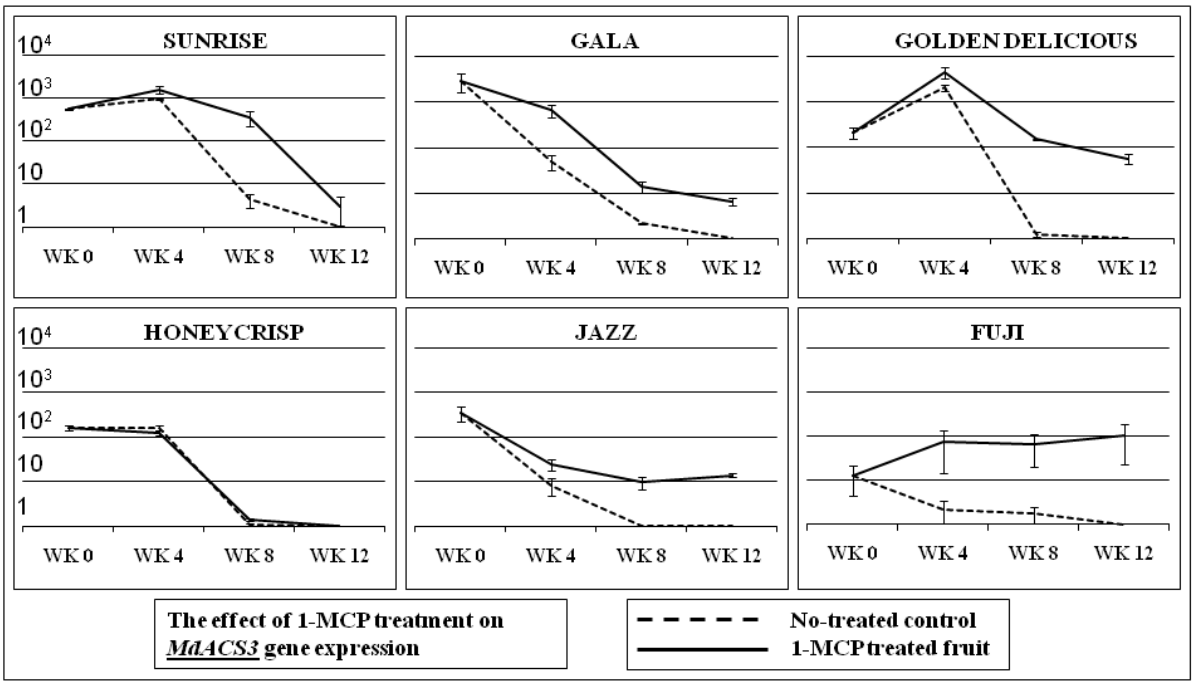
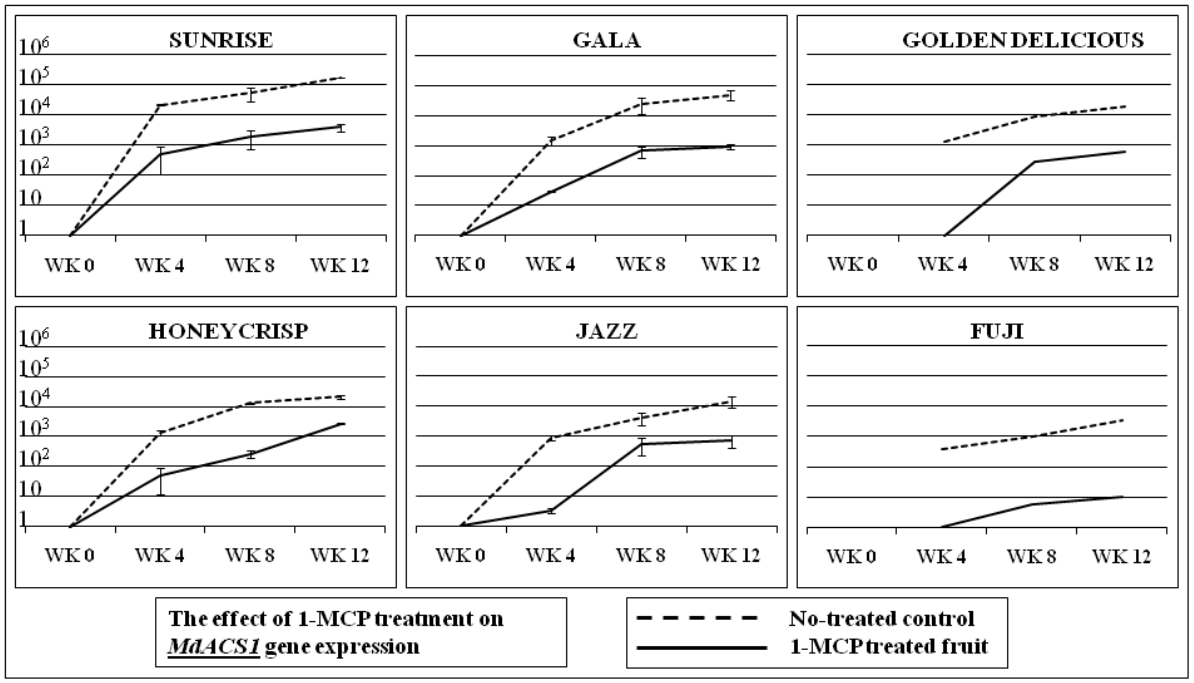


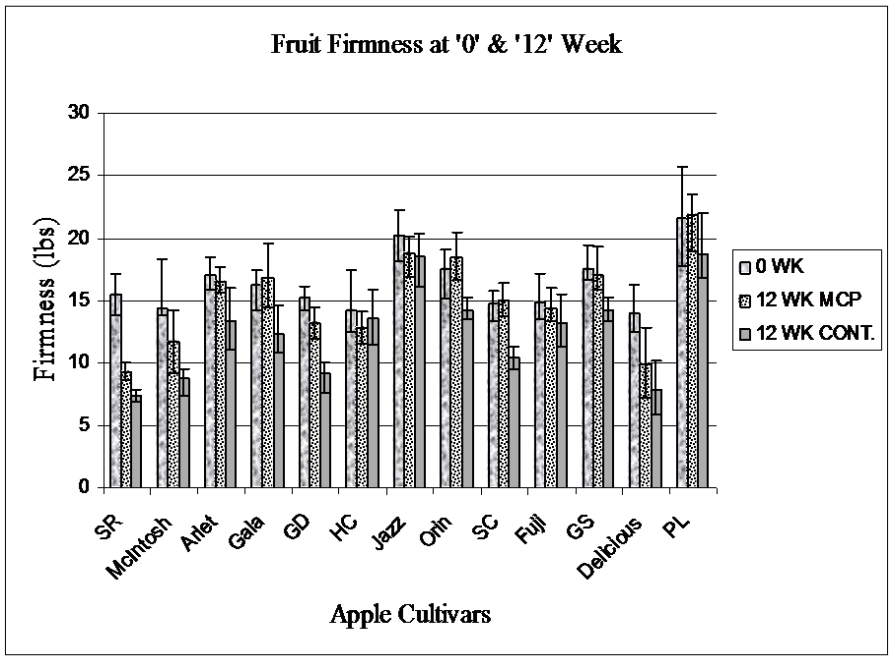
Figure 3B. Treatment with 1-MCP suppressed on *MdACS1* expression for all cultivars.



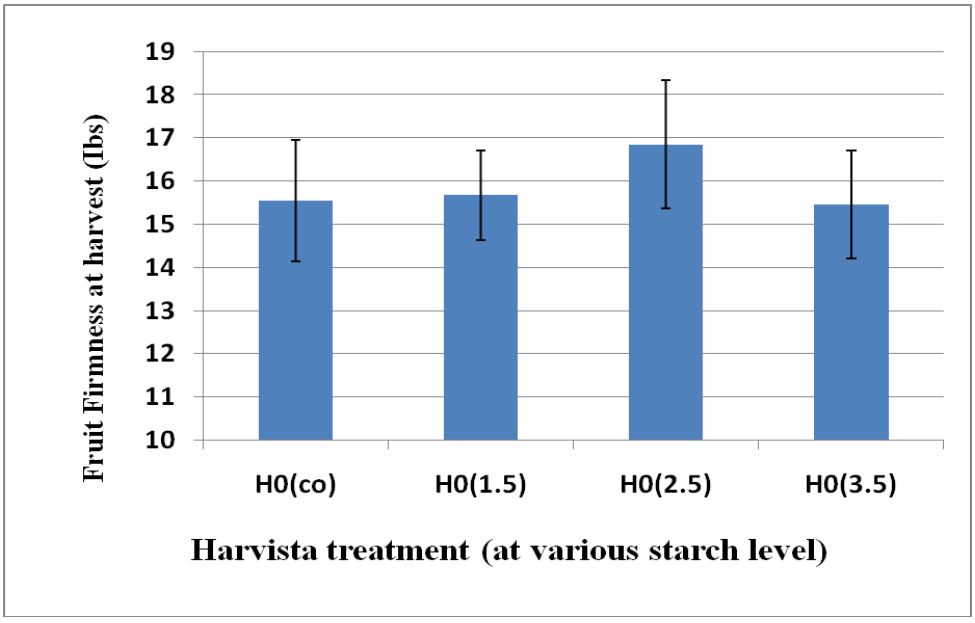
4. The cultivar-specific on-tree firmness loss during the six weeks before physiological maturity usually correlated with fruit ripening season and *MdACS3* expression level. Firmness loss was greater for the early ripening cultivars such as 'Golden Delicious', 'Sunrise' 'Honeycrisp', 'Gala', and smaller decreases were observed for later ripening cultivars such as 'Fuji', 'Pink Lady', , 'Granny Smith', and 'Suncrisp'.

	Apple Cultivar	Firmness at -4 week (lbs)	Firmness at +2 week (lbs)	Firmness loss (lbs)
Early ↑	Sunrise	20.3 ± 0.9	12.1 ± 0.6	8.2
	McIntosh	23.7 ± 4.0	14.7 ± 1.1	9.0
	Arlet	20.6 ± 0.9	15.7 ± 0.9	4.9
	Gala	22.1 ± 1.4	14.6 ± 1.6	7.5
	Golden Delicious	19.6 ± 1.3	14.5 ± 0.9	5.1
	Honeycrisp	17.7 ± 1.5 (-5 week)	13.3 ± 1.5	4.4
	Jazz	26.0 ± 0.9 (-5 week)	17.8 ± 1.1	8.2
	Orin	21.8 ± 1.7	17.0 ± 1.2	4.8
	Suncrisp	18.6 ± 1.3 (-5 week)	16.0 ± 0.8	2.6
	Fuji	18.1 ± 1.0 (-5 week)	15.4 ± 1.2	2.7
	Granny Smith	19.5 ± 1.4 (-5 week)	16.8 ± 1.4	2.7
	Delicious	16.0 ± 0.7 (-5 week)	12.8 ± 1.1	3.2
Late ↓	Pink Lady	23.8 ± 2.1 (-5 week)	20.9 ± 1.4	2.9

5. No clear relationship between 1-MCP treatment efficacy and *MdACS3* expression pattern was observed (Figure 4B), suggesting efficacy is dependent on factors other than *MdACS3* expression alone. However, early-ripening cultivars had higher average firmness retention (3.1 pounds) than the late-ripening group (1.5 pounds).

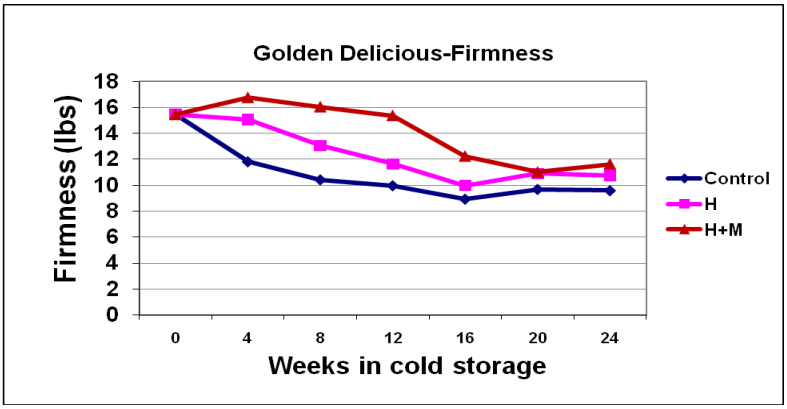


6. The timing of Harvista® treatment indicated that the best timing is at the starch level of 2.5, presumably when the ethylene receptor genes expression initiated.

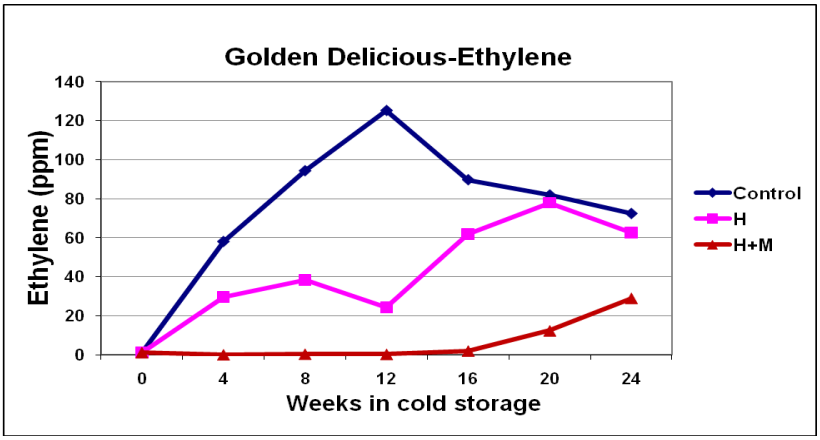


Harvista® was applied at the different time according to starch level (1.5, 2.5 and 3.5; co represents the no-applied control); then the fruit were harvested at the same time when physiological maturity has acquired.

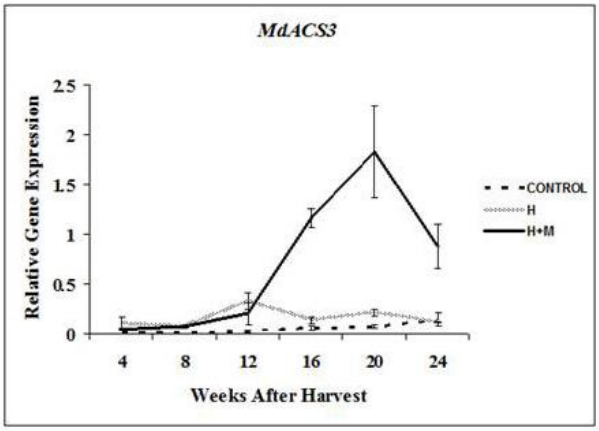
7. The post-harvest firmness change after Harvista® treatment at starch level of 2.5.

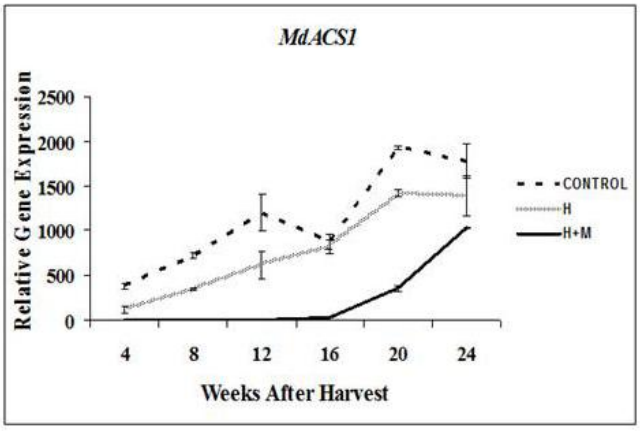


8. Suppression of ethylene evolution by spraying Harvista® during postharvest storage.

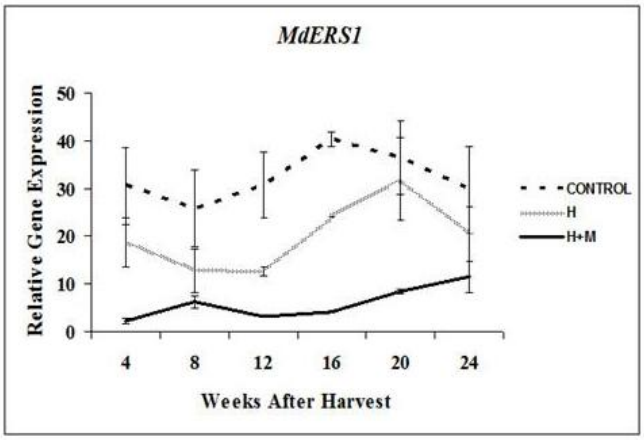


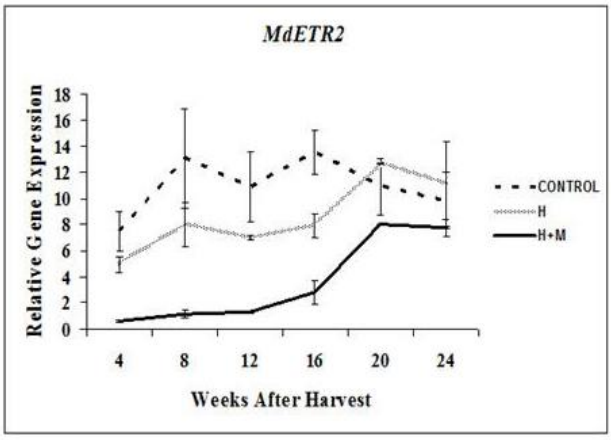
9. Suprresion of ethylene biosynthesis gene expression compared with non-sprayed control.

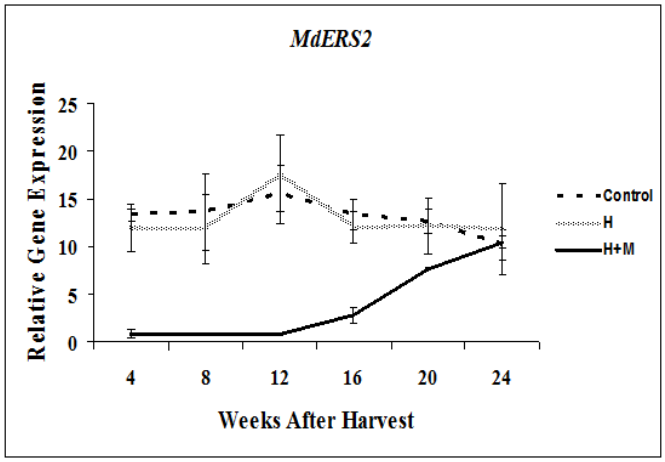




10. All major ethylene receptor genes showed down-regualted expression during postharvest storage upon the application of Harvista®.







EXECUTIVE SUMMARY

Apple fruit ripening is a tightly-regulated genetic process, and ethylene is known to play a pivotal role in regulating apple fruit ripening. While many studies on ethylene's roles focused on the ethylene biosynthesis upon climacteric ripening at or after harvest, little information is available on the roles of a pre-climacteric ethylene biosynthesis gene, i.e. *MdACS3*. In particular, how this low-level expressed, early-activated ethylene biosynthesis gene is related to the timing and strength of the major *MdACS1* gene is unknown. The relationship between *MdACS1* and *MdACS3* is important for understanding apple ripening patterns, developing strategy of fruit quality management and molecular tools for breeding practices.

We utilized 14 cultivars/breeding parents to investigate the expression patterns of *MdACS3* during apple fruit maturation, ripening and postharvest storage. The projected ripening season of these apple cultivars range from August to November in central Washington State. Our results indicated that *MdACS3* has significant influence on apple fruit ripening season (or the timing of *MdACS1* gene activation), fruit firmness and postharvest storability including 1-MCP treatment efficacy. Our results, for the first time clearly demonstrate the relationship between these two genes, i.e. *MdACS3* stimulates and triggers the activation of *MdACS1* genes. Based on the differential responses to 1-MCP treatment, it can be concluded that *MdACS3* expression is system 1 ethylene biosynthesis gene and *MdACS1* the system 2 ethylene biosynthesis gene. Furthermore we also tested a reported *MdACS3* gene-specific marker based on the allelotypes of *MdACS3a* allele. However, our results indicated there is no good association with our observed expression patterns. A further refined marker is needed.

Second part of the project focused on the effect of a new spraying formula of 1-MCP, i.e. Harvista®, its pre-harvest application and its effect on postharvest storability. Our result indicated that application of Harvista® at proper pre-harvest maturity (starch level of an average of 2.5) showed obvious effect on suppressing *MdACS1* gene expression, and fruit firmness retention during storage, although less effective than postharvest 1-MCP treatment. The suppressing effects on all major ethylene receptor genes were also observed.

Overall this is a systematic investigation on ethylene pathway which critically impacts apple fruit ripening, quality and storability. Our results elucidated the relationship between *MdACS3* and *MdACS1* genes, as well as its roles on apple fruit ripening season and on-tree firmness retention. These results set the foundation to develop a DNA marker for predicting the ripening season and fruit storability, for potentially selecting individuals in breeding population with desired ripening time.

FINAL PROJECT REPORT

Project Title: Developing an online toolbox for tree fruit breeding

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Cooperators: Amy Iezzoni (MSU), Gennaro Fazio (USDA-ARS), Gayle Volk (USDA-ARS)

Other funding Sources

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $2,000,000 plus equal amount matching from universities and industry (Sep 2009 – Aug 2013)
Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture". PI: Dorrie Main. Co-PIs include Jung, Evans, Peace and Oraguzie. Synergistic project for practical application of bioinformatics to tree fruit crops.

Agency Name: USDA-CSREES, Specialty Crops Research Initiative
Amount awarded: $7,200,000 plus equal amount matching from universities and industry (Sep 2009 – Aug 2013)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Amy Iezzoni. Co-PIs include Peace, Main, Evans and Oraguzie. A synergistic project to establish sustainable marker-assisted breeding infrastructure for U.S. Rosaceae crops, using the Marker-Assisted Breeding Pipeline concept that involves Pedigree-Based Analysis.

Agency Name: WTFRC Apple Review
Amount requested: $635,201 (2009-2011)

Notes: "Apple Scion Breeding" PI: Kate Evans. Co-PIs: Peace, Ross, Zhu. The foundation program which this project supplements.

Agency Name: WTFRC Apple Review
Amount requested: $121,500 (2009-2010)
Notes: "Genetic marker assistance for the Washington apple breeding program" PI: Cameron Peace. Co-PIs: Evans, Olmstead , Mattheis. Fundamental development of seedling database for incorporation in the breeders toolbox.

Agency Name: MARS Inc. through USDA-ARS
Amount awarded: $550,000 (Jan 2008 – Jan 2012)
Notes: "The Cacao Genome Database. PI: Dorrie Main. Synergistic project for practical application of bioinformatics to tree fruit crops.

Total project funding:

Item	2009	2010
Salaries	26000	26000
Benefits	8808	8808
Wages	3000	3000
Benefits	0	0
Equipment	0	0
Supplies	0	0
Travel	1000	1000
Miscellaneous	0	0
Total	$38,808	$38,808

RECAP ORIGINAL GOALS

1. Integrate publicly available gene, trait, QTL and breeding data for apple and cherry with the current genomics and genetics data and tools in the Genome Database for Rosaceae.
2. Develop web interfaces for molecular geneticists and breeders to upload new data.
3. Develop web interfaces and online tools suitable for accessing and mining all breeder relevant data.

Year One Goals and Activities:

- Design data schema for new features
- Collect gene, trait, QTL and breeding data for apple and cherry and upload to database.
- Design queries for data retrieval
- Start web interface/tool design process

Year Two Goals and Activities:

- Continue to collect to gene, trait, QTL and breeding data for apple and cherry and upload to database.
- Complete web/interface tool design process

Anticipated Accomplishments:
After this two year project is complete the apple and cherry breeding program will have a robust, and secure data management and data querying database that will link directly to the integrated genomics and genetics data housed in the Genome Database for Rosaceae (GDR). GDR will have a publicly available breeding toolbox that is integrated with existing genomics and genetics data. This breeders database and toolbox will be part of a larger open-source genomics, genetics and breeding database for Rosaceae, that can also be utilized by other plant communities.

SIGNIFICANT FINDINGS

1. Developed a new diversity module for Chado, the open-source genomics database schema, in collaboration with bioinformaticists working on other databases including SGN (Solanaceae Genomics Network), VectorBase, and KnowPulse. Paper being prepared for submission to peer-reviewed Databases journal.

2. Excel templates for breeders to record and upload their data have been created.

3. Existing data from Washington Apple Breeding Program (WABP) have been converted into template, and the data from Pacific North West Sweet Cherry Breeding program (PNWSCBP) in the process of being converted into template.

4. Scripts created to upload data in template to database.

5. Data from WABP has been uploaded to database.

6. Held multiple online conferences between bioinformatics team and breeders to design the web interfaces.

7. Interfaces for browse, search and data download under development.

8. Private web management system created for breeding programs using the Drupal Groups function.

9. Following a presentation at the 5th International Rosaceae Genomics Conference by Dr. Evans on the "Development of an Online Tree fruit Breeders Toolbox", several breeders from International programs contacted Drs. Main/Evans about also wanting to use the toolbox system, indicating utility and expandability to other breeding programs

METHODS

1. A new module of Chado for breeding data has been developed in collaboration with bioinformaticists working on databases for multiple projects with multiple species to ensure the development of a module that can handle data from various projects.

2. The templates and the uploading scripts for breeding data have been designed and developed to have two different levels so that breeders in different programs can easily upload their data and yet the same main uploading scripts can be used in uploading data from multiple breeders. The main uploading scripts were developed in modules (ontology, contact, location, cross, germplasm, project, propagation, and phenotype) so that the common modules can be reused for uploading different types of breeding data. For example, all the modules above, except the phenotype module, can be used when uploading breeding data with genotype (Figure 1).

3. We are employing various tools and technologies that comes with Drupal, a pioneering Content Management System, to build a web site with various functionalities with minimum work for initial building and maintenance. For example, we used a feature called 'Organic Group' in Drupal to develop a private web management system for each breeder. We have also started a collaboration with a group in University of Saskatchewan to use a tool called 'View' in Drupal to develop versatile query and data detail pages that require minimum coding for maintenance and future modifications.

RESULTS AND DISCUSSION

We are on track to meet almost all our year 2 goals (end of March 2011), with the exception perhaps of not having all the trait data we'd like available by that date. One of the major findings to date is that we have successfully modeled a database system that will accommodate not just the data and analysis needs for the apple and cherry breeding programs in Washington State but also other crops and any other species for which phenotyping data are available. We have also developed data templates and data uploading system that are flexible for various breeding program. Another major work is that we identified the format of browse/query/download web interfaces that will be most useful for breeders (Figure 2). The development of these web interfaces are well underway and we anticipate it to be completed by the end of our year 2. In addition, we have developed a easier-than-wiki-type system where individual breeders, without any experience in creating a web site, can easily create their own web site, create users with different roles and directly link to their breeding database.

The provision of breeder focused web-databases with various browse, queries and download functionality will greatly accelerate breeders' work in evaluating breeding selections, comparing various lines and identifying elite lines for further testing. Our system allows breeders to directly upload the outputs of analytical tests whenever possible, reducing the time and labor managing the

data. The breeding data reside within the same schema where the genomic and genetic data of GDR are stored, enabling the future connection between the genomics and genetics data with the actual improvement of cultivars. The two breeding programs in Washington are participants of RosBREED Project and they will utilize DNA markers to test the genotype of their breeding selections. When the genotyping data become available and uploaded to the breeding database, the data will be easily integrated with the genetics and genomics data of GDR.

The breeding data integrated within GDR will significantly accelerate identification and application of the genes and markers underlying important economic traits such as pre and post harvest fruit quality, and pest and disease resistance. Improvement of metric traits through the application of bio-informational methods will give a more predictable outcome to plant breeding than is currently the case with conventional one-gene-at-a-time genetics or phenotypic selection approaches. This database will allow the collection, storage and analysis of appropriate DNA, RNA, phenotype and germplasm datasets which can then be linked to traits that are of interest to breeders and industry stakeholders. This database resource will aid marker-assisted tree fruit breeding and facilitate the creation of new cultivars which meet consumers' needs and sustainable agricultural practices in the Pacific North West.

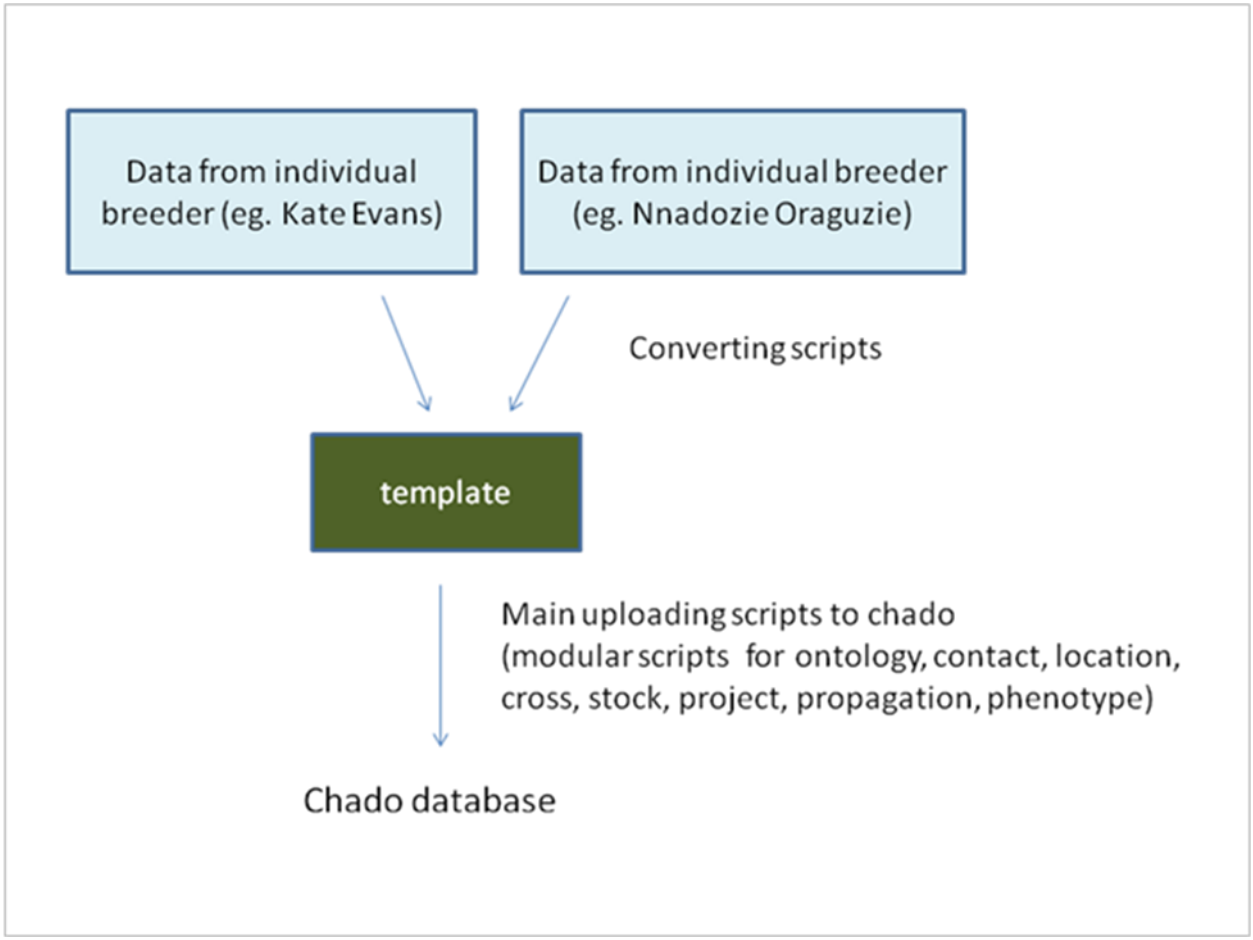

Figure 1. The scheme of data uploading pipeline for breeding data

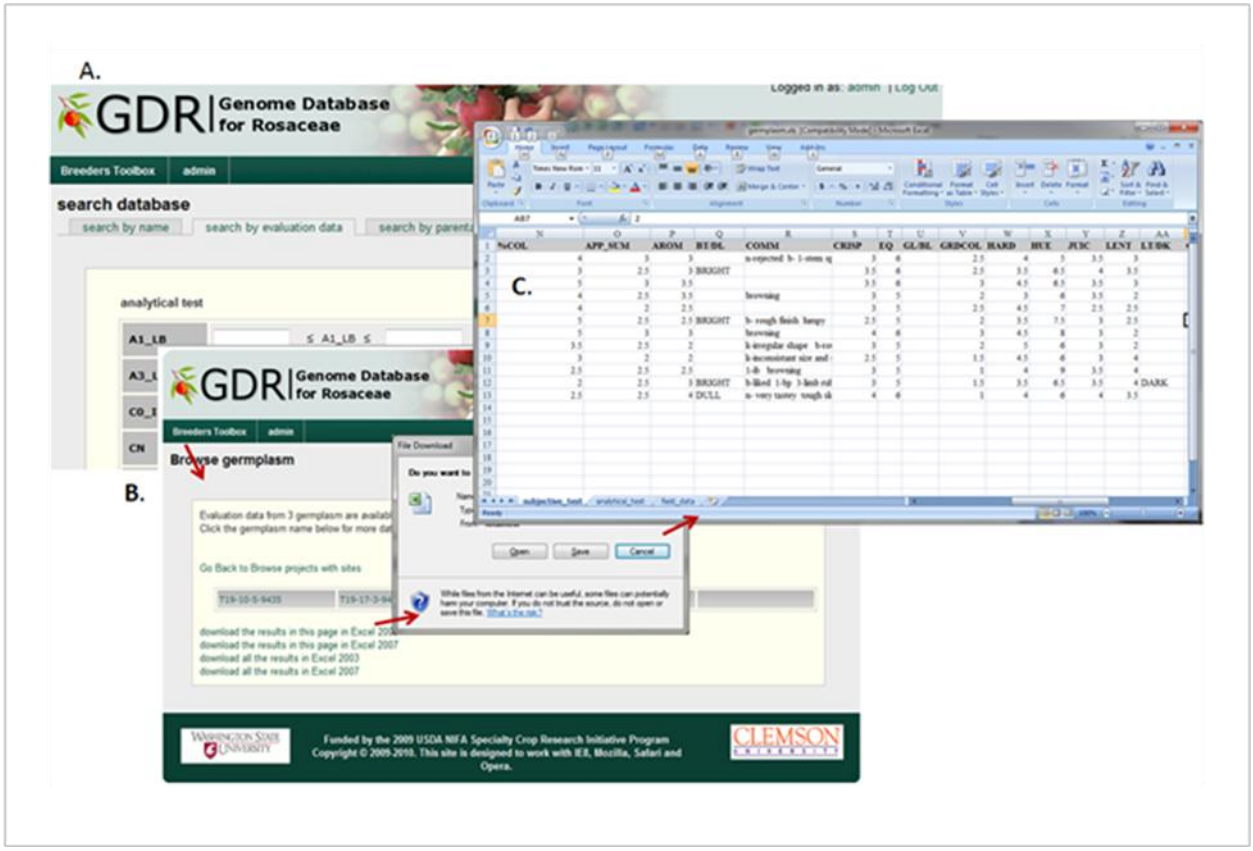

Figure 2. Screen Shots of the web interfaces of breeders' toolbox. A. Users can browse by projects (not shown) or search by name, evaluation data, or parentage. B. Users get a result page with list of germplasm names corresponding to various queries or browse selections made along with the options to download evaluation data in Excel. C. An example of downloaded Excel document with the evaluation data. In search pages, users can select the types of evaluation data that they want to include in the downloaded data.

EXECUTIVE SUMMARY

This project was initiated to provide the Washington Apple Breeding Program (WABP) and the Pacific North West Sweet Cherry Breeding program (PNWSCBP) with a database resource to house and manage their voluminous data within an easy-to-use, secure, online system that seamlessly integrates with all the available genomics and genetics data available in the Genome Database for Rosaceae (GDR). This required that gene and trait data continue to be updated in GDR while also building the private data management system and online toolbox for the individual breeding programs. This involved in-depth discussions so the development team could fully understand each program's breeding practices to ensure they were able to capture every relevant data point within the generic database structure (Chado) they decided to use. This was followed, in collaboration, which we initiated, with several other bioinformatics groups to remodel Chado and add a natural diversity module (currently being written up for publication) facilitating a more comprehensive genomics, genetics and breeding data structure.

Having modeled the database structure, an excel template was devised to house all the breeding data in sheets according to the breeders needs and scripts were written to (1) convert the data from the WABP Access database into this template and (2) upload the data from this template into the individual program databases. Interfaces are being developed specifically for the WABP to provide the required functionality, still an ongoing process. This was presented by Dr. Evans at the 5th International Rosacaeae Conference in South Africa, and was very well received by the community. Several breeders from outside the US have since expressed interest in also adopting this system if it is made available to them through GDR in the future.

By the end of year 2 we anticipate having the PNWSCBP data uploaded and available for browsing/querying, similar to the WABP functionality. Further developments will include providing an automated data upload and editing function for new data, the addition of genotyping data provided through the RosBREED and WTFRC projects, adding trait data and linking to the apple and peach gene and genome sequences available in GDR, and providing advanced querying and reporting functionality as required for the breeding programs.

We are confident that the interdisciplinary, team-based approach to creating this database and toolbox will prove to be a fundamental resource to our WSU managed tree fruit breeding programs and greatly facilitate the development of new and improved cultivars that will enhance the competitiveness and sustainability of our local tree fruit growers and producers.

FINAL PROJECT REPORT

Project Title: Genetic marker assistance for the Washington apple breeding program

PI:	Cameron Peace	**Co-PI(2):**	Kate Evans
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Cooperators: Jim McFerson (WTFRC), Fred Bliss (Davis, California), Dorrie Main (WSU Pullman), Bruce Barritt (WSU, ret.), Yanmin Zhu and Dave Rudell (USDA-ARS Wenatchee), Deven See (USDA-ARS Pullman), Jim Luby (U Minnesota), Eric van de Weg and Marco Bink (Plant Research International), Fabrizio Costa, Sara Longhi, and Riccardo Velasco (IASMA), Sue Gardiner, Stuart Tustin, and David Chagne (HortResearch), Susan Brown (Cornell U), Phil Forsline and G. Fazio (USDA-ARS Geneva), Walter Guerra (Laimburg), Francois Laurens (INRA), Rozemarijn Dreesen, Wannes Keulemans, and Mark Davey (KULeuven), Amy Iezzoni (MSU), and others.

Other funding sources

Agency Name: USDA-CSREES National Research Initiative
Amount awarded: $400,000 (2009-2011)
Notes: "Functional gene markers for Rosaceae tree fruit texture" PI: Peace. Co-PIs: Costa, van de Weg, Luby, McFerson, Gardiner, Hamblin, and Oraguzie. Closely coordinated with activities 2-4 of this WTFRC apple project.

Agency Name: WTFRC Apple Review
Amount requested: $635,201 (2009-2011)
Notes: "Apple Scion Breeding" PI: Evans. Co-PIs: Peace, Ross, Zhu. The foundation program on which this reported WTFRC project builds.

Agency Name: WTFRC Apple Review
Amount awarded: $77,616 (2009-2010)
Notes: "Developing an online toolbox for tree fruit breeding" PI: Main. Co-PIs: Evans, Oraguzie, Peace, Jung. Establishment of bioinformatics and databasing support to facilitate the translation of genomics information into application in WSU tree fruit breeding programs. Synergistic with activity 5 of this reported WTFRC project and SCRI project "Tree Fruit GDR" below.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $2,000,000 + equal matching from universities, industry (Sep 2009 – Aug 2013)

Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture". PI: Main. Co-PIs include Evans and Peace. Synergistic project for application of bioinformatics to tree fruit crops.

Agency Name: USDA-CSREES, Specialty Crops Research Initiative
Amount awarded: $7,200,000 + equal matching from universities, industry (Sep 2009 – Aug 2013)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace, Main, and Evans. A synergistic project to establish sustainable marker-assisted breeding infrastructure for U.S. Rosaceae crops, using the Marker-Assisted Breeding Pipeline concept that involves Pedigree-Based Analysis.

Agency Name: WTFRC Cherry Review
Amount awarded: $45,000 (2009)
Notes: "Establishing the Marker-Assisted Breeding Pipeline for sweet cherry" PI: Peace. Co-PIs: Olmstead, Iezzoni, and Oraguzie. Synergistic project for establishing equivalent marker-assisted breeding infrastructure for the PNW sweet cherry breeding program.

Agency Name: WTFRC Cherry Review
Amount awarded: $88,600 (2010-2011)
Notes: "Marker-assisted breeding strategies for large fruit and self-fertility" PI: Peace. Co-PIs: Oraguzie, Iezzoni, and Whiting. Synergistic project for conducting marker-assisted breeding in the PNW sweet cherry breeding program.

Agency Name: WTFRC Cherry Review
Amount awarded: $56,000 (2010-2011)
Notes: "Targeting the ethylene biosynthetic pathway to improve cherry quality" PI: Peace. Co-PIs: Wiersma, Oraguzie, and Whiting. Synergistic project investigating role of ethylene genes in cherry.

Agency Name: WTFRC Technology Review
Amount awarded: $165,743 (2011-2012)
Notes: "Breeding in the 21st Century: Technology platform for fast breeding" PI: Dhingra. Co-PIs: Peace, Evans, Oraguzie. Synergistic project to assess methods of faster breeding.

Agency Name: WTFRC Technology Review
Amount awarded: $9500 (2011)
Notes: "Ultra-low freezers for genomics, genetics, and breeding labs" PI: Peace. Co-PI: Dhingra. As the project title states.

Agency Name: WTFRC Technology Review, Washington Wheat Commission
Amount awarded: $100,000 (2009)
Notes: WTFRC: "ABI 3730 DNA Analyzer to augment tree fruit breeding and research" PI: Peace. WWC: PI: Deven See. WTFRC and WWC funding matched to obtain a refurbished ABI 3730 DNA Analyzer for high-throughput genotyping of tree fruit and cereals, based at Pullman.

Agency Name: WSU Agricultural Research Center
Amount awarded: $100,000 (2009)
Notes: Additional support to C. Peace for high-throughput DNA extraction and genotyping equipment, complementing the ABI 3730 and removing technical bottlenecks for routine tree fruit genotyping. Part of this support was used to leverage a further $50,000 from the Washington Wheat Commission and $8,000 from D. See's USDA base funding to obtain a BioMek Laboratory Automation Station – a "robot" for high-throughput DNA extraction and genotyping sample preparation – operational since September 2009.

Total Project Funding: $121,500 – exclusive of collaborative expenses

Budget History:

Item	**Year 1: 2009**	**Year 2: 2010**	
Salaries			
Benefits			
Wages	$21,186	$22,034	
Benefits	$ 3,814	$ 3,966	
Supplies	$10,000	$10,000	
Travel - In-state - Other	 $ 2,000 $13,500	 $ 2,000	
Outreach	$ 5,000	$ 5,000	
Miscellaneous	$13,000	$10,000	
Collaborative Expenses - Stemilt RCA room rental - Crew labor	 $ 6,000 $13,000		
Total	**$68,500**	**$53,000**	

Collaborative Expenses	**Year 1: 2009**	**Year 2: 2010**	
Stemilt RCA room rental	$ 6,000		
Crew labor	$13,000		
Total	**$19,000**		

RECAP ORIGINAL OBJECTIVES

The overall goal was to provide comprehensive molecular genetics support for the WABP utilization of marker-assisted breeding. Specific objectives were to:

1) Establish a world-class long-term reference apple germplasm planting in Washington.
2) Obtain comprehensive fruit quality phenotypic data on representative industry and breeding stock of Washington and the nation.
3) Ensure Washington fruit quality phenotyping is contemporary and coordinated with national and international collaborators.
4) Enhance outreach efforts to demonstrate local impacts of genetic marker use for apple.
5) Create a high-throughput seedling genotyping database for the WABP.
6) Develop a DNA fingerprinting system for new cultivar releases from the WABP.
7) Continue to pipeline new markers for high priority traits into the WABP.

SIGNIFICANT FINDINGS

All seven Objectives were accomplished to some degree: 1) Completed, 2) Completed, 3) Completed, 4) Only half-completed, 5) Still in early stages, 6) Completed, 7) Completed, with massive infrastructure enhancement underway through the RosBREED project.

- Obj 1. The Apple Germplasm Library, a collection of up to 2700 trees serving as the germplasm base of the Washington apple breeding program and various genetic studies, was established, maintained, added to, presented, reported on, and studied.

- Obj 2. Fruit quality evaluations were successfully conducted by the WTFRC lab on hundreds of breeding and collection trees over three storage durations to dissect the genetic control of crispness, firmness, juiciness, sweetness, acidity, and storage disorders. Data collected is being used in an international USDA-funded National Research Initiative (NRI) project examining and exploiting the genetic control of apple texture (led by Peace).

- Obj 3. We established an international network of apple breeders, geneticists, collection curators, and fruit quality experts, and developed protocols for standardized fruit harvest and quality evaluation for internationally coordinated, powerful research studies.

- Obj 4. The success stories of use of the ethylene genes and other genetic marker applications in the Washington apple breeding program have been and continue to be described at various venues with diverse audiences. Efforts have integrated with the larger federally funded RosBREED project, with our local experiences providing the major MAB examples to date.

- Obj 5. Funding and expertise for developing a streamlined seedling genotyping database was increased with new collaborative partnerships (with Drs. Dorrie Main and Deven See).

- Obj 6. The foundation was developed for a DNA fingerprinting system that determines uniqueness and parentage of WSU selections to better characterize and protect selections and new cultivar releases. This system was used to deduce the parentage of ‘WA 2’ (‘Splendour’ × ‘Gala’), confirm parentage of ‘WA 5’, and describe uniqueness of both. Genetic markers were also used to fix a nursery mix-up prior to Phase 2 planting, and to ensure relocated mother trees from TFREC to Columbia View were identical to their originals.

- Obj 7. Several promising genetic tests are at various stages in the MAB Pipeline for the highest priority breeding trait categories of texture, flavor, and appearance. Out the downstream end of the Pipeline, two ethylene gene markers for fruit storability are now routinely used support breeding parent and seedling selection decisions. Gene markers for flavor, appearance, and further texture components are poised for routine use from 2011.

- The existence of this industry-supported WTFRC-funded project provided a critical foundation for $7.2 million to be awarded by the USDA for the RosBREED project, of which the Washington apple breeding program and its supporting molecular genetics program are major participants and beneficiaries – substantially multiplying the investment by Washington apple growers in new cultivar development.

RESULTS & DISCUSSION

Significance to industry and potential economic benefits

Germplasm is the basis and future – and innovative limit – of any breeding program. We now have a comprehensive locally accessible "library" of germplasm at the Sunrise Research Orchard, which among other familiarizing opportunities is showcased to industry each July since 2008 in annual Field Days. The Apple Germplasm Library includes diverse material harboring a wide spectrum of valuable traits that will be drawn from to produce WA's future cultivars that sustain the industry. The Library also contains material that through genetic studies is aiding efforts to reduce the time it takes to incorporate novel traits into new cultivars. Growing this material locally finally ensures that local environmental adaptability and local industry suitability are entwined in this process.

Fruit quality evaluation data obtained will be mined in 2011 within our internationally networked USDA-funded National Research Initiative (NRI) project to refine genetic tests for storability, texture, and flavor optimization. These genetic tests will be applied to informing postharvest management through placement of cultivars into genetic potential performance groups, and to breeding decisions in crossing and seedling selection. Already, previous genetic knowledge on two genetic tests for fruit ethylene production has been put to practical use in breeding, and represents a world first among apple and related crops for routine marker-assisted seedling selection for fruit quality. However, interactions between these two tests, and interactions with other genetic components of texture and flavor, remain unclear. The NRI project began two years ago to address these issues and involves experts across the U.S., Europe, and New Zealand. These data and those from collaborating institutions will be used to determine the joint influence of key texture/storability genes, investigate their impact on flavor retention and storage disorders, and apply resulting knowledge in supporting breeding and industry management decisions – including refining the specific situations in which applications of the genetic tests are appropriate.

The most important feature of the ethylene genes, used now in parent selection, seedling selection, and to describe the genetic potential of new cultivars, is that they represent the first markers to be used routinely in this way. We do not intend that they be used always, and the main outcome of the NRI project will be to refine their application based on the extent to which they can predict fruit quality. Thanks to the path forged by the trail-blazing ethylene gene markers, more markers for further traits are now coming into use.

Data management tools completed (calculator of cultivar parentage and uniqueness) and being developed (database for high-throughput seedling genotyping) link in with a wider range of computer-based tools, databases, and interfaces being established: Dr. Dorrie Main's "Breeders Toolbox", RosBREED's Breeding Information Management System, Dr. Main's Tree Fruit Genome

Database Resources, and our seedling selection efficiency tool that is also being enhanced in RosBREED. Breeding and genomics are huge data generators, yet previously existing management tools were insufficient and inefficient to extract the full value from these data so that breeding decisions can be optimally informed. Our efforts in this area, and those of critical collaborators such as Dorrie Main, place the WABP atop the wave of data rather than awash in the ocean. Specifically from this project, we can now accurately confirm parentage of breeding selections, or deduce it where necessary. Parentage is one of the pieces of information that growers use when considering new cultivar adoption, yet this information is not always known from breeding records and rarely confirmed. DNA fingerprinting to establish cultivar uniqueness is now routinely performed, facilitating the cultivar release process, ensuring protection of outstanding selections in trials, and enabling rapid verification of identity during repropagation within the breeding program.

MAB has progressed over the last two years of this project from a promising means of improving breeding efficiency to a routine enhancer of various breeding operations in the WABP. The WABP applies DNA-based genetic markers in marker-assisted parent selection, parentage verification, assessment of crossing success, marker-assisted seedling selection on thousands of seedlings at a time, marker-assisted advanced selection description, genetic identity confirmation during repropagation, and fingerprinting of new cultivar releases – the first Rosaceae breeding program in the world to conduct routine MAB on so many fronts and the first for apple and related tree fruit for many of these applications.

Visits and discussions with apple breeders around the world have highlighted reasons for our success here. Foremost is the local industry support for the breeding program and its supporting science. A common feature elsewhere is that local industries are small, disconnected, and unclear about their cultivar and research needs. In some cases, breeding programs exist where local industries do not want or would not be able to adopt new cultivars. Another reason for our success is that there is a demand from the WA industry for fast, efficient return on investment in breeding. This demand has manifested in explicit requests for support from modern DNA-based technologies and expertise. In some other places, MAB is pushed upon breeders by molecular lab-based scientists, and in all places a huge chasm in understanding exists between genomics research and breeding application. From such requests has grown our apple genomics, genetics, and breeding team of advisors, technicians, and scientists. A third success criterion in our case is the focused perseverance of all involved.

Details on results

Obj 1. Establish a world-class long-term reference apple germplasm planting in Washington.

- 2041 trees are now in the Apple Germplasm Library (Figure 1), representing four germplasm "sets" (Parent, Pedigree, Mapping, and Diversity). 168 Parent Set trees planted in 2008 (67% of full capacity), 466 Pedigree Set trees planted 2008 (full capacity), 182 Mapping Set trees planted 2009 and 771 in 2010 (full capacity), and 227 Diversity Set trees planted 2010 (57% capacity).
- Library is spread across approximately 3.5 acres
- Several hundred trees planted in spring 2010 did not survive, primarily due to the Oct 2009 freeze event. These trees, primarily Mapping Set, will be replaced – providing the opportunity for a "staggered start" to improve the ability to identify true genetic effects.

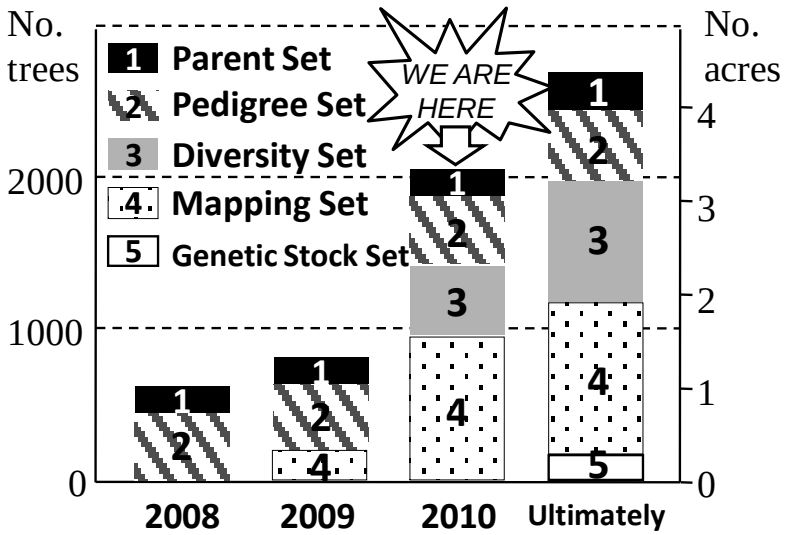

Figure 1. The Apple Germplasm Library will ultimately consist of about 2700 trees (with >2000 unique individuals) grouped into five Sets. The first planting was in spring 2008. The largest additions were planted in spring 2010. Maximum capacity will be reached gradually.

- Library's full capacity is about 2700 trees across 4.5 acres. Different components, with their varied uses, will be recycled at different rates in future years.
- NRI project used 7 cultivars of the Parent Set in 2009 season; most trees not yet fruiting then.
- RosBREED project is using 240 Pedigree Set seedlings and 24 Parent Set cultivars in 2010-2012.
- Flowering and fruiting dates and levels were recorded for all trees in 2009 and 2010.
- Parent Set trees used for crossing in WABP in 2010. Pedigree Set trees evaluated as Phase 1 seedlings in WABP in 2010.
- Sister plantings – "Apple Diversity Block", "World of Apples" and "Student-Led Fruit Breeding Program" – for educational and public use was established in 2010 at the Tukey Research Farm in Pullman, using extra trees (after nursery propagation) of the Diversity Set and more than 800 *Malus sieversii* trees donated by G. Fazio, with planting conducted by undergrad and grad students and with financial support from the Dept Horticulture & Landscape Architecture.
- Presentations on Library in 2009: Good Fruit Grower article ("In search of superior apples", pp14-15, July 2009), on-site – 1st Annual Sunrise Orchard Field Day (color 2-page flyer provided), on-site – 2009 annual Apple Crop Germplasm Committee meeting (color 2-page flyer provided).
- Presentations on Library in 2010: on-site – 2nd Annual Sunrise Orchard Field Day.
- Plan to evaluate fruit quality traits of Diversity and Mapping Sets and non-RosBREED Pedigree Set, alongside Parent Set cultivars, starting in the 2012 season, for enhanced genetic dissection, fishing for novel traits, and breeding application.
- Plan to evaluate insect resistance in Diversity Set in 2011.
- Plan to begin to populate the Genetic Stock Set in 2012 with seedlings from "fast breeding" (funded Technology Review project).
- Future funding requirements: ongoing funds for maintenance (currently approx. $2000/acre/year) and for propagation and planting to fill capacity and replace dead trees. After 2011, plan to apply for federal funding to help offset these costs that to date have been borne by WTFRC.

Personnel involved: *Plant material choice*: C. Peace and B. Barritt, and J. Olmstead. *Provision of trees*: B. Barritt, K. Evans, P. Forsline, G. Fazio, Y. Zhu. *Planting*: Sunrise Research Orchard field crew, B. Konishi, L. Brutcher, J. Olmstead, K. Evans, C. Peace, CP's Pullman crew of T. Rowland, D. Edge-Garza, S. Haldar. *Plant maintenance*: Sunrise Research Orchard field crew, B. Konishi, L. Brutcher, J. Brunner (supervision). *Flyers*: C. Peace, J. McFerson.

Obj 2. Obtain comprehensive fruit quality phenotypic data on representative industry and breeding stock of Washington and the nation.

- Comprehensive fruit quality evaluations were conducted by the WTFRC crew on 130 cultivars and selections and 260 pedigree-linked seedlings growing in Washington and at the USDA-ARS apple collection in Geneva (Plant Genetic Resources Unit, PGRU).
- Measurements included harvest date, starch index, weight, crispness (instrumental Mohr Digi-Test Cn value and sensory 0-150 quantitative scale), firmness (instrumental Mohr Digi-Test penetrometer and sensory 0-150 quantitative scale), juiciness (sensory 0-150 quantitative scale), sweetness (instrumental for SSC), acidity (instrumental for TA), internal ethylene content (instrumental), and storage disorders (0-4 visual scale for decay, superficial scald, soft scald, shrivel, bitter pit, russet, lenticel disorders, watercore, internal browning, and greasiness). All traits were measured for each of 5 fruit at 3 evaluation times (harvest, 10 wks storage + 1 wk ripening, 20 wks storage + 1 wk ripening). Sensory evaluations were conducted by two panelists for each fruit.
- The WTFRC crew also measured SSC and TA for fruit juice samples of 190 cultivars, selections, and seedlings from the University of Minnesota breeding program (major NRI collaborator).
- The single-season dataset is being used, in combination with similar datasets from breeding programs of Minnesota, Italy, New Zealand, and Belgium (Figure 2), in our NRI project to refine understanding of the genetic control of apple texture and apply to industry and breeding.

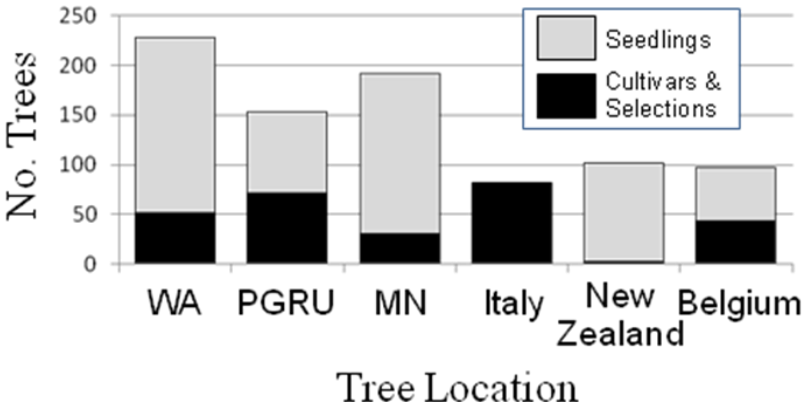

Figure 2. Scope of the fruit quality evaluation. A total of 861 apple cultivars, selections, and seedlings were evaluated, representing the apple breeding stock of the U.S. and the world. The WTFRC fruit lab crew conducted phenotyping for the samples from WA (WABP) and PGRU (Plant Genetic Resources Unit, USDA-ARS Geneva), as well as sugar and acid measurements for all samples from MN (Univ. Minnesota apple breeding program).

- Subsequent seasons (2010-2012) of fruit quality evaluation using many of the Parent Set and Pedigree Set trees of the Apple Germplasm Library are underway in the federally funded RosBREED project, enabled by the WTFRC investment in this 2009 effort.
- Future funding requirements: Fruit quality evaluation of remaining trees of the Parent and Pedigree Sets not covered by RosBREED is recommended for several seasons starting in 2012 (in combination with evaluation of trees of the Diversity and Mapping Sets).

Personnel involved: *Standardized phenotyping protocol development*: C. Peace, J. Luby, S. Brown, F. Costa, R. Dreesen, K. Evans, I. Hanrahan, P. Forsline, W. Guerra, J. Johnston, F. Laurens, J. Mattheis, J. McFerson, S. McKay, N. Oraguzie, J. Palmer, T. Schmidt, S. Tustin, E. van de Weg (that's a lot of experts to reach consensus with!). *WA germplasm choice*: C. Peace, K. Evans, B.

Konishi. *Harvest of WA fruit*: B. Konishi, K. Evans, WTFRC crew. *Harvest of NY fruit for WA evaluation*: P. Forsline, N. Gutkin. *WA fruit quality evaluations*: T. Schmidt, I. Hanrahan, M. Bell, F. Castillo, B. Konishi, K. Evans, J. Mattheis.

Obj 3. Ensure Washington fruit quality phenotyping is contemporary and coordinated with national and international collaborators.

- Washington fruit quality phenotyping is contemporary and coordinated with national and international collaborators. Standardized fruit quality phenotyping protocols for apple were developed in summer 2009 with an international network of experts. These protocols were also adopted in the RosBREED project in 2010.
- In Feb 2009, C. Peace visited three Plant and Food Research (PFR) stations on New Zealand's North Island (Auckland, Palmerston North, and Hawke's Bay), forging new opportunities for phenotyping technologies and standardization, molecular genetics, and functional genomics of apple fruit quality.
- In summer 2010, C. Peace visited the apple breeding programs of the University of Minnesota in St. Paul, MN, Cornell University in Geneva, NY (scion and rootstock), Katholieke Universiteit Leuven in Leven, Belgium, and the Laimburg Research Centre for Agriculture and Forestry in Laimburg, Italy, in addition to NRI-funded visits to IASMA, Italy, and Plant Research International in Wageningen, Netherlands. Numerous discussions were also held at conferences with these and other apple breeders and germplasm curators of the world during the last two years regarding the status quo and future of apple breeding, fruit quality evaluation and genetics, MAB opportunities and challenges, and further prospects for collaboration.
- Future funding requirements: Nothing specific; we will continue to work with our collaborators in the U.S. and abroad on joint projects that amplify the WA apple industry's return on investment in genomics, genetics, and breeding research.

Obj 4. Enhance outreach efforts to demonstrate local impacts of genetic marker use for apple.

- The success stories of use of the ethylene genes and other genetic marker applications in the WABP were described at various venues with diverse audiences: WSHA annual conferences in 2009 and 2010, North Central Washington Apple Day 2010, Sunrise Research Orchard field days, United Fresh 2009 Convention in Las Vegas, NV, a popular science journal article (*Kean S. 2010. Besting Johnny Appleseed. Science 328: 301-303*), and Apple IAC/GGB (industry advisory committee / genomics, genetics, and breeding team) meetings, as well as recent WTFRC Apple Reviews.
- More outreach activities involving industry were planned from the outset, but the departure from WSU of co-PI J. Olmstead left us without a devoted Extension Specialist for this part of the project.
- Successes with the ethylene genes and other genetic marker applications in the WABP have also been described at many research venues in the last two years – most recently at the 5th International Rosaceae Genomics Conference in Stellenbosch, South Africa (C. Peace presented "DNA-informed breeding for high-impact fruit quality and productivity traits in Washington, USA").
- Outreach efforts will continue to be integrated with the RosBREED project. Our local experiences with apple and sweet cherry have provided the major MAB examples to date. For example, the *Md-ACS1* ethylene genetic test for apple was used as the first of the "Jewels in the genome", an ongoing article in the RosBREED quarterly Newsletter by RosBREED project director A. Iezzoni (described on p9 of Feb 2010 issue, which can be found at: www.rosbreed.org/resources/newsletters).
- Future funding requirements: Nothing specific. We will continue to take advantage of RosBREED resources and momentum to support outreach efforts in demonstrating and documenting local impacts of genetic marker use for apple.

Personnel involved: *Sunrise Field Days*: K. Evans, C. Peace, B. Konishi. *WSHA presentations*: K. Evans, C. Peace, and CP's Pullman crew of D. Edge-Garza, S. Haendiges, T. Rowland, S. Haldar, G. Lightbourn, S. Verma, and C. Starr.

Obj 5. Create a high-throughput seedling genotyping database for the WABP.

- Streamlined database not yet developed – awaits fruition of a collaboration with D. See and D. Main to hire a programmer for one year to develop the database and software tools. WTFRC funds already allocated here will provide $10K, while D. See and D. Main will provide another ~$45K.
- In the interim, we created a spreadsheet for automatic summarizing of data for managing genotypic data on several thousand WABP seedlings with the ethylene genes. This spreadsheet was used during seedling culling in spring and early fall of 2010.
- Marker-assisted seedling selection continues to be informed by our Seedling Selection Efficiency Tool (Excel spreadsheet). 2010 experiences used to update costs and durations involved.
- High-throughput seedling genotyping in 2011 will use the interim spreadsheet if necessary.
- Future funding requirements: D. Main seeks WTFRC funds in 2011 to develop high-throughput seedling genotyping database as extension of "breeders' toolbox". Development of RosBREED's Breeding Information Management System in 2011-2013 will also include efforts in this area.

Personnel involved: *Databasing and software needs*: D. Main. D. See, K. Evans, C. Peace. *Interim spreadsheet*: D. Edge-Garza, T. Rowland, C. Peace, K. Evans.

Obj 6. Develop a DNA fingerprinting system for new cultivar releases from the WABP.

- An Excel spreadsheet-based calculator of cultivar parentage and uniqueness was developed. The system primarily uses simple sequence repeat (SSR) markers, which are excellent for such purposes, and also includes available data on functional markers such as the *Md-ACS1* and *Md-ACO1* ethylene genes for storability and SSRs near genes controlling other traits of interest.
- The system was applied to new cultivar releases from the WABP, 'WA 2' and 'WA 5' (Table 1; *Evans KM, Barritt BH, Konishi BS, Dilley MA, Brutcher LJ, Peace CP. 2010. 'WA 2' apple. HortScience 45:668-669*). The previously unknown pollen parent of 'WA 2' was determined to be 'Gala', and the parentage of 'WA 5' according to breeding records was confirmed (Table 1).

Table 1: Parentage analysis of 'WA 2' and 'WA 5' with nine apple genetic markers. Alleles inherited from 'Splendour', the mother of both 'WA 2' and 'WA 5', are shown in **bold**. The other allele must have therefore been inherited from their respective fathers ('Gala' for 'WA 2', 'Co-op 15' for 'WA 5').

WA cultivar	Marker genotype				
Parent cultivar	*Md-ACS1*[a]	*Md-ACO1*[b]	*Md-Exp7*[c]	*Md-Exp2*[c]	*Md-PG2*[c]
'WA 2'	**2**:2	**2**:2	**202**:202	**295**:450	**359**:363
'WA 5'	**2**:2	**1**:1	**202**:214[d]	**295**:450	**363**:363
'Splendour'	2:2	1:2	202:202	295:295	359:363
'Gala'	2:2	1:2	202:202	450:450	363:363
'Co-op 15'	1:2	1:2	202:214[d]	450:450	358:363

WA cultivar	Marker genotype			
Parent cultivar	Hi01b01[c]	CH02b03[c]	CH05c06[c]	Hi04e04[c]
'WA 2'	**162**:189	79:**99**	**118**:126	**216**:228
'WA 5'	**162**:162	79:**107**	**104**:126	216:246[e]
'Splendour'	162:189	99:107	104:118	216:246
'Gala'	189:189	75:79	118:126	228:246
'Co-op 15'	153:162	75:79	118:126	216:246

[a] Allele 2 of *Md-ACS1* is associated with low ethylene production

[b] Allele 1 of *Md-ACO1* is associated with low ethylene production

[c] Allele size is measured in base pairs

[d] Allele 214 is associated with scab resistance

[e] Cannot determine which allele was inherited from 'Splendour'

- Unique DNA profiles were established for both new cultivar releases. For the nine genetic markers reported, the probability of another tree having the same genotype as 'WA 2' is 1 in 8 million. For 'WA 5', this measure of uniqueness is 1 in 667 million (because 'WA 5' carries a rare allele associated with scab resistance).
- The DNA fingerprinting system was also used to ensure that during the repropagation of 23 WABP selections, to move them from TFREC to Columbia View, subsequent clones remain identical to their original mother trees.
- In another case on spring 2010, the system was used to sort out a nursery mix-up of several budded seedlings prior to Phase 2 planting.
- The system will continue to be used to establish uniqueness and verify or deduce parentage of advanced selections and new cultivar releases of the WABP, and for reliably tracking individuals through multiple rounds of propagation during breeding operations.
- Future funding requirements: None.

Personnel involved: *SSR choice*: C. Starr, C. Peace, E. van de Weg, G. Fazio. *Uniqueness and parentage spreadsheet*: C. Starr, C. Peace, S. Verma.

Obj 7. Continue to pipeline new markers for high priority traits into the WABP.

- For converting reported trait-specific markers into tools that can be used in the WABP, we use the 8-stage "MAB Pipeline" that is also the basis of enabling MAB for other Rosaceae breeding programs in the RosBREED project (www.rosbreed.org/breeding/community-breeders). RosBREED is currently improving the infrastructure underlying this Pipeline so that future efforts will be streamlined.
- The ethylene genes *Md-ACS1* and *Md-ACO1* are the first markers to be validated and converted into routine genetic tests for the WABP. This year, by spending $10,000 on genetic screening, marker-assisted seedling selection provided an estimated net savings of $62,000 in present and future costs for the WABP.
- Field planting in spring 2010 was eased after screening 2600 seedlings for *Md-ACS1* and *Md-ACO1*, which resulted in the culling of 1690 predicted inferior trees and avoids their future resource-consuming tree maintenance and fruit assessment.
- Integrating into an earlier stage of the breeding scheme, expensive nursery propagation and subsequent maintenance and assessment was avoided by culling 2900 seedlings (of 5300 screened in summer 2010) that carried inferior alleles for *Md-ACS1* and/or *Md-ACO1*.
- A marker for degree of skin blush (*Md-MYB1*) was assessed for utility to the WABP, with a resulting scientific publication (*Zhu Y, Evans K, Peace C. 2010. Utility testing of an apple skin color MdMYB1 marker in two progenies. Molecular Breeding 10.1007/s11032-010-9449-6*). This marker may be used to guide crossing decisions in future years, but is unlikely to be used for seedling selection in the foreseeable future because seedlings with non-blushed fruit are not currently culled.
- A marker for crispness, acidity, and juiciness (targeting the *Ma* locus) was assessed for utility in the WABP using a meta-analysis of performance data for advanced and elite selections over the last decade. This marker guided crossing decisions since 2010, and will be used to cull seedlings beginning in spring 2011. This marker being investigated in greater detail in the RosBREED project, and outcomes will immediately inform WABP breeding decisions.
- A marker for firmness (*Md-Exp7*) is currently undergoing assessment of utility for the WABP. This marker has secondary use as a good indicator for apple scab resistance because it is closely linked – 'WA 5', for example, is probably scab resistant according to this marker.
- Several other markers are further back in the MAB Pipeline, including the *2MBAc* locus for "ripe apple flavor". A Pullman-based PhD student, S. Verma, is investigating the interaction between extreme genotypes for this aroma-controlling locus and various genotypic categories of the ethylene genes for their joint ability to predict consistency of flavor after storage.

- Future funding requirements: About $5000 annually for genetic screening consumables, with the availability of at least a part-time lab technician or student, will ensure that adequate attention is given to miscellaneous lab activities involved in Pipelining specific markers for the WABP.

Personnel involved: *Prioritization*: K. Evans, F. Bliss, C. Peace. *Genotyping efficiency*: D. Edge-Garza, T. Rowland, D. See, C. Peace. *Marker improvement*: D. Edge-Garza, S. Haendiges, D. See, C. Peace. *Validation and Utility*: C. Peace, E. van de Weg, M. Bink, Y. Zhu, K. Evans, S. Verma, Y. Guan. *MAPS*: K. Evans, C. Peace. *MASS cost-efficiency*: C. Peace, D. Edge-Garza, J. Olmstead, K. Evans, F. Bliss. *MASS trial use*: D. Edge-Garza, T. Rowland, B. Konishi, L. Brutcher, K. Evans, C. Peace. *New markers to enter the Pipeline*: C. Peace.

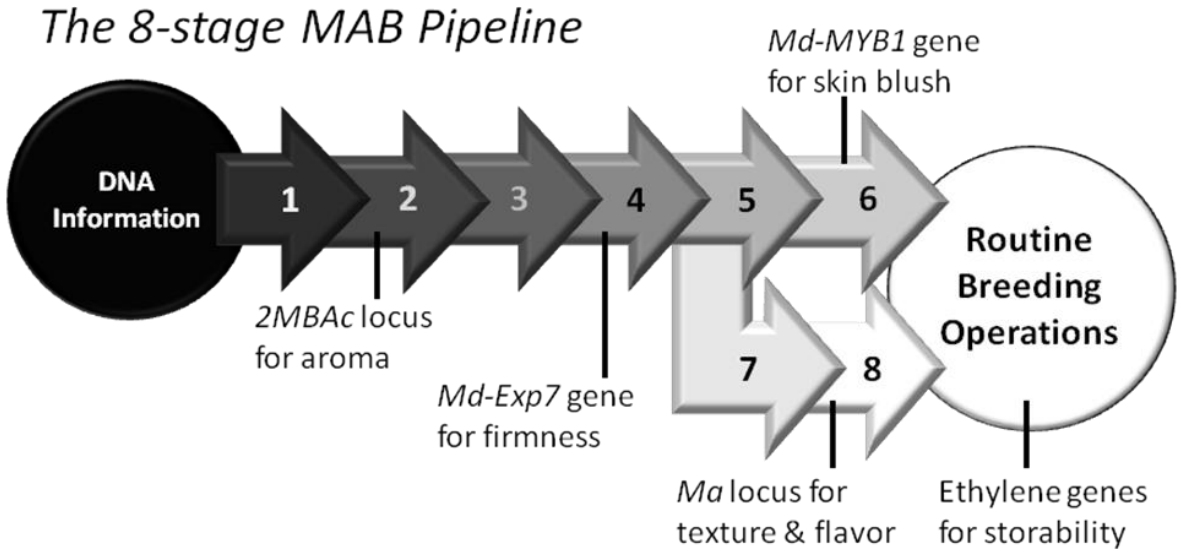

Figure 3: Current status of translating reported DNA information into routine applications in the Washington apple breeding program. The breeding "outlet" from stage 6 involves DNA-informed crossing decisions, while the breeding outlet from stage 8 is for seedling selection.

EXECUTIVE SUMMARY

Our 2009-2010 project on genetic marker assistance for the Washington apple breeding program (WABP) achieved many useful advances. Marker-assisted breeding (MAB) has progressed over the last two years of this project from a promising means of improving breeding efficiency to a routine enhancer of various breeding operations in the WABP. The WABP now applies DNA-based genetic markers to enhance breeding operations in several ways: Marker-assisted parent selection, parentage verification, assessment of crossing success, marker-assisted seedling selection on several thousand of seedlings at a time, marker-assisted advanced selection description, genetic identity confirmation during repropagation, and fingerprinting of new cultivar releases. The WABP the first Rosaceae breeding program in the world to conduct routine MAB on multiple fronts and the first for apple and related tree fruit for many of these applications.

Using markers for seedling selection is only one MAB application, as noted above, yet often attracts lots of attention because of the large numbers of seedlings and large amount of genotyping involved. However, we like to emphasize that using markers for wise parent selection is even more efficient. Our approach is that markers are only used for seedling selection when we are unable to choose parent combinations to avoid inferior seedlings in the first place.

The first markers to come out the application end of the "MAB Pipeline" (which is our systematic means of translating DNA information into practical tools for routine use) are those for the ethylene genes. The markers for the two targeted genes help predict fruit storability. These markers are used now in the WABP in parent selection, seedling selection, and to describe the genetic potential of new cultivars. However, the markers are applied on a case-specific basis. We do not intend for them to be used always, and the main outcome of our concurrent federally funded "NRI" project on apple fruit texture will be to refine their application based on the extent to which they can predict fruit quality. This prediction and utility depends on how they interact with other traits and the environment and how those traits meet industry demands. Thanks to the trail-blazing ethylene gene markers, more markers for other industry-prioritized traits are now coming into use.

Deliverables include:

- Apple Germplasm Library at the Sunrise Research Orchard established and used.
- Fruit quality evaluations on hundreds of breeding and collection trees successfully conducted.
- Protocols for standardized fruit harvest and quality evaluation developed and used.
- An international network of apple breeders, geneticists, collection curators, and fruit quality experts established and engaged.
- Success stories of first markers off the rank delivered to diverse audiences.
- Interim spreadsheet for automated management of seedling marker genotypes created and used.
- Cost-efficient seedling selection schemes using our Seedling Selection Efficiency Tool identified and conducted.
- Saved est. $62K net from $10K spent: spring field planting eased (1690 of 2600 seedlings culled); nursery propagation and future breeding costs avoided (2900 of 5300 seedlings culled).
- Unique DNA profiles for 'WA 2' and 'WA 5' to protect intellectual property rights established.
- Funding for multi-million dollar RosBREED project leveraged with WTFRC support.

Future Directions:

Evaluation, maintenance, and capacity-filling of Apple Germplasm Library; Open door for wider germplasm use in breeding and faster response to industry needs via fast-breeding methods; Improve release and adoption decisions about WABP's new cultivars by revealing and communicating genetic potential for commercial performance; Enhance and utilize bioinformatics support for maximized access to performance and DNA-based data; Establish a full-time genotyping technician/manager in Pullman; Coordinate with RosBREED project to maximize WA tree fruit industry benefit.

FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Crop load and canopy management of apple

PI: Tory Schmidt
Organization: WTFRC
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Cooperators: Jim McFerson, Ines Hanrahan, Felipe Castillo, Tom Auvil - WTFRC

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

Item	2008	2009	**2010**
Salaries	23,230	26,220	25,000
Benefits	6,770	7,650	7,200
Wages	27,150	25,700	24,000
Benefits	12,750	12,100	11,500
Equipment	2,500	3,000	3,000
Supplies	2,500	3,000	3,000
Travel	2,000	2,000	2,000
RCA rental	1,200	4,200	4,200
USDA facilities fee	750	750	750
Total gross costs	76,850	84,620	80,650*
Reimbursements	(27,600)	(25,000)	(36,450)*
Total net costs	$49,250	$59,620	$44,200*

Footnotes: RCA rental based on fiscal year billing cycle
Travel includes fuel costs for driving to trial sites
USDA facilities fee covers storage space and use of research packing line
* Figures do not include $72,000 for contracted chemical thinning trials (confidential)

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

OBJECTIVES:

1) Evaluate pre-bloom, bloom, and post-bloom chemical thinning agents and mechanical thinning technologies with particular focus on complete programs to achieve three goals:
   a) Minimize costs of green fruitlet thinning
   b) Maximize fruit quality
   c) Encourage annual bearing
2) Investigate influence of important variables (drying conditions, spray technology, carrier volume) on chemical thinner efficacy and fruit finish
3) Develop practical PGR programs to manipulate floral initiation and promote annual bearing
4) Evaluate horticultural effects of reflective materials (Extenday, mylar products)
5) Profile natural tree-to-tree variation in long-term cropping patterns in a newly planted apple block
6) 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 3,5)

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)

Endothall (ThinRite) has been as effective as Crocker's Fish Oil + lime sulfur in recent trials and may provide a viable alternative for chemical bloom thinning of apple (Table 2)

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. evening sprays) of chemical thinning programs (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 4, 5)

Crops may be effectively thinned chemically without use of carbaryl; BA + NAA programs demonstrate positive results (Tables 4, 5) with no deleterious effect on fruit quality

Apogee shows no clear, consistent effect on the efficacy of chemical bloom or postbloom thinners in the first year of testing (Tables 2, 4)

Summer applications of NAA have not increased return bloom in WTFRC trials; GA trials to inhibit return bloom show promise for mitigation of biennial bearing (Figure 1, Table 6)

Extenday products improve yields of target fruit in apple by:

1) Increasing fruit set without sacrificing fruit size (Tables 7, 8)
2) Increasing fruit size without reducing fruit set (Tables 7, 8)
3) Increasing fruit color (Tables 7-9)

Trees treated with Extenday products over multiple seasons demonstrate increasing capacity to carry high quality fruit (Tables 7, 8)

Mylar products increase apple fruit color, but not as dramatically as Extenday in WTFRC trials (Table 9)

Long term study of tree-to-tree variability in cropping and growth is underway at WSU Sunrise research orchard (data not shown)

Ongoing collaborative efforts across disciplines, institutions, and regions leverage funding and increase relevance and impact of research (Table 10)

BACKGROUND:
We have scaled back internal research efforts in chemical thinning to accommodate more collaborative work in other areas, but also in part because of the success of earlier work. Many programs and principles put forward by our research, especially aggressive bloom thinning with lime sulfur, are now firmly established across the Washington industry. We will continue screening new materials and programs for crop load management (see new McArtney metamitron proposal), but our focus is now increasingly on collaborative projects exploring mechanical thinning techniques (see Lewis/Schupp technology committee project report) and increasing the precision and predictability of crop load management programs through web-accessible developmental models and decision systems (see Yoder project report on pollen tube growth model and Schmidt project report on bloom phenology and fruit growth models).

We continue to evaluate the relative success of chemical and mechanical thinning programs through 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 assume 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 in 2010 are reflected in Table 1; in those 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 treatments were left to the discretion of individual grower-cooperators, provided that each experimental plot receives the same programs.

Table 1. Chemical thinning programs evaluated. WTFRC 2010.

BLOOM THINNERS
2% Crocker's Fish Oil (CFO) + 2-3% LS
2% Crocker's Fish Oil (CFO) + 2% LS preceded by 12 oz/A Apogee
24-32 oz ThinRite/A 1x
16-24 oz ThinRite /A 2x
0.5% GSL 90 + 1-2% Sulforix
6% NC99
POSTBLOOM THINNERS
48 oz Sevin (carbaryl) + 3 oz NAA/A
48 oz Sevin (carbaryl) + 128 oz BA/A
48 oz Sevin (carbaryl) + 3 oz NAA/A preceded by 12 oz/A Apogee 2x
48 oz Sevin (carbaryl) + 128 oz BA/A preceded by 12 oz/A Apogee 2x
128 oz BA + 3 oz NAA/A preceded by 12 oz/A Apogee 2x

BLOOM THINNING:
Even though we conducted several contract thinning trials subsidized by private chemical companies, we carried out only one independent chemical bloom thinning trial in 2010. Our second year of evaluation of Sulforix plus a non-ionic surfactant confirmed some potential for reduced fruit set, but fruit marking (Table 2) continues to be a concern and may preclude this material from providing a viable sulfur alternative to standard lime sulfur programs.

Ongoing trials with endothall (ThinRite) have yielded some modestly encouraging results in that some programs (Table 2), especially those utilizing two applications, have been as effective as standard lime sulfur treatments. The material's registrant, United Phosphorus, believes ThinRite will be fully registered and available for commercial use in 2012 and we anticipate further trial work in 2011 to fine tune effective programs.

Several researchers in Europe and the Eastern United States have reported reduced chemical thinner efficacy in the context of standard prohexadione-Ca (Apogee) programs. Despite pre-treating trial plots with Apogee, we were unable to detect any effect on the efficacy of a standard fish oil + lime sulfur program in Gala (Table 2).

Table 2. Crop load effects of bloom thinning programs. Gala/M.9, Manson, WA. WTFRC 2010.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		%	%	g		%
Apogee; 2% CFO + 2% LS	69 b	44 a	43 ns	164 ns	116	72 a
2% CFO + 2% LS	84 ab	34 ab	50	162	118	71 ab
0.5% GSL 90 + 2% Sulforix	66 b	47 a	40	168	114	77 a
0.5% GSL 90 + 1% Sulforix	76 ab	40 ab	47	163	117	65 abc
5% NC99	96 a	27 b	50	158	121	52 bc
24 oz ThinRite 1x	76 ab	40 ab	45	162	118	59 abc
16 oz ThinRite 2x	69 b	42 ab	48	164	116	65 abc
Control	84 ab	34 ab	49	160	119	50 c

Intrigued by results from European thinning trials, we attempted to utilize a food grade black food dye as a chemical bloom thinner. After consulting with Carolyn Ross (Food Science Dept, WSU), we procured some powdered food dye which the manufacturer felt had the most potential for such an application. Unfortunately, we were unable to discover a spray solution that would adequately adhere to plant material. Laboratory assays of several surfactants, bases, and acids mixed with the dyewere unsuccessful at allowing the initial black hue to persist once the spray solutions had dried.

Even though we have reduced our work in bloom thinning, we continue to corroborate prior results of ATS and oil + lime sulfur programs in the context of other experiments. No thinning program we have evaluated to date 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 1999-2010.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	15 / 57 (26%)	10 / 60 (17%)	4 / 52 (8%)
NC99	15 / 32 (47%)	7 / 34 (21%)	2 / 28 (7%)
Lime sulfur	25 / 54 (46%)	12 / 48 (25%)	9 / 47 (19%)
CFO + LS	61 / 106 (58%)	26 / 97 (27%)	21 / 93 (23%)
JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	14 / 27 (52%)	4 / 26 (15%)	4 / 26 (15%)
VOE	13 / 29 (45%)	4 / 28 (14%)	2 / 30 (7%)

[1]Does not include data from 2010 trials.

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

POSTBLOOM THINNING:

As with bloom thinning trials, we also designed a 2010 trial to assess the effects of Apogee on the efficacy of standard postbloom thinning programs. Results were mixed and non-significant when comparing results with and without pretreatments of Apogee, but all programs demonstrated effective thinning as compared to the untreated control (Table 4).

Results from 2010 trials are consistent with prior outcomes which demonstrate that 1) tank mixes of carbaryl and BAare at least as effective as tank mixes of carbaryl and NAA (Tables 4, 5) and 2) BA + NAA programs are equal or superior to any standard postbloom thinning programs utilizing carbaryl (Tables 4, 5). Perhaps most striking about Table 5 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 4. Crop load effects of postbloom thinning programs with and without Apogee. Fuji/M.26, Quincy, WA. WTFRC 2010.

Thinners	Apogee	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
			%	%	g		%
BA + NAA		70 ab	60 ns	21 ns	186 ns	103	51 ab
BA + NAA	Y	60 b	59	26	181	105	56 ab
Carbaryl + BA		66 ab	58	23	190	100	60 ab
Carbaryl + BA	Y	75 ab	52	28	185	103	51 ab
Carbaryl + NAA		60 b	57	29	189	101	44 b
Carbaryl + NAA	Y	76 ab	52	26	206	93	45 b
Control		95 a	48	24	201	95	69 a

Table 5. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2010.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	2 / 18 (11%)	0 / 19 (0%)	0 / 19 (0%)
Carb + BA	29 / 78 (37%)	9 / 77 (12%)	9 / 73 (12%)
Carb + NAA	12 / 52 (23%)	7 / 52 (13%)	5 / 50 (10%)
BA + NAA	5 / 15 (33%)	3 / 15 (20%)	1 / 11 (9%)
Carb + NAA + Ethephon	0 / 5	0 / 5	2 / 5
Carb + NAA + BA	0 / 8	0 / 8	3 / 8

[1]Does not include data from 2010 trials.

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

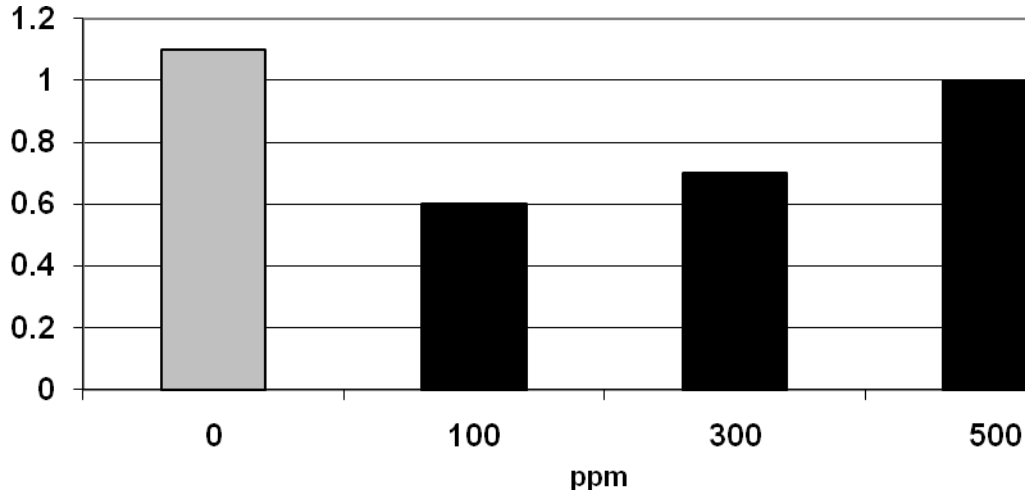
At this stage, we are confident that BA + NAA programs can provide satisfactory, if not superior, alternatives to postbloom thinning programs which rely on carbaryl. We have not observed any pygmy fruit in any of our 11 trials evaluating that combination, nor any other harmful effects to fruit quality; language on product labels warning against tank mixing of BA and NAA products is likely an artifact of historic concerns of NAA causing pygmy fruit in isolated cases and has little relevance to combining the two chemistries.

Reports from Europe suggest that low doses of the herbicide metamitron provide effective postbloom chemical thinning; we were unable to procure any material for Washington trials in 2010, but will seek out new sources for material samples in 2011. Further, we have also begun evaluation of another novel postbloom thinner which has shown promise in European studies and could potentially be approved for use in organic orchards. Results of these trials are protected by a confidentiality agreement and cannot be shared at this juncture.

RETURN BLOOM PROGRAMS:

After several years of unimpressive trial results, we discontinued our efforts to develop programs to improve return bloom with ethephon and NAA. Our focus recently has been to use gibberellic acid (GA) products to suppress flowering of the on year of biennial bearing cycles in pursuit of consistent annual cropping. In several recent trials, we observed the interesting trend that higher concentrations of GA do not amplify treatment effects (Figure 1).

Figure 1. 2009 return bloom effects (flower clusters/cm^2 TCSA) of 10mm applications of GA_3. Fuji/M.26, Orondo, WA.WTFRC 2008.



After observing these effects, we assayed a broader range of GA concentrations, as well as multiple applications of lower rates. Table 6 reflects successful reduction of return bloom from four weekly applications of 200 ppm GA_3 starting at 10 mm fruitlet size in two trials without deleterious side effects on shoot growth or fruit size. Trials launched in 2010 focus more heavily on programs based on multiple applications; if results from these trials are as compelling as those initiated in 2009, we are hopeful that key GA_3 product registrants will pursue label changes for their materials to accommodate these new use patterns.

Table 6. Key effects of WTFRC 2009 GA_3 return bloom trials.

GA_3 concentration	*2009 shoot length*	*2009 fruit weight*	*2010 return bloom*
ppm	***cm***	***g***	***flower clusters/cm^2 LCSA***
Braeburn/M.7 - George			
200	***36.5 ns***	***251 a***	***4.1 bc***
400	***35.0***	***229 b***	***6.0 a***
800	***34.9***	***246 a***	***4.5 bc***
4 x 200 (weekly apps)	***34.1***	***233 b***	***3.5 c***
Control	***34.2***	***234 ab***	***5.1 ab***
Gala/M.26 - George			
200	***31.2 ns***	***171 ns***	***4.3 ab***
400	***30.4***	***170***	***4.4 ab***
800	***30.1***	***180***	***4.0 ab***
4 x 200 (weekly apps)	***30.9***	***180***	***3.2 b***
Control	***31.9***	***170***	***4.7 a***

REFLECTIVE MATERIAL TRIALS:

Since 2005, we have conducted approximately30 trials evaluating reflective materials in commercial Washington apple orchards. Products tested have included the woven plastic fabrics Extenday, Daybright, and Daywhite, all distributed by Extenday USA, as well as Brite N'Up, a Mylar-based material. The Extenday products are designed for use throughout the growing season and may be reused for 6-8 years with good maintenance, while Mylar products cannot be reused and are generally only deployed 2-3 weeks before harvest.

Each material we tested is designed to reflect sunlight striking the orchard floor back up into plant canopies. Increased light saturation as harvest approaches can increase red color development, while increased light saturation throughout the growing season is associated with enhanced carbon fixation (photosynthesis), cell division, and cell expansion. While all products tested improved apple fruit color when deployed shortly before harvest (Table 9), Extenday products have also consistently increased fruit set and/or fruit size in WTFRC apple (Tables 7, 8), pear, cherry, peach, and nectarine trials. Because these materials specifically promote the production of high yields of large, well-colored, high quality fruit, they have tremendous potential to significantly improve grower returns.

Table 7 reflects four years of results from a Honeycrisp block treated with Extenday from bloom until harvest. Due to concerns about poor fruit color in this high value block, the grower-cooperator deployed reflective Mylar film 3-4 weeks before harvest in control plots during each year of the study. Fruit color was similar in Extenday and Mylar plots in all years of the study, except the first season when Extenday plots improved yields of the premium color grade by 30%.Overall yields were significantly higher in Extenday plots in all 4 years, whether due to increased fruit set and/or improved individual fruit size.

Table 7. Fruit yield and color effects of full-season Extenday vs. late-season Mylar control. Honeycrisp/Sup.4, Selah, WA. WTFRC 2007-2010.

	YIELD			*COLOR GRADE*	
	Fruit set	*Fruit wt.*	*Yield*	*WAXF*	*WAF*
	(#/tree)	***(g)***	***(kg/tree)***	***(%)***	***(%)***
2007					
Extenday	***496 ns***	***206 a***	***98 a***	***39***	***49***
Mylar control	***469***	***182 b***	***86 b***	***30***	***46***
2008					
Extenday	***202 ns***	***219 a***	***39 a***	***28***	***69***
Mylar control	***198***	***187 b***	***35 b***	***27***	***70***
2009					
Extenday	***510 a***	***193 a***	***99 a***	***42***	***25***
Mylar control	***442 b***	***174 b***	***71 b***	***44***	***28***

2010					
Extenday	***472 a***	***228 a***	***97 a***	***52***	***47***
Mylar control	***361 b***	***209 b***	***70 b***	***53***	***46***

Increased yield differentiations in later years of a trial are not unique; we have frequently observed cumulative increases in yields over the course of multiple year studies. Table 8 summarizes the average effects of Extenday in each season of every full-season apple trial we have conducted since 2005. While modest yield gains are typical in the first year of trials, the effects are more dramatic in subsequent seasons, likely due to increased carbohydrate reserves and renewed fruiting wood, especially in lower, shaded portions of tree canopies.

Table 8. Mean cumulative yield effects relative to untreated controls of full-season multiyear use of Extenday in all WTFRC apple trials. 2005-2010.

Trial age	n	*Fruit set (harvested fruit/tree)*	*Individual fruit size (g)*	*Total yield (kg/tree)*
1st year	***12***	***+ 9%***	***+ 6%***	***+ 15%***
2nd year	***7***	***+ 24%***	***+ 2%***	***+ 26%***
3rd year	***4***	***+ 17%***	***+ 8%***	***+ 23%***

Reflective materials deployed late in the growing season have little effect on apple fruit set or size, but can improve fruit color in red or partially red cultivars. Table 9 shows effects of Extenday and Brite N'Up on Gala fruit color; both materials were deployed at the same timings using equal material widths. While the Mylar product improved color, Extenday was more effective in all three seasons.

Table 9. Effects of reflective materials deployed 4 weeks prior to harvest on harvest sequence and fruit color. Gala/M.9, Othello, WA. WTFRC 2007-2009.

	TOTAL YIELD HARVESTED				*COMMERCIAL COLOR GRADE*		
	1st pick	*2nd pick*	*3rd pick*	*4th pick*	*WAXF*	*WAF*	*US#1*
	(%)	***(%)***	***(%)***	***(%)***	***(%)***	***(%)***	***(%)***
2007							
Extenday	39 a	40 ns	19 b	2 b	***92***	***7***	***1***
Brite N' Up	21 b	42	30 a	7 a	***82***	***17***	***1***
Control	16 b	40	35 a	8 a	***78***	***21***	***1***
2008							
Extenday	32 a	59 ns	9 b	***na***	***99***	***1***	***0***
Brite N' Up	19 b	63	19 b		***96***	***4***	***0***
Control	14 b	56	30 a		***95***	***5***	***0***
2009							
Extenday	68 a	26 b	6 b	***na***	***87***	***12***	***1***
Brite N' Up	38 b	40 a	22 a		***65***	***30***	***6***
Control	24 c	47 a	29 a		***49***	***46***	***5***

COLLABORATIVE RESEARCH:

In the last three years, we have continued to build productive and dynamic research and outreach partnerships with a number of cooperators (Table 10). These working relationships bring in outside funding for our work (e.g. SCRI projects), attract elite scientists to focus on WA issues, elevate our profile nationally and internationally, and lay the foundation for synergistic collaborations which will be crucial to the future success of our program.

Table 10. Significant WTFRC collaborations on external crop load and canopy management projects 2008-2010.

COLLABORATOR(S)	*PROJECT*	*COMMENTS*
Yoder, Combs	*Pollen tube growth model*	*WA field testing, flower style sampling*
Olmstead, Lewis	*Bloom phenology& fruit growth models*	*See project report AP-09-908 for details*
Lewis, Schupp	*Mechanized thinning*	*Field support for WA portion of SCRI project*
Lewis, Singh	*CASC*	*Field support for WA portion of SCRI project*
McArtney	*Novel chemical thinners*	*WA testing of alternative sulfur products*
McArtney, Greene	*Return bloom programs*	*Bud sampling, WA testing of NAA programs*
Elfving	*Return bloom programs*	*Field support for GA trials*
Rom, McAfee	*Novel chemical thinners*	*WA testing of organic thinning agents*
Elfving	*PGRs for shoot growth*	*Field support for Apogee, ABA trials*

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

Project Title: Modeling Washington apple bloom phenology and fruit growth

PI:	Karen Lewis	**Co-PI (2):**	Tory Schmidt
Organization:	WSU Extension, Ephrata	**Organization:**	WTFRC
Telephone/email:	(509) 760-2263 kmlewis@wsu.edu	**Telephone/email:**	(509) 665-8271 tory@treefruitresearch.com
Address:	P. O. Box 37	**Address:**	1719 Springwater Ave.
Address 2:	Courthouse	**Address 2:**	
City:	Ephrata	**City:**	Wenatchee
State/Zip:	WA 98823	**State/Zip:**	WA 98801

Co-PI(3):	Nairanjana Dasgupta
Organization:	WSU Dept. of Statistics
Telephone/email:	(509) 335-8645 dasgupta@wsu.edu
Address:	Neill Hall #403
City:	Pullman
State/Zip:	WA 99164-3144

Cooperators: Tim Smith (WSU Extension, Chelan County), Gwen Hoheisel (WSU Extension, Prosser), Norman Suverly (WSU Extension, Okanogan), Tom Auvil (WTFRC), Ines Hanrahan (WTFRC), Felipe Castillo (WTFRC), Mark Bell (WTFRC), Ute Chambers (WSU-TFREC), Monte Shaffer (WSU, Pullman)

Total project funding request: **Year 1:** $4,180 **Year 2:** $7,938 **Year 3: $5,690**

Other funding sources: None

WTFRC Collaborative expenses:

Item	2009	2010	2011
Stemilt RCA room rental			
Crew labor[1]	5,000	5,000	7,000
Shipping			
Supplies			
Travel[2]	1,800	1,800	2,400
Miscellaneous			
Total	$6,800	$6,800	**$9,400**

[1] Labor calculated as 2 persons at $16.00/hr working 12 hrs per week for 13 weeks during the growth season.

[2] In-state travel to research plots.

NOTE: 2011 budget increased from $6800 to reflect additional sites handled by WTFRC staff

Budget 1
Organization Name: WSU Extension **Contract Administrator:** M.L. Bricker
Telephone: (509) 335-7667 **Email address: mdesros@wsu.edu**

Item	2009	2010	2011
Salaries[1]		2,941	3,059
Benefits		847	881
Wages[2]	1,000	1,000	1,000
Benefits	180	150	150
Equipment			
Supplies			
Travel[3]	3,000	3,000	600
Total	$4,180	$7,938	**$5,690**

[1] Salary (benefits at 28.8%) for Nairanjana Dasgupta
[2] Wages (benefits at 15%) for part-time help in Wenatchee for bloom observations.
[3]Cooperator in-state travel for bloom observations (5 persons at $600 each)
NOTE: 2011 budget decreased from $8090 to reflect reduced participation from Extension staff (Olmstead, Suverly, Lewis, Hoheisel)

Objectives:

1. Develop functional models for apple bloom development from bud break to petal fall for three cultivars: 'Red Delicious' (standard with historic data), 'Cripps Pink' (early bloomer), and 'Gala' (mid-late bloomer).
2. Develop fruit growth models for the same three cultivars from petal fall until harvest.
3. Incorporate models into WSU DAS system.

Significant Developments:

- Bloom phenology observations successfully recorded at 11 location nodes throughout Central Washington, including 11 Red Delicious, 11 Gala, and 9 Cripps Pink blocks (Table 1)

- Time course photographs taken of same bud/flower/fruitlet from green tip to 20mm fruitlet size at most sites

- Fruit growth measured throughout growing season at 11 Red Delicious, 10 Gala, and 9 Cripps Pink blocks; fruit diameter recorded at all sites, as well as fruit length for Red Delicious blocks (Table 1)

- Streamlined data forms facilitated data entry and analysis; sampling protocols were universally standardized to improve data consistency and statistical robustness

- Field testing of autonomous digital cameras yielded mixed results; cameras successfully captured good quality images at preset intervals, but limited field of view and inability to focus on buds in camera foreground impaired ability to discern precise morphology (Figures 2, 3)

- WTFRC crew will assume all data collection for Prosser and Royal Slope sites in 2011; Omak site will be dropped due to loss of local cooperator (Suverly)

- Preliminary statistical models have been built for bloom phenology (Table 2) and fruit growth (Figure 1) by statisticians with 2009 data; incorporation of 2010 data is ongoing

- Initial discussions held with Dasgupta and Ute Chambers to begin preparations for incorporation of models into WSU Decision Aids System (DAS)

Methods:

Bloom phenology: Team members from WSU Extension and WTFRC internal program observed and evaluated flagged apple blocks around the state (Table 1) at regular intervals from bud break until mean fruitlet size reached 20mm. Representative buds/clusters at chest level on the northwest side of trees of each cultivar were categorized by phenologic stage and digital pictures were taken of representative buds/flower/fruitlets. Based on input from WSU statisticians, observation intervals were shortened to 2-3 days (2-5 days in 2009) and sample size was increased to 30 buds for the 2010 season (20 buds in 2009). Data were recorded on a tally sheet by each individual and eventually submitted to the WTFRC internal program for collation. Hobo data loggers were deployed at each site to record ambient temperatures throughout the season.

Fruit growth: After June drop and hand-thinning, 50 surviving fruit were tagged in the same blocks used for the bloom phenology observations. All fruit were measured by WTFRC staff for diameter and Red Delicious was additionally measured for length as an indicator of fruit type at weekly intervals until the blocks were harvested in the fall. As with bloom phenology, fruit growth protocols (sample size and intervals) were modified for 2010 based on recommendations from statisticians.

Table 1. Roster of sites utilized for apple bloom phenology observations and fruit growth measurements. 2010. (RD = Red Delicious, CP = Cripps Pink, G = Gala)

LOCATION	GROWER	CVs	ELEV (ft)	STAFF	FRUIT GROWTH
Omak	Root	RD, G	1250	Suverly/Crew	RD only
S Shore Chelan	Easley	CP	1120	Auvil/Crew	Y
	Sunshine	RD, G	1450	Auvil/Crew	Y
Brays Landing	Podlich	RD, CP, G	900	Auvil/Crew	Y
S Orondo	C & O Nursery	RD, CP, G	755	Crew	Y
E Wenatchee	Gausman	RD, CP	910	Esteban	Y
	Witte	G	1025	Esteban	Y
Rock Island	WSU-TFREC	RD	910	Crew	Y
	WSU-TFREC	G	880	Crew	Y
	Zirkle CRO	CP	775	Crew	Y
Royal Slope	Delay	CP	1095	Lewis/Crew	Y
	Delay	RD, G	1055	Lewis/Crew	Y
Naches	Rowe	RD, G	1580	Crew	Y
Parker	Brandt	RD, CP, G	879	Crew	Y
Sawyer	WTFRC Rootstock	G	870	Crew	Y
	Badgely	RD	870	Crew	Y
	Weippert	CP	870	Crew	Y
Prosser	Ballard	RD, CP, G	681	Hoheisel/Crew	Y

Results & Discussion:

The contributions of our cooperating statisticians brought immediate and meaningful impact to the project which has improved both our resource efficiency and robustness of results. Based on their recommendations, the following changes were adopted in our second season of data collection:

- Shorter and more regular sampling intervals
- Increased sample size for bloom observations
- Decreased sample size for fruit measurements
- Standardized data collection protocols

These changes not only improved the statistical strength of our 2010 data sets, but helped reduce confusion and error in field data collection. Sampling protocols for 2011 may be further revised to optimize accuracy and efficiency.

Hobo data loggers were again deployed at all nearly all sites to record ambient temperatures in the immediate microclimate of the sampled trees; most sites were selected due to their proximity to AWN stations (usually within a mile), and models using temperatures from both systems will be evaluated for the best statistical fit. Potential discrepancies between temperatures recorded by AWN and individual data loggers could have many explanations, but may be instructive regarding broader extrapolation of weather readings from either system.

In an effort to explore options for reducing time commitments for our field personnel, we tested autonomous digital cameras designed for monitoring big game trails to assess their utility for making routine observation of bloom development. A camera deployed at the WSU Sunrise orchard near Rock Island performed flawlessly, capturing and storing hundreds of good quality images Figures 2, 3). Unfortunately, the camera's effective focal range is 5+ feet; images of branches inside that range were too blurry to prove useful and images of branches that were in focus were too small to discern individual buds or flowers. We have been unable to locate alternative cameras that accommodate better near-range focus within a reasonable price range.

Statistical analysis was done separately for bloom phenology and growth data. For the growth data we used a Non-linear regression model to model the growth pattern for the different locations for the 2009 data. We used the Richards's curve formulation (model given below):

$$Y_{ji} = \frac{\beta_i}{(1 + e^{\delta_i(X_{ji} - \tau_i)}}$$

Where: Y_{ji} represents the growth for apple *j* at location *i*, X_{ji} denotes the time in Julian Days, the parameter β represents the maximal growth reached by the fruit, δ represents the growth rate and τ represents time when maximum growth occurred. As these parameters all have physical meaning in the context of apples we decided to use this model. From the model it is evident that we used location-specific parameters indicating that the model allowed the parameters to be different across locations. Table 2 represents some of our findings showing significant differences across sites. To compare across sites we used a technique that we developed (Many-to-one comparisons for Apple Growth, Dasgupta and Shaffer, submitted to *Journal of Agricultural, Biological, and Environmental Statistics*). At this point we did not use weather information across sites to explain the difference; our hope is to incorporate weather information in the models with 2010 data that will make the model more relevant for DAS users. To delineate our results we attach Figure 1 which shows the growth data for the Red Delicious across all the locations and our overall fit from the non-linear model. We also overlay the LOESS non-parametric (data driven) fit on the graph to see how closely our non-linear models fit what the data represents.

We are still in the process of analyzing bloom data as we investigate how to best convey relevant information to growers. One issue with the bloom data is that our response is categorical (stage of bloom) and the data for a particular bloom is auto-correlated over time. To investigate how to deal with binary auto-correlated data we pursued some research and have submitted the paper "Many-to-one comparisons in the longitudinal Binary Data set up" co-authored by Dasgupta, Sutradhar, and Yang to *Sankhya* to address the statistical methodology for this issue. However we also did some simple analysis and Table 2 shows the most likely bloom stage over the time of data collection in Julian Days for the three cultivars.

Figure 1: Plot of Apple growth over Time with our non-linear fit and loess fit

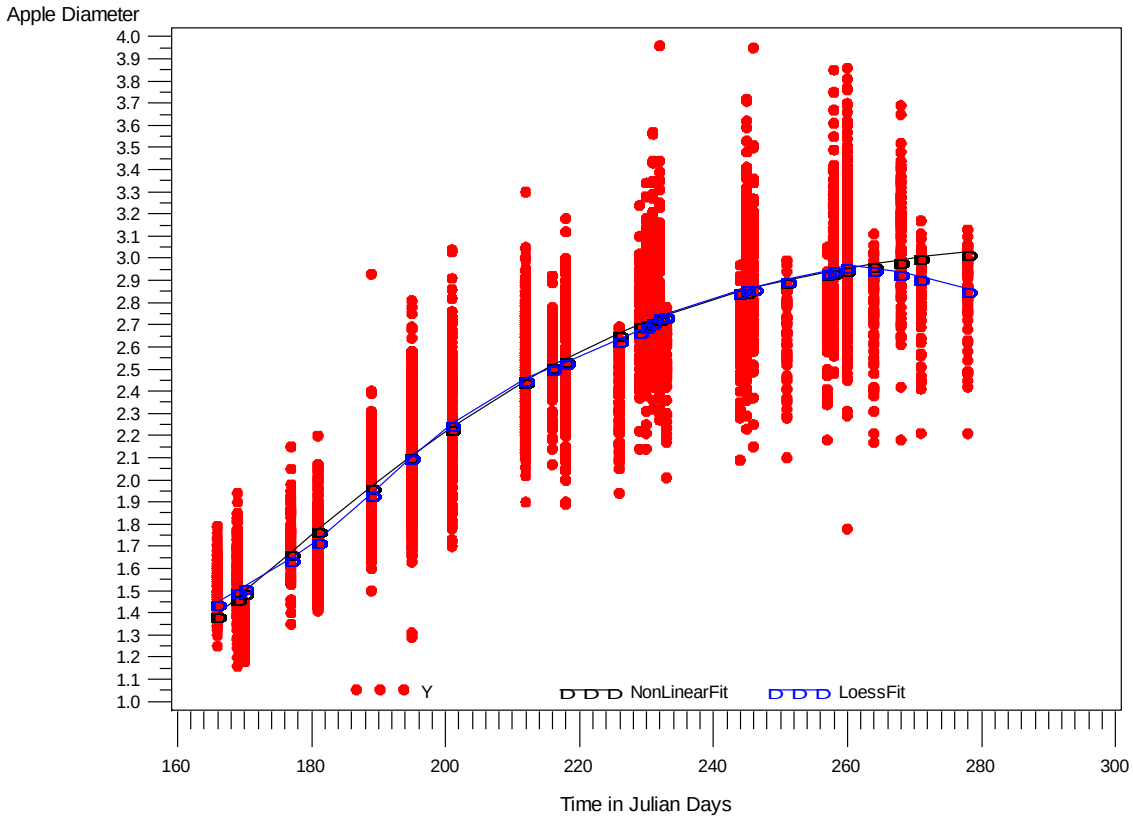


Table 2: Probable Julian dates for phenologic stages of three apple cultivars based on initial analysis of 2009 and 2010 data.

Cultivar STAGE	Red Delicious	Gala	Cripps Pink
GreenTip	72-79	72-79	72-74
½ Inch Green	81-88	81-96*	75-85*
Tight Cluster	89-96	97-104*	86-90*
First Pink	97-105*	105-106*	92-97*
Full Pink	106-107*	107-110	98-104
First Bloom	108-109	111-113	105-107
Full Bloom	109-113	111-113	108-110
Petal Fall	>113	>113	>110

The "*" indicates that some of the range is not continuous, but we report the most likely range. Results are confounded by location to location variability.

Statistical followup and future plans for analysis:

1. Fit growth models to 2010 data
2. Model bloom data with multinomial models
3. Incorporate weather information into both models

Figure 2. Sample photo taken by autonomous trail camera at WSU Sunrise research orchard. Note images of buds in foreground are too blurry to discern between tight cluster and first pink.

Figure 3. Sample photo taken by autonomous trail camera at WSU Sunrise research orchard. Note king bloom is open in two clusters on branch in foreground, but it is difficult to assess phenology of other clusters facing away from the camera.

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

Project Title: Development of pollen tube growth model for Washington State growers

PI: Dr. Keith Yoder **Co-PI(2):** Dr. Rongcai Yuan (deceased)
Organization: Virginia Tech
Telephone: (540)-869-2560, X21
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Address 2: Va. Tech AHS-AREC
City: Winchester
State//Zip: VA 22602

Cooperators: Leon Combs, Research Specialist, Virginia Tech AHS-AREC; Winchester, VA; E-mail: lecombs@vt.edu
Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA
Dr. Vincent P. Jones, Washington State University TFREC

Total Project Request: Year 1: $39,867.00 **Year 2:** $45,904 **Year 3: $47,729**

Other funding Sources: None; applying for a related SCRI grant, amount unknown

WTFRC Collaborative expenses:

Item	2009	2010	2011
Salaries		2000	3000
Benefits		600	1000
Wages			5000
Benefits			800
Shipping			100
Supplies			100
Travel		200	500
Total		$2800	**$10,500**

Footnotes:

Budget 1
Organization Name:
Virginia Polytechnic Institute
and State University (Virginia Tech)
Telephone: (540)-231-3659

Contract Administrator:
Paige Selvey, Grant Administrator

Email address: pselvey@vt.edu

Item	2009	2010	2011
Salaries*	23,900	27,980	29,213
Benefits	11,467	13,424	14,016
Supplies	750	750	750
Travel (to Wash. St. orchard sites)	3000	3000	3000
Contractual services & repairs	750	750	750
Total	$39,867	$45,904	**$47,729**

***Note:** Salary for Research Specialist Leon Combs.

OBJECTIVES:

Our overall goal for 2009-11 is to collaborate with the Washington Tree Fruit Research Commission and Washington State University Tree Fruit Research and Extension Center in the development of a computer generated pollen tube growth modeling program.

The specific objectives are:

1) Validate our prior work on pollen tube growth and thinning by conducting field studies at selected cooperating orchard sites in Washington State. Assist, if needed, with field implementation of beta testing of the modeling program with cooperating growers.

2) Repeat Washington State "in-orchard" tests conducted in 2008 to accumulate multiple-year data on commercially important cultivars. Try to collect more "normal year" data (as compared to 2008) on Red Delicious, Golden Delicious, Fuji, Gala, Honeycrisp, Jonagold, Pink Lady, Granny Smith, and Braeburn.

3) Assimilate data into development of a functional model of pollen tube growth for growers to test on selected specific apple cultivars.

4) Continue studies of pollen germination/tube growth under natural field temperature and light conditions compared to 2005-08 laboratory and field experiments, expanding studies to additional commercially important cultivars.

5) Further develop reliable laboratory techniques for the study of a wide range of constant and variable temperatures on pollen germination and tube growth.

2011 Objectives:

- Develop model parameters for Red Delicious, Pink Lady, Honeycrisp.
- Continue collaborative effort to develop computer model program and incorporate into DAS (working with Vince Jones and Ute Chambers, WSU).
- Provide continued training to recognize desirable amount of king bloom open to "start the clock".
- Expand beta field testing of models for Gala, Fuji, and Golden Delicious (3rd year).

SIGNIFICANT FINDINGS:

(What we added in 2010)

- Added to database to include effects of high temperatures (75-95°F) on pollen tube growth.
- Better understanding of actual time required for fertilization.
- Better awareness of cultivar differences in time required for fertilization.
- Refining "triggering method" for start of bloom thinning applications.
- Augmented style length database for various apple cultivars.
- Developed preliminary pollen tube growth modeling program for beta testing on Cripps Pink (Pink Lady).
- Expanded testing of pollen tube growth modeling program in Washington State sites.
- Added plantings of Jonagold, Granny Smith, and Cameo for future testing in Virginia.
- Continuing controlled growth chamber studies of pollen germination/tube growth.
- Purchased new growth chamber for expanding controlled temperature tests (Virginia Tech Equipment Trust Fund - $28,000).

METHODS:

A. Computer Modeling Program

Assisted with initial implementation by helping the model programmer with interface, and dissemination of relevant data for computer generated output programs.

B. Field Studies

Controlled pollination studies were conducted by Leon Combs in selected Washington orchards and sampled flowers were preserved in vials, refrigerated, and transported to be examined in our laboratory at Winchester using previously developed techniques. Flowers were harvested from cooperating orchards at designated times after pollination, petals removed, flower styles placed in a solution of sodium sulfite (5%) and stored at 40°F. Prior to microscopic examination, samples were boiled for 15 minutes. Pistils were dissected from the remaining flower tissue, rinsed with distilled water and squashed under a coverslip or slide and stained with 0.01% aniline blue in 0.067M K_2HPO_4. Slides were stained for 24 hours before examination at 100X under fluorescent light using a Zeiss HBO-50 high-pressure mercury vapor light source and a Nikon Optiphot microscope. Collected data include abundance of pollen germination/ tube growth on the stigma surface (0-10 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.

C. Additional Field Studies

Controlled bloom thinning tests were conducted at beta-test sites using field data model program developed from previous research project data. These studies will be used as a preliminary guide in estimating % king bloom open, pollen tube growth, and when bloom thinners should be applied according to model parameters. Data will be recorded on fruit size, yield, and quality. By actually using the program concept, growers can evaluate the program and suggest improvements or modifications that would help refine the model into a more grower-friendly tool.

RESULTS AND DISCUSSION:

Proper bloom thinning produces the largest fruit, the most return bloom and reduces biennial bearing. This 3-year study continues to apply and build on findings from our previous research project: "Temperature Effect on Pollen Germination/Tube Growth in Apple Pistils". To import our data into a predictive model to be used by Washington growers, extensive field research studies using previous experimental data are needed to validate and justify use of this program as an aid in bloom thinning of apples. Understanding the progression of pollen tube growth after pollination is critical for proper timing of bloom thinner applications. Crop loads not sufficiently thinned can result in trees being thrown into biennial bearing with little or no crop in the "off year". Our goal is to assimilate data into development of a functional model of pollen tube growth to be used on specific apple cultivars, and to continue developing the laboratory and orchard database on additional varieties/cultivars now being tested and other varieties yet to be tested.

Washington Field Testing - 2010

In 2010, we appreciated the participation and years of experience of the following key individuals as beta-testers for this research: Tom Butler (Washington Fruit & Produce Co.), Kevin Larson (Roche Fruit), Harold Ostenson (Stemilt Fruit), Darin Case (Dovex), and Tory Schmidt (Washington Tree Fruit Research Commission). With the cooperation of this pollen tube growth model beta-testing group, we conducted research studies at several locations across the Washington apple-growing region. Harold Ostenson (Stemilt Fruit), also conducted limited testing of the model during their current growing season in Chile. Field

testing of the pollen tube growth model, enables us to better analyze, evaluate, and develop this tool for apple crop load management by the industry. Practical utilization of our experimental pollen tube model was demonstrated on Imperial Gala **(Figures 1, 2, 3)**.

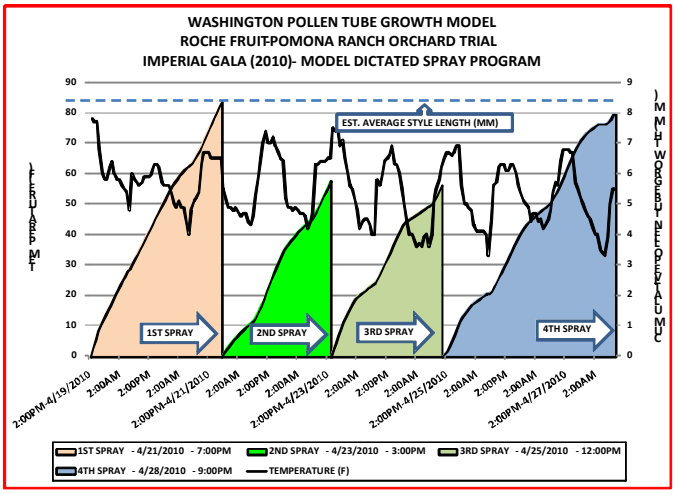

Figure 1. Shown are hourly temperatures (dark irregular line across the center) and the predicted progress of pollen tubes in Imperial Gala styles based on those temperatures, Pomona Ranch. The broken line near the top indicates mean style length (8.50 mm), at which point fertilization is assumed to occur. The arrows indicate the timing of the four lime sulfur (3%) bloom thinning sprays. The curve at the left shows the pollen tube growth after the desired % of king bloom clusters were open (April 19). The first bloom thinning application was April 21. Additional bloom thinning sprays were applied on April 23, 25, and 27. These later applications were intentionally timed to prevent set of pollinated blossoms before they were fertilized, thereby reducing the need for hand thinning. An additional petal fall spray (Sevin 3 pt/acre) was applied on May 6 (not shown on graphics).

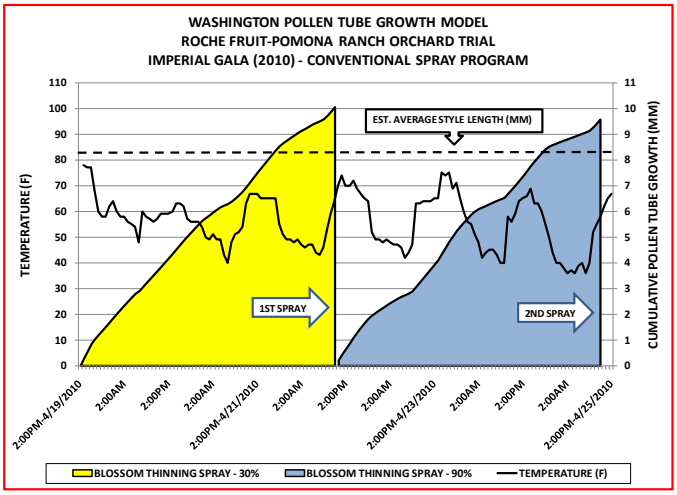

Figure 2. The conventional spray program (3% Lime Sulfur) applied at 30% bloom open (April 22) and at 90% bloom open (April 25), Pomona Ranch. A petal fall spray (Sevin, 3 pt/acre + NAA 200, 1.5oz /acre) was applied to the conventional test block and also a fruitlet thinning spray (same as petal fall spray) was applied at 12-15 mm fruit diameter. Some hand thinning was done to both test blocks, and the cost of thinning the conventional block was approximately one dollar/tree more than pollen model test block (not shown in graphics).

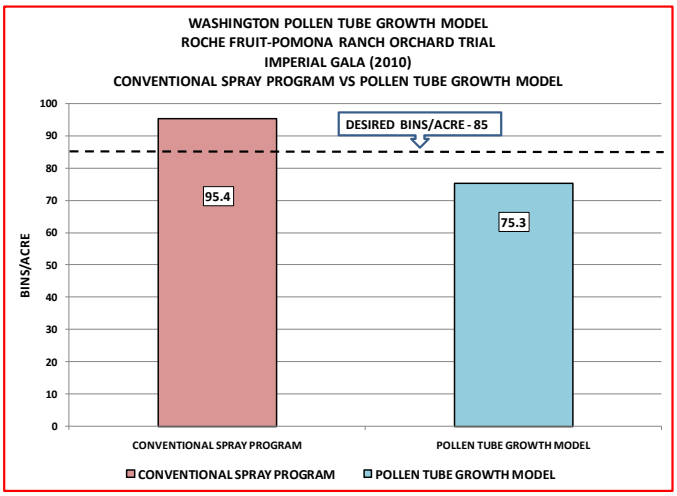

Figure 3. Pomona Ranch harvest data for the conventional block shows 95.4 bins/acre vs 75.3 bins/acre for the model test block. The desired harvest for the test blocks would have been 85 bins/acre. It will be interesting to compare return bloom in these test blocks in 2011.

Additional Field and Laboratory Tests

We are continuing to add to the cultivar database for predictive models to be used by Washington apple growers **(Figures 4, 5)**. Cultivar differences must be recognized and must be compensated for in models, and eventually we would like to see a separate model for each important cultivar.

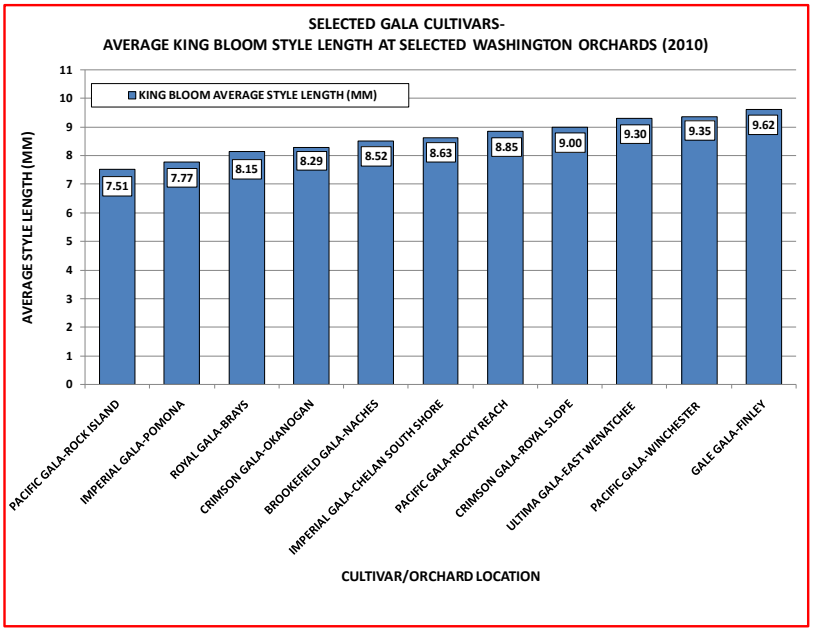

Figure 4. Gala king bloom style length samples from selected locations across the Washington apple growing region illustrate not only differences in average style length between strains, but also differences in style length of the same Gala strain or Cripps Pink (Figure 5) grown at different locations. Style length is one of the factors affecting length of time required for fertilization.

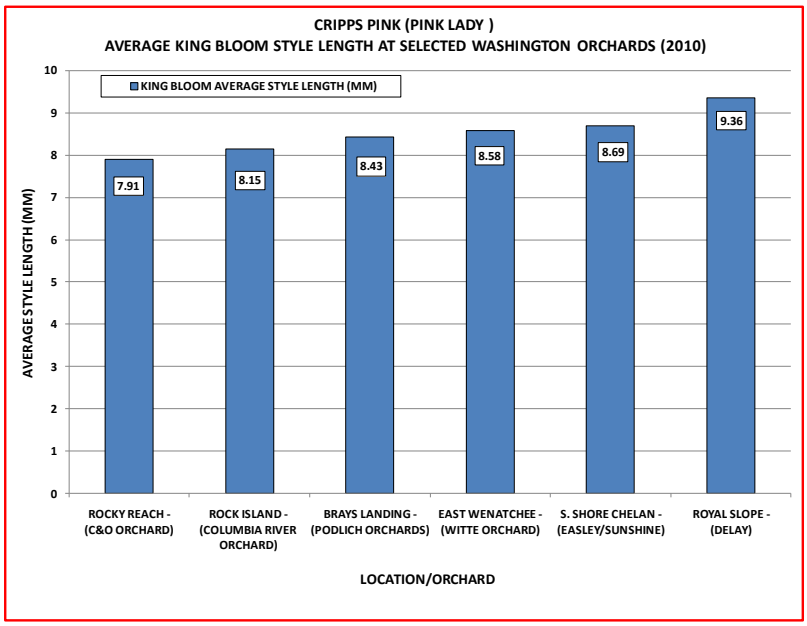

Figure 5. King bloom style lengths of Cripps Pink (Pink Lady) cultivar in selected orchards also show apparent style length differences from different locations.

Growth Chamber Studies, Virginia Tech, Winchester, VA

We expanded controlled growth chamber tests on pollen tube growth to include tests at 85°F and 95°F **(Figures 6, 7)**. This was in response to concerns voiced by beta-testers who had experienced temperatures in that range during bloom periods while testing in 2009.

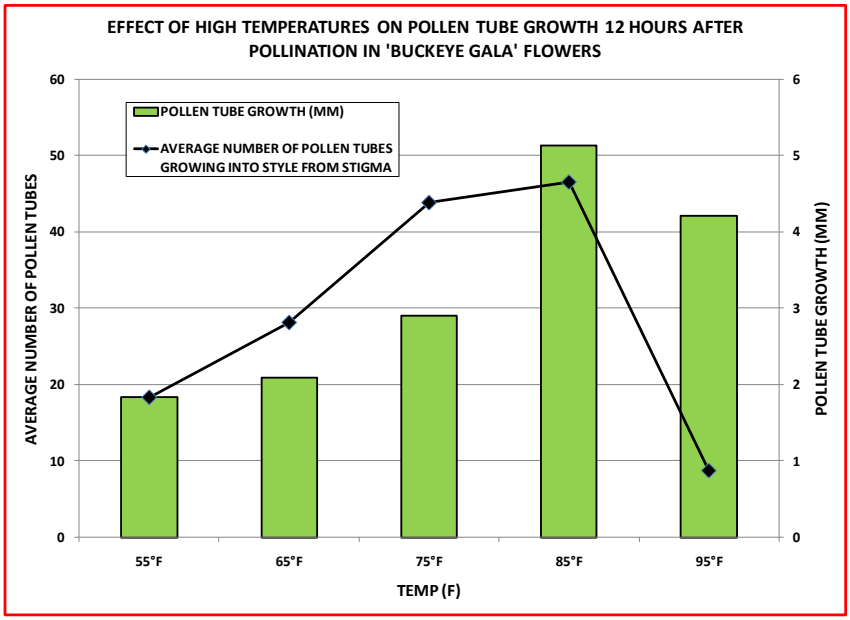

Figure 6. Average pollen tube growth and number of pollen tubes growing into Buckeye Gala styles from stigmas 12 hours after pollination. These tests show that pollen tube growth increases up to 85°F but declines at 95°F. The number of pollen tubes entering the style from the stigma at 95°F decreases significantly (approx. 80%) compared to the number entering

the style at 85°F. This could be a result of fewer pollen grains germinating on the stigma surface due to the higher temperature.

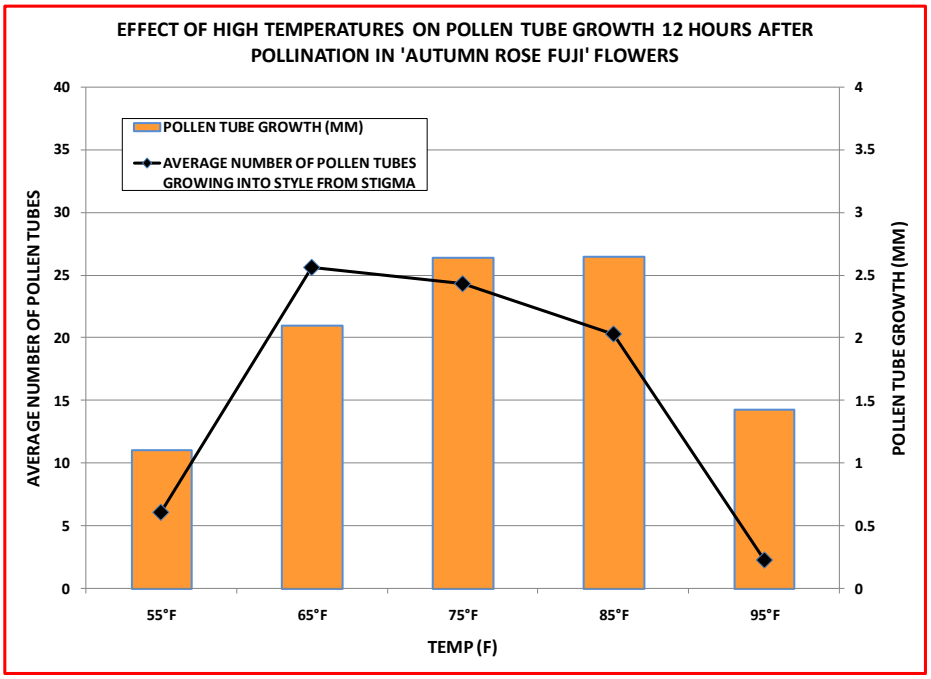

Figure 7. High temperature experiment on Autumn Rose Fuji. Unlike Gala, there is a decline in number of pollen tubes growing into the style at 85°F, and, like Gala, a greater decline at 95°F. There is very little difference in pollen tube growth rate at 75° and 85°F. The average number of pollen tubes penetrating the stigma base (approx. 3) at 95°F shows a significant reduction in the likelihood of fertilization occurring at the higher temperature. This observation is also true for the Gala test.

SUMMARY

The ideal approach for bloom thinning would be to track fertilization of the desired percent of king bloom needed to make the crop, then apply bloom thinning sprays, as needed, to prevent set of later bloom thus reducing or eliminating the need for expensive fruitlet sprays and hand-thinning. Critical needs to accomplish this goal are knowing how much time is needed to fertilize the desired king bloom of each cultivar after pollination, and then applying bloom thinners at the proper time to prevent fertilization of later unwanted bloom.

We will continue to pursue the following areas that must be addressed to enhance the use of the modeling program and make it a more useful aid in bloom thinning:

- Determining the timing of king bloom set and starting the clock toward fertilization.
- A way of measuring the percent of fruit set in the field.
- Streamline the process to determine fruit set, real time.
- Pollen and pollen tube viability.
- More work needs to be done on varietal tube growth and style length differences.

We will continue to address these and other questions as we continue to work with WTFRC, beta testers and Vince Jones and Ute Chambers, WSU, to develop a pollen tube growth computer model for integration into DAS.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-10-102

Project Title: Management of vegetative growth in apple trees with bioregulators

PI: Donald C. Elfving
Organization: Tree Fruit Research & Extension Center
Telephone: 509-663-8181 ext. 252
Email: delfving@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: T.R. Schmidt, WTFRC, Wenatchee; T.D. Auvil, WTFRC, Wenatchee; Dr. T. Einhorn, MCAREC, Oregon State University.

Total Project Request: Year 1: 4,070 **Year 2: 5,250**

Other funding sources:

Agency Name: Valent BioSciences, Agro-K
Amt. awarded: (will partially defray harvest costs)
Notes:

WTFRC Collaborative expenses:

Item	2010	2011
Stemilt RCA room rental	0	0
Salaries	1,000	1,000
Benefits	300	300
Crew labor	600	700
Shipping	0	0
Supplies	0	0
Travel	100	100
Miscellaneous	0	0
Total	**2,000**	**2,100**

Budget 1
Organization Name: WSU-TFREC Contract Administrator: ML Bricker/K. Larson
Telephone: 335-7667 / 663-8181 **Email address:** **mdesros@wsu.edu /kevin_larson@wsu.edu**

Item	2010	**2011**
Salaries[1]	-0-	-0-
Wages[2]	2,500	3,000
Benefits[2]	370	450
Equipment	-0-	-0-
Supplies[3]	200	300
Travel[4]	1,000	1,500
Miscellaneous	-0-	-0-
Total	4,070	**5,250**

[1] No technical help indicated since Technician position no longer exists. Time-slip help is absolutely essential to collect the volume of data needed to set up trials and evaluate growth responses to the various bioregulator applications involved.

[2] Time-slip help substitutes for unfilled Technician position. Time-slip benefit rate is calculated at 15% for 2011.

[3] This category includes miscellaneous supplies, non-capital equipment, consumables, repairs, etc. that are needed to carry out the research project. Cell phone charges are allowed under this grant.

[4] Treatment application and data collection at distant sites, all off-station. Includes vehicle lease-to-purchase, operating, repair costs.

Objectives:

1. Explore possible methods for improving the efficacy of prohexadione-Ca (Apogee) for control of unwanted vegetative vigor, including application timing, concentration and combinations of Apogee with abscisic acid (ABA).
2. Examine whether ABA, the dormancy-inducing hormone, can be used either alone or in combination with other bioregulators to force terminal bud set or otherwise control extension growth in actively-growing apple shoots.
3. Determine if any successful growth-control treatments compromise fruit load, fruit growth or fruit quality in any way.

Significant findings:

1. ABA (100 mg/liter a.i.) applied all season (six applications over 4 months beginning on 23 April) had no effect on shoot growth at all. ABA applied in a similar manner at 500 mg/liter a.i. paralleled the control shoot expansion until warm weather started in June, after which the shoot elongation rate was reduced by ABA such that the ABA 500 trees showed a reduction of about 26% in terminal shoot extension over the last half of the season (not statistically significant).
2. The full label rate of Apogee was applied in two sprays 22 days apart in late April and again in mid-May (total of 99 oz. Apogee/acre). Shoot growth was strongly inhibited until the first week of July, when regrowth began that brought final terminal shoot extension on trees receiving Apogee only to within 87% of the growth of untreated trees (not statistically significant).
3. Apogee-treated trees received one of several follow-up applications of either abscisic acid (ABA) or a chemically similar analog of ABA to assess if any combination of Apogee plus follow-up treatments could help control the persistent problem of late summer regrowth observed even when full label rates of Apogee are used.
4. The three combination treatments that resulted in a significant reduction in total shoot extension regrowth were the following: Apogee applied twice as described above followed by 1) ABA (100 mg/liter a.i.) applied once (1 June), or 2) ABA (500 mg/liter a.i.) applied once (1 June), or 3) ABA 500 applied four times, on 1 June, 29 June, 27 July and 24 August.
5. Although the analog is estimated to be 10-fold more active than ABA itself, treating previously Apogee-treated trees with the analog (50 ppm) once or 10 ppm monthly did not significantly reduce terminal shoot extension growth.

Methods:

Three trials were initiated in 2010 to examine effects of cytokinins vs. gibberellins along with scoring vs. surfactant treatments on branch induction on two-year-old wood. Two additional trials were initiated to examine in greater detail the potential for surfactants to substitute for scoring or nicking cuts in one-year-old wood in stimulating lateral branch development. The trials focused on whether surfactants could substitute for cutting the bark on two-year-old wood for encouraging penetration of bioregulators into active tissues, whether GA alone could induce branching on two-year-old wood as has been demonstrated for such treatments on one-year-old wood, and whether the distance between scores or banded bioregulator treatments on two-year-old wood had any beneficial effect on branch induction.

Results and discussion:

One goal of the 2010 program was to determine whether gibberellic acid (GA) alone can induce lateral branching in two-year-old wood of sweet cherry. Previous research has clearly shown that GA alone is about as effective as cytokinin for branch induction in one-year-old wood. One advantage this finding confers is that GA products are OMRI-approved, and thus can be used in organic orchards. They are also a bit cheaper than Promalin. Unfortunately, this year's test of GA vs. cytokinins on two-year-old wood failed due to cold weather injury that no clear conclusions can be drawn, and we need to do this trial again next year, hoping for no fall cold damage in 2011.

In several of the 2010 trials, comparisons of surfactant concentrations vs. using scoring cuts to improve bioregulator penetration were undertaken. Despite some cold damage effects in these trials, it was clear that when we applied Promalin to scoring cuts, branching was improved to some extent. These results showed that if there were live buds present on two-year-old wood and that wood had not been killed outright by last fall's cold event, those living buds could be activated if the Promalin could penetrate into active tissues. Results of the two trials with one-year-old wood confirmed this observation.

Few of the surfactant-supplemented treatments showed significant branching activity on either two-year-old or one-year-old wood, but again, the degree to which these observations might have been affected by vascular or bud cold injury is unclear. In the case of the one-year-old wood, killing the terminal portion of those shoots altered the apical dominance situation by producing the equivalent of a heading-back cut. This physiological change resulted in a certain amount of increased branching, thus limiting the degree to which additional branching could be induced by the bioregulator applications themselves.

The question comes up from time to time as to how close scoring cuts should be made to ensure a maximum branching response. In one-year-old wood, we found several years ago that scoring cuts closer than 30 cm (12 Inches) did not increase branching at all. In 2010, one of the experiments was designed to compare several Promalin/surfactant combinations and Promalin/scoring cuts with making scoring cuts or banding bioregulators at 15 cm (6 inches) vs. 30 cm (12 inches) intervals on two-year-old wood. Distance between scores or bands made absolutely no difference in terms of branching on older wood, just as was previously observed on one-year-old wood. Applying treatments every foot (30 cm) along a branch section appears to be quite adequate for branch induction, regardless of the age of the treated wood.

Acknowledgements:
The assistance and support of the following people and organizations is gratefully acknowledged: Tom Auvil, Felipe Castillo, Dr. Greg Clarke, Ken Dart, Tom Gausman, Jeff Henry, Dr. Peter Petracek, Tory Schmidt, Richard Scranton, Agro-K, Valent BioSciences, Washington Tree Fruit Research Commission and the WSU Agricultural Research Center.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

Project Title: Greater system efficiency and fruit quality via soil microbiology

PI:	Mark Mazzola	**Co-PI (2):**	James Mattheis
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Address:	1104 N. Western Ave	**Address:**	1104 N. Western Ave
City:	Wenatchee	**City:**	Wenatchee
State/Zip	WA 98801	**State/Zip:**	WA 98801

Total Project Request: $208,547 **Year 1:** 66,927 **Year 2: 69,830** **Year 3:** 71,790

Other funding sources

Agency Name: National Institute of Food & Agriculture, Agriculture and Food Research Initiative • Foundational Program (Microbial Communities in Soil Panel)
Amt. requested: $490,862
Notes: pending

Agency Name: National Institute of Food & Agriculture, Agriculture and Food Research Initiative •(Oomycete panel)
Amt. requested: $369,343
Notes: pending

WTFRC Collaborative expenses: None

Budget 1
Organization Name: **Contract Administrator:**
Telephone: **Email address:**

Item	2010	2011	2012
Salaries[a]	47,690	49,120	50,594
Benefits	15,737	16,210	16,696
Wages			
Benefits			
Equipment			
Supplies	3,500	4,500	4,500
Travel			
Miscellaneous			
Total	$66,927	**$69,830**	$71,790

Footnotes: [a]Funding is requested to support a postdoctoral research associate.

OBJECTIVES:

The current research program directly addresses the 2010 apple research priority indicating the need to develop a "quantitative systems approach to soil ecology and biology with verifiable results in terms of high production of highest quality fruit with application in both organic and conventional cropping systems". The specific objectives of this program are to:

1. Assess the rooting behavior of apple as affected by different resource inputs (e.g. mineral fertilizer, compost, seed meal) and determine the relative contribution of soil biology and input to root development.

It is known that root production can be influenced by orchard management practice, with the lowest rate of root development observed in systems maintaining a weed free herbicide strip (Atkinson, 1983). *Development of a healthy and functional root system is the central factor determining the effective and efficient use of nutrient and water inputs in plant production systems.* As various inputs display a negative effect on soil biology, a determination of factors directing root proliferation (amendment or biology) will be instrumental in optimizing this process.

2. Examine the effect of fertility management options on the dynamics of nematode and protozoan communities in the apple rhizosphere through application of DNA-based studies including T-RFLP and real-time quantitative PCR.

Soil fauna have demonstrable effects on plant root development. While numerous studies have examined the effect of fertilizers on bacterial community size and function (Hallin et al., 2009), few have assessed these same effects on the soil fauna. Synthetic fertilizers commonly depress soil microbial diversity and activity. Development of treatment strategies to augment rather than suppress the soil fauna populations will contribute positively to tree productivity both directly through biotic mechanisms and indirectly via mineralized nitrogen production.

3. Quantify the key genes driving microbial nitrogen cycling in the apple rhizosphere under different resource input programs and link to the efficiency of use in the orchard ecosystem.

Sustainable management of fertility inputs should seek to obtain a highly efficient turnover of minerals, with particular emphasis on minimizing nitrogen loss due to leaching or release of gaseous N through denitrification. In order to better define the multiple transformation events within the nitrogen cycle, we plan to test the effects of different fertility amendments in orchard soils and to determine the specific microbial populations and processes involved.

4. Determine the effect of altered soil biology on fruit quality characteristics including ripening; coloring; and long term storage quality.

Fertility inputs significantly influence fruit quality through its effect on fruit ripening and capacity to retain desired eating characteristics under long-term storage. Such differences have been linked to differences in soil fertility, which particularly in organic systems is regulated by orchard soil biology. The effect of different fertility programs on the resident soil biology will be determined using DNA fingerprinting techniques (T-RFLP) and linked to the resulting effects on overall fruit quality.

SIGNIFICANT FINDINGS:

- In a sandy texture orchard soil, root development of M9 rootstock was enhanced or depressed in a nitrogen amendment-dependent manner

- In this same orchard soil it was apparent that the effect of nitrogen amendments on M9 lateral root development were indirect, being determined by the orchard soil biology

- Isolates of *Streptomyces* recovered from the rhizosphere of M9 apple rootstock enhanced root and overall plant development

- The type of nitrogen amendment significantly altered abundance of microbial genes involved in N cycling, and thus could result in altered retention or loss of N from orchard soils

GOALS & ACTIVITIES

During year two of this project a primary objective will be to determine whether the amendment-based but biology driven manner modulation of M9 rootstock root development, as observed in WSU-Sunrise orchard soil, is a general phenomenon across orchard soils. Should this be demonstrated, further characterization of the functional biology responsible for enhanced or suppressed root development will be conducted with the goal of a prescriptive basis for use of inputs to enhance root proliferation. Should funds become available (see pending proposals above), a high throughput DNA sequencing approach will be used to address this objective. Secondly, studies will continue to examine the effect of fertility management options on retention or loss of nitrogen from orchard soils with the goal of reducing microbial driven nitrogen loss through input management. Thirdly, a substantial population of *Streptomyces* isolates from the rhizosphere of M9 rootstock will be evaluated for the capacity to produce nitric oxide and assays will be conducted to determine if this quality determines the ability of these bacteria to enhance apple development.

METHODS:

Studies conducted in WSU-Sunrise Orchard soil (sandy texture; 1.2% organic matter[OM]) during 2010 will be repeated in two additional orchard soils of varying texture and OM content (sandy loam, 4.2% OM; gravelly sandy loam, 3.3% OM). Amendment type and rate will be as in the 2010 studies described in the results and discussion below. Soils will be collected and one-half of each sample will be heat-pasteurized for 90 min at 70^{o}C on two consecutive days to depress native populations of the resident soil biota. Nitrogen amendments will be applied to achieve a rate of 70 kg N ha^{-1} and treated soils will be incubated for two weeks prior to planting with M9 rootstock. Apple rootstocks will be grown for six to nine months in native and pasteurized soil with or without application of soil amendments. At harvest, root systems will be evaluated to assess the effect of soil treatments on root architecture. For each treatment, half of the plant root samples with adhering soil will be used for microbial analyses should data indicate a need for such assessments. Microbial populations will be characterized using both culture-based and molecular methods, including real-time quantitative polymerase chain reaction and T-RFLP analysis.

These same soils will be used to monitor the effect of different fertilizer regimens on multiple processes within the nitrogen cycle. Root samples with adhering rhizosphere soil will be collected at monthly intervals. Rhizosphere soil will be collected from roots and ammonium (NH^{+}_{4}) and nitrate (NO^{-}_{3}) concentrations will be determined. Bacterial genes encoding the nitrogenase reductase (*nifH*; process nitrogen fixation), ammonia monooxygenase (*amoA*; process nitrification), nitrite reductase

(*nirK* and *nirS*; involved in the process of denitrification) as well as the archaeal *amoA* genes will be quantified by real-time PCR and linked to the soil property and emission data for the denitrification intermediate, nitric oxide (NO). Quantification of the investigated genes will be conducted on an ABI StepOne Plus real time PCR system.

Streptomyces will be recovered from the rhizosphere of M9 apple rootstocks and isolates will be analyzed for the production of nitric oxide using a quantification of NO production will be conducted using the Sievers NOA 280i nitric oxide analyzer. Isolates will be grouped by NO production level and used in plant assays to evaluate the role of NO in the capacity of *Streptomyces* isolates to induce root development in apple. M9 rootstock will be planted into sterile soils treated with a single *Streptomyces* isolate with five replicates per isolate. Rootstocks will be grown for six months and root biomass and root architecture will be assessed at harvest.

RESULTS & DISCUSSION:

A sandy, low organic matter content (1.2%) orchard soil (WSU-Sunrise orchard) was fumigated by a commercial operator with 1,3-dichloropropene-chloropicrin (Telone-C17) in October 2009 and soil was collected from fumigated plots in March 2010. Soils were treated with urea or canola (*Brassica napus*) seed meal to attain equivalent nitrogen rates (70 pounds per acre) and were applied with or without compost (3 ton per acre). In this sandy orchard soil, urea nitrogen application had a negative effect on overall lateral root development for the rootstock M9 (**Fig. 1**). Urea application significantly reduced lateral root formation relative to all other soil treatments including the no treatment control soil. Application of compost in concert with urea eliminated the negative effect on root development observed in soils treated with urea alone, but lateral root formation was not improved relative to the control. All other soil treatments significantly increased M9 lateral root formation with average lateral root numbers highest in soils treated with compost or seed meal alone, and no additional benefit was observed when these amendments were applied in combination. Enhanced root formation has the potential to encourage tree development in newly established orchards and to improve use efficiency of fertility inputs.

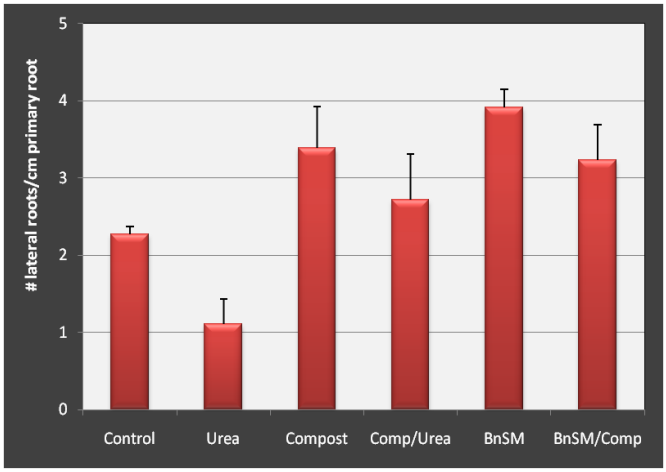

Figure 1. Effect of amendments on M9 rootstock lateral root development when trees were grown in a Telone-C17 fumigated sandy orchard (WSU-Sunrise) soil. BnSM=canola seed meal; Comp=compost

Interestingly, in this initial experiment, the results described above were only observed in the native fumigated orchard soil. That is to say, when studies were conducted in this same fumigated orchard soil but which had been pasteurized immediately prior to planting M9 rootstock, no significant

differences, either positive or negative, were observed among soil treatments. Urea application did not depress root formation and seed meal or compost amendment did not enhance root development. These findings strongly suggest that resident soil biology was functional in the observed amendment-derived effects on rootstock root development in this particular orchard soil and that the responses were not stimulated directly by the amendment. The question still remains as to whether this response is a general phenomenon across orchard soils, or whether particular soil attributes such as organic matter content or soil texture will influence root development in response to these treatments. The identification of materials that differentially enhance or diminish microbe-directed root development will be extremely useful in conducting studies to garner a more complete understanding of the soil biology that ultimately affects rootstock lateral root proliferation. Thus, should funds become available (see pending proposals above) comparative analysis of the rhizosphere microbial populations will be conducted using high-throughput sequencing methods.

Studies were conducted in this same orchard soil to monitor the presence of specific microbial genes that function in the cycling of nitrogen. The goal is to gain an understanding of the active microbial populations responsible for nitrogen cycling in orchard soil, the effect of different fertility inputs on the abundance of these microbial groups, to relate the comparative abundance of these populations to potential loss of nitrogen from orchard soils and to identify inputs or methods that could minimize such loss. All soil treatments increased the abundance of the bacterial *nirK* gene in soil relative to the non-treated control soil (**Fig. 2**). However, significant differences in abundance of this gene were detected among soil treatments. *nirK* abundance was highest in soils treated with urea, and significantly lower in soils amended with canola seed meal. Addition of compost in concert with either nitrogen input resulted in a significant decrease in *nirK* abundance. Suppression of *nirK* abundance by compost treatment may be of significance as *nirK* encodes an important step in the denitrification process leading to loss of nitrogen from orchard soils through volatilization. These data suggest that orchard management practices, such as an increase in soil organic matter content, could demonstrably reduce the loss of nitrogen from this orchard soil system.

Soil amendments had significant effects on the abundance of ammonia monooxygenase gene (*amoA*) detected in the rhizosphere of M9 rootstock. This gene encodes the enzyme involved in the first step in conversion of ammonia to nitrate. Interestingly, amendments utilized in this study only influenced the level of the bacterial *amoA* gene (**Fig. 3**) and not the archaeal *amoA* detected in the rhizosphere of M9 rootstock in the study orchard soil. These data indicate that bacteria are the primary contributors to the process of nitrogen mineralization in this particular orchard soil system.

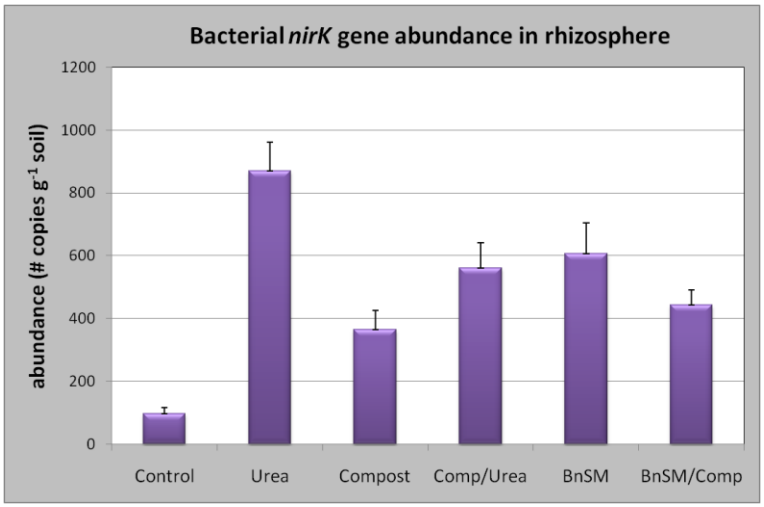

Figure 2. Effect of soil amendments on relative abundance of the bacterial gene *nirK* in the rhizosphere of M9 rootstock when trees were grown in a Telone-C17 fumigated sandy orchard (WSU-Sunrise) soil. The bacterial gene *nirK* functions in the process of nitrate reduction resulting in the loss of nitrogen from soil through volatilization. BnSM=canola seed meal; Comp=compost

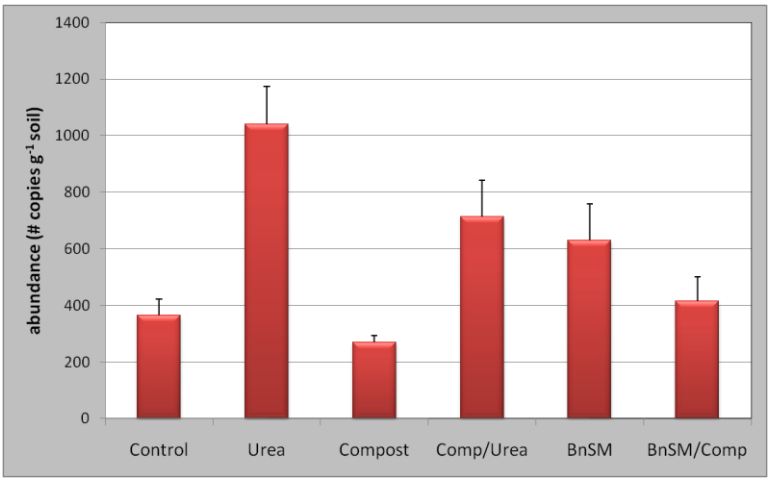

Figure 3. Relative abundance of bacterial ammonium monooxygenase gene detected in the rhizosphere of M9 apple rootstock grown in a Telone-C17 fumigated sandy orchard (WSU-Sunrise) soil. BnSM=canola seed meal; Comp=compost

As indicated above, when assayed in Telone-C17 fumigated orchard soil that was pasteurized immediately prior to planting M9 rootstock lateral root development was not modified by any of the test amendments. However, when any one of four specific isolates of the bacterium *Streptomyces* were added to the sterile soil, apple root development was dramatically enhanced when plants were initiated from seed (**Fig. 4**).

Figure 4. Effect of *Streptomyces* isolates on root development of apple seedlings grown from seed in a **sterile** soil system.

Interestingly, in the same soil system when 8-week-old seedlings were planted into soils treated with the individual *Streptomyces* isolates, only those that produced the gas nitric oxide (also an

intermediate in the nitrogen cycle) were able to enhance growth of apple in the sterile orchard soil (**Fig. 5**). Current year studies will evaluate whether this response is directly associated with nitric oxide production and whether a similar response can be realized when using M9 rootstock as the test plant material.

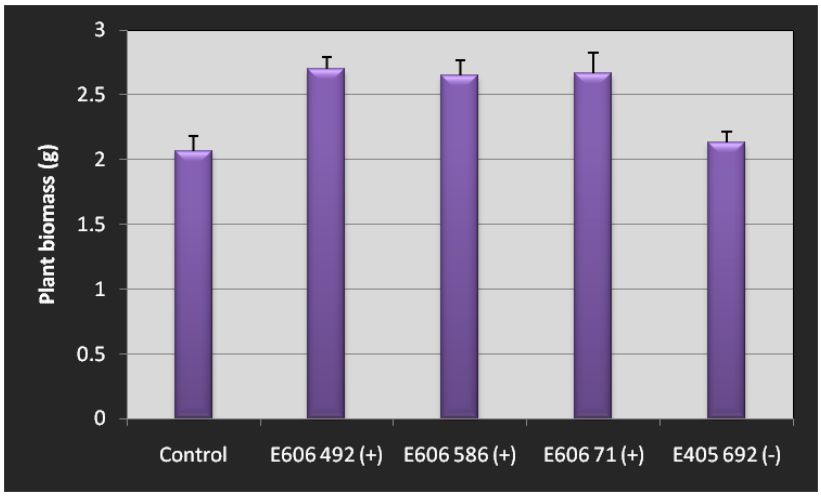

Figure 5. Effect of *Streptomyces* sp. isolates on growth of apple planted in a pasteurized Telone-C17 fumigated orchard soil . (+) indicates the isolate produces nitric oxide; (-) indicates the isolate does not produce nitric oxide.

CONTINUING PROJECT REPORT **YEAR**: **1 of 2**

Project Title: Genetic controls of apple fruit-specific auxin metabolism

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Co-PI: Kate Evans
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City/State/Zip: Wenatchee, WA 98801

Total Project Request: **Year 1:** $65,000 **Year 2: $66,000**

Other funding sources: None

WTFRC Collaborative expenses: None

Budget:

Contract Administrator: Charles Myers, Extramural Agreements Specialist		
Email:cwmyers@pw.ars.usda.gov		
Item	**2010**	**2011**
Salaries *	35,000	36,000
Benefits	14,000	14,000
Wages		
Benefits		
Equipment		
Supplies	15,000	15,000
Travel		
Miscellaneous	1,000	1,000
Total	$65,000	**$66,000**

*The **salaries and benefits** are budgeted for a postdoc dedicated to this project.
The **supplies** include reagents for molecular study, software and fruits from commercial orchards.

OBJECTIVES:

1. Elucidate roles for previously identified candidate genes in ethylene, auxin, gibberellin, jasmonate and brassinosteroid metabolism and response during apple fruit ripening.

2. Characterize the relationship between gene expression patterns and specific fruit ripening phenotypes (ripening season, fruit size, fruit texture) in a cross population of 'Honeycrisp' x 'Cripps Pink', as well as other germplasm.

3. Develop a shortlist of candidate genes for hormone metabolism for further validation for use in marker assisted selection.

METHODS:

1. Phenotyping fruit ripening and quality for 'Honeycrisp' (HC) x 'Cripps Pink' (CP) population: Characterize the phenotypes of a non-selected population of 170 siblings from HC X CP cross for their fruit ripening season and fruit quality attributes including fruit firmness, crispness, and fruit size. Phenotypic data and fruit tissues have been collected for at least 3 time-points around physiological maturity (arbitrarily set at starch level of 3.5). The two parents 'Cripps Pink' (or Pink Lady) and "Honeycrisp", have unique and distinct ripening behaviors (ripening season) and (texture attributes), and they were subjected to a parallel comparative transcriptome analysis. Within HC x CP population, a wide-spectrum of phenotype variations are observed for ripening season, fruit texture, fruit size among siblings.

2. Quantitative gene expression analysis: Selections from HC x CP population with distinct phenotypes will be categorized and utilized for gene expression pattern studies. Sequence analysis and gene-specific primer design will be conducted, then gene expression patterns for the selected 20-25 previously identified candidate genes will be characterized using Quantitative Reverse Transcription Real-Time PCR (Q-RTPCR). Selections with defined phenotype from this cross population will be analyzed against the gene expression patterns of selected candidate genes for "associated segregation" between specific phenotypes and gene expression patterns.

SIGNIFICANT FINDINGS

Gene-trait association analysis (associated segregation) among eleven candidate genes/phenotype combinations examined suggested that the expression levels of several candidate genes between specific paired phenotypes.

1. **Genes potentially associated with fruit ripening season**: ACS3 (a pre-climacteric ethylene biosynthesis gene), JOM (jasmonate O-methyl transferase) and AIP (an auxin induced protein) showed strong correlation with fruit ripening season.

2. **Genes potentially associated with fruit firmness**: The expression patterns of an AUTR (auxin transporter) encoding gene showed good correlation with fruit firmness.

3. **Genes potentially associated with fruit size**: The expression pattern of a JOM (jasmonate O-methyl transferase) gene showed good correlation with fruit size.

RESULTS AND DISCUSSION

1. Phenotyping fruit ripening and quality traits within a HC x CP cross population

Fruit ripening date, fruit firmness, fruit size were phenotyped based on weekly maturity data for all fruiting trees within HC x CP cross population. Phenotypic data among individuals were categorized based on the values of ripening date, fruit firmness and fruit size at physiological maturity (based on starch level at an average of 3.5). Fruits from about 12 individual trees in each phenotypic group (early or late-ripening; firm or soft fruit; small or large fruit) were selected for gene expression analysis.

A. Individuals sorted by early or late ripening phenotypes

Early-ripening group			Starch values at harvest date		
Individual	Starch	Ethylene	8/26	9/2	9/9
157	3.67	0.00	**3.67**		
97	3.83	31.83	**3.83**		
140	3.00	1.97		**3.00**	
56	3.33	4.20		**3.33**	
139	3.33	5.61		**3.33**	
162	4.17	0.03		**4.17**	
23	4.50	1.23		**4.50**	
124	3.17	0.28			**3.17**
123	3.50	5.27			**3.50**
14	3.67	0.00			**3.67**
136	4.00	1.07			**4.00**
142	4.67	0.10			**4.67**
Late ripening group			Starch values at harvest date		
			10/15		
70	2.67	n/a	**2.67**		
85	2.83	n/a	**2.83**		-
74	3.00	n/a	**3.00**		
171	3.17	n/a	**3.17**		
3	3.33	0.22	**3.33**		
46	3.33	n/a	**3.33**		
137	3.33	n/a	**3.33**		
84	3.50	n/a	**3.50**		
86	3.50	n/a	**3.50**		
168	3.50	n/a	**3.50**		
41	3.67	n/a	**3.67**		
173	2.17	0.01	**2.17**		

B. Individuals sorted by soft or firm phenotypes

Soft group				Starch values at harvest date						
Individual	Starch	Ethylene	Firmness (lb)	9/2	9/9	9/16	9/23	9/30	10/7	10/14
174	3.50	0.07	**10.73**			3.50				
13	3.33	0.13	**10.91**						3.33	
138	2.17	0.52	**12.03**		2.17					
86	3.50	n/a	**12.09**							3.50
121	2.67	0.00	**13.32**					2.67		
70	2.67	n/a	**13.59**							2.67
159	3.17	0.97	**13.59**			3.17				
85	2.83	n/a	**13.65**							2.83
47	3.67	0.20	**14.61**			3.67				
133	3.33	0.03	**14.68**						3.33	
56	3.33	4.20	**14.72**	3.33						
46	3.33		**14.90**							3.33
17	4.33	0.00	**12.15**			4.33				
41	3.67	n/a	**14.82**							3.67
98	3.50	0.08	**14.86**				3.50			
Firm group				Starch values at harvest date						
					9/9	9/16	9/23	9/30	10/7	
5	4.33	n/a	**18.23**				4.33			
14	3.67	0.00	**18.25**		3.67					
104	4.00	0.06	**18.52**				4.00			
88	3.50	0.00	**19.87**					3.50		
18	4.00	0.00	**20.79**			4.00				
122	3.50	0.30	**21.09**						3.50	
89	3.67	0.00	**21.19**			3.67				
129	4.50	0.00	**21.24**				4.50			
37	3.83	0.33	**21.55**						3.83	
92	3.50	0.07	**21.64**					3.50		
115	3.67	0.00	**22.11**			3.67				
60	3.50	0.44	**22.99**			3.50				

C. Individuals sorted by fruit diameters

Early-ripening group			Starch values at harvest date		
Individual	Starch	Ethylene	8/26	9/2	9/9
157	3.67	0.00	**3.67**		
97	3.83	31.83	**3.83**		
140	3.00	1.97		**3.00**	
56	3.33	4.20		**3.33**	
139	3.33	5.61		**3.33**	
162	4.17	0.03		**4.17**	
23	4.50	1.23		**4.50**	
124	3.17	0.28			**3.17**
123	3.50	5.27			**3.50**
14	3.67	0.00			**3.67**
136	4.00	1.07			**4.00**
142	4.67	0.10			**4.67**
Late ripening group			Starch values at harvest date		
			10/15		
70	2.67	n/a	**2.67**		
85	2.83	n/a	**2.83**		
74	3.00	n/a	**3.00**		
171	3.17	n/a	**3.17**		
3	3.33	0.22	**3.33**		
46	3.33		**3.33**		
137	3.33	n/a	**3.33**		
84	3.50	n/a	**3.50**		
86	3.50	n/a	**3.50**		
168	3.50	n/a	**3.50**		
41	3.67	n/a	**3.67**		
173	2.17	0.01	**2.17**		

2. Preliminary test on the correlation between the transcript levels of identified candidate genes and sorted fruit ripening phenotypes

	Early	Late	P value
ACS3	20.8 ± 3.5	28.7 ± 3.3	3.20E-05
JOM	25.6 ± 2.3	31.9 ± 2.3	3.80E-06
AIP	24.3 ± 1.2	28.3 ± 1.5	1.70E-06
AUTR	24.5 ± 1.1	22.0 ± 1.2	4.80E-05
	Soft	Firm	
JOM	25.6 ± 2.8	28.8 ± 3.5	0.0467
AUTR	23.0 ± 0.9	24.8 ± 1.2	0.0023
XTH7	24.4 ± 2.4	26.2 ± 2.8	0.1092
BRIP	26.1 ± 3.0	28.4 ± 3.7	0.1495
AQUAPIP	29.3 ± 3.4	29.4 ± 2.1	0.9505
	Small	Large	
JOM	31.2 ± 2.3	27.9 ± 2.5	0.003
AUTR	23.9 ± 1.1	24.2 ± 1.3	0.4477

Values in 2nd and 3rd column are the average and standard deviation of normalized Ct values for tested candidate genes, which represent the relative abundance of transcripts or the expression level. Each value is the average based on the gene expression data from the fruits of 10-12 individual trees within the same phenotypic group. Fruit cortex tissues of 3 apples were pooled for RNA isolation and qPCR were carried out in triplicate. Anova analysis were performed between the paired groups, and $P<0.01$ would normally be considered significant and $P<0.001$ highly significant.

A. For sorted group with early- or late-ripening phenotypes:

Among four candidate genes tested, all of them, i.e. ACS3 (a pre-climacteric ethylene biosynthesis gene), JOM (a jasmonate O-methyl transferase encoding gene), AIP (an auxin induced protein) and AUTR (an auxin transporter component), showed strong correlation with apple fruit ripening dates. In other words the differences of expression levels of these genes between phenotypic groups are highly significant.

B. For sorted groups with soft or firm fruits

Among five candidate genes tested, only AUTR (an auxin transporter component) showed significant difference in expression levels between the two groups.

C. For sorted groups of small and large fruit size

JOM (a jasmonate O-methyl transferase encoding gene) showed significant difference of gene expression levels between the phenotypes of fruit size.

3. Future directions

Similar test will be run for a few more selected candidate genes; and the expression patterns of selected candidate genes will also be examined among several other cultivars and advanced selections in current breeding program. Such extended analysis would better define the roles of these candidate genes for their specific role regulating apple ripening and quality.

4. What this study means to tree fruit industry?

Plant hormonal regulation is a major factor on fruit ripening and quality. Elucidating the specific mechanism of how plant hormone interactions contribute to cultivar-specific fruit quality and maturation/ripening patterns would be the first step in developing breeding tools, such as gene-specific functional markers, for improved precision and efficiency in apple breeding. From current analysis, several candidate genes based on our previous study showed strong association with apple fruit ripening season. These results could be utilized to develop tools for predicting fruit ripening patterns. Furthermore, understanding the molecular bases of plant hormone regulations in apple fruit could also potentially generate innovative technology to manage ripening and quality.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

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

PI:	Carolyn Ross	**Co-PI (2):**	Kate Evans
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Total Project Request: **Year 1:** 27,895 **Year 2:** $**28,851** **Year 3:** $29,846

Other funding sources None

WTFRC Collaborative expenses: None

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

Item	**2010**	**2011**	**2012**
Salaries[1]	22,014	**22,895**	23,811
Benefits[2]	1,881	**1,956**	2,035
Wages			
Benefits			
Supplies[3]	4,000	**4,000**	4,000
Travel			
Total	$27,895	**$28,851**	$29,846

Footnotes:
1 Salaries: One MS graduate student will be supported by this research (9 month salary)
2 Benefits: includes health insurance and medical aid
3 Supplies: includes chemical reagents, sensory panels supplies (consumables consisting of paper plates, towels, cuspidors, forks, plastic wrap, tape, saltines, photo copies, participation incentives).

OBJECTIVES:
The overall objective of this study is to characterize the sensory properties of newly developed selections from WSU Apple Breeding Program (WABP) and determine the preference of these various apple selections. The sensory properties of these apple selections will then be related to consumer acceptance. Specific objectives are to:

Objective 1: Perform trained sensory panel analysis to characterize new selections of WABP
Objective 2: Perform consumer sensory panel evaluation to determine preference (overall and specific attributes) of new selections of WABP.

SIGNIFICANT FINDINGS:
Apples varied in their sensory properties and consumer acceptance based on apple selection, pick date and growing site

Day 1 Results:

- WSU5 (Brewster Pick 2), WSU5 (Quincy Pick1) and Honeycrisp were rated highly for the trained panel for texture attributes and by the consumer panel.
- For WSU5 (Brewster), harvest time had a significant impact on texture attributes, with Pick2 being significantly higher in texture intensity. Consumers found significant differences in texture attributes and overall acceptance were observed (Pick 2 higher).
- For WSU5 (Quincy), harvest time had a significant impact on sourness (Pick 2 lower)
- Growing location may influence consumer acceptance - WSU5 (Pick1), apples grown in Quincy had higher acceptance for crispness and firmness compared to those grown in Brewster.

Day 2 Results:

- Honeycrisp (Pick2) was the highest in all sensory attributes as evaluated by the trained panel, except for mealiness and astringency.
- While WSU7 (Quincy, Pick1 and 2) were high in juiciness and firmness, moderately high in sweetness, high in sourness and high in apple flavor intensity as evaluated by the trained panel.
- WSU5 (Quincy Pick2) was moderately high in crispness and juiciness, and also in sweetness, sourness and apple flavor intensity.
- For WSU7 (Quincy), harvest time had a significant impact only on juiciness acceptance, with Pick2 having a higher juiciness acceptance compared to Pick1.

METHODS:
Apples: Two WSU elite selections were tested in November, WSU 5 and WSU 7. Fruit was harvested in two picks from the Brewster and Quincy Phase 3 sites aiming for Cornell scale starch stage 3 maturity. Samples of Gala and Honeycrisp were harvested from adjacent sites to the Quincy Phase 3 site. Fruit was stored at Stemilt in regular atmosphere at 33^{o}F. All harvesting, storage and sorting of the fruit was handled by the WTFRC staff lead by Tom Auvil and Ines Hanrahan. Each stage of the fruit handling was recorded on a chain of custody sheet developed for the project. Fruit was sorted and delivered to Pullman 3 days before the scheduled sensory testing. In Pullman, apples were stored at 33^{o}F in regular atmosphere. Small samples of each batch of fruit were delivered to the TFREC where the standard analytical quality measures were performed (full data presented in the Evans WABP report).
Day 1 apples: Honeycrisp, WSU5 (Brewster Pick 1), WSU5 (Quincy Pick 1), WSU5 (Brewster Pick 2), WSU5 (Quincy Pick2), Gala
Day 2 apples: Honeycrisp (Pick2), WSU7 (Brewster Pick2), WSU7 (Brewster Pick1), WSU7 (Quincy Pick1), WSU7 (Quincy Pick2), Gala (Pick2)

Trained Panel Evaluation: The trained panel was composed of 10 individuals. The panelists were trained over 11-15 hours using techniques described by Meilgaard et al. (1999). The panel training sessions were also broadcast through Skype (software program which allows video conferencing) to the TFREC in Wenatchee. The WABP's Industry Advisory Committee and the WTFRC were informed of dates and times of the training sessions and were encouraged to attend. The WABP team attended all of the initial training and the apple discussion sessions. They also tasted apple varieties from the same source as those used for the training sessions in Pullman and provided evaluation scores. The apple attributes were selected using reported literature and previous studies performed in our lab. Panelists were trained to recognize apple flavor (sweetness, sourness, apple flavor intensity and astringency) and texture (firmness, crispness, juiciness and mealiness).

Panelists were trained to recognize the attributes using specific evaluative techniques and assign an intensity rating to each attribute using a 15-cm unstructured line scale. Evaluations took place in individual sensory booths equipped with laptop computers for recording data. Following training sessions, apple selections were presented to each panelist for evaluation in replicate. Panelists were presented with 1/8 of the apple under study. The apple selections were randomly presented to the panelists at room temperature and under white lighting conditions. Panelists were asked to indicate the intensity of the apple attributes described above. Results were collected using Compusense 5.0 software (Guelph, ON) and analyzed using ANOVA and Tukey's HSD.

Consumer Panel: The panel took place in the sensory evaluation facility at WSU using 100 consumers on each of 2 days. Evaluations took place in individual sensory booths equipped with laptop computers. Consumers were presented with 1/8 apple of the apple selections. Honeycrisp and Gala were presented as controls. Consumers indicated their overall acceptance and the acceptability of flavor (sweetness, sourness, and apple flavor intensity) and texture (firmness, crispness, and juiciness) attributes for each apple selection. All attributes were evaluated by the panel using a 7-point scale (1 = dislike very much, 7 = like very much). Results were collected and analyzed as described above.

RESULTS and DISCUSSION:

Day 1 Apples

As shown in **Table 1**, specific differences in sensory attributes intensities were found between apples. Based on crispness, the WSU5 apples did not significantly differ from the Honeycrisp apple, with the Gala apple having the lowest intensity. WSU5 (Brewster, Pick2), WSU5 (Quincy, Pick1) and Honeycrisp had the highest firmness. For juiciness, the greatest differences were observed between Gala and Honeycrisp. WSU5 (Quincy Pick1) and WSU5 (Quincy Pick2) having the highest intensity and Gala having the lowest intensity. Based on sweetness, Honeycrisp and Gala were significantly higher than all of the WSU5 apples. For sourness, Gala and WSU5 (Quincy Pick2) had the lowest intensity while Honeycrisp,WSU5 (Quincy Pick1) and WSU5 (Brewster, Pick1 and Pick2) had the highest intensity. For apple flavor intensity, Honeycrisp and Gala were the most intense with the WSU5 apples having lower intensity. The separation of these apples is shown in **Figure 1**.

Consumer acceptance of apple varieties are shown in **Table 2**. Based on acceptance of crispness, Gala and WSU5(Brewster Pick1) were the lowest while WSU5 (Brewster, Pick 2), WSU5 (Quincy, Pick1) and Honeycrisp were the highest. A similar result was observed with firmness, with Gala being the lowest. For juiciness, Honeycrisp, WSU5 (Quincy Pick1), and WSU5 (Brewster Pick2) were rated as the most acceptable, with WSU5 (Brewster, Pick1) and Gala being the lowest. Based on sweetness, the apples with the highest acceptability were WSU5 (Brewster Pick2), WSU5 (Quincy Pick1), Honeycrisp and WSU5 (Brewster Pick1). For sourness, the most acceptable apples were WSU5 (Brewster, Pick2), WSU5 (Quincy, Pick1) and Honeycrisp. A similar result was observed

with apple flavor intensity. Overall, the most accepted apples were WSU5 (Brewster, Pick2), WSU5 (Quincy, Pick1) and Honeycrisp. Gala was the least accepted. Separation of the apples is shown in **Figure 2**.

The results of the consumer panel may be explained by the results of the trained panel. The three most accepted apples by the consumers, WSU5 (Brewster, Pick2), WSU5 (Quincy, Pick1) and Honeycrisp were all rated highly by the trained panel for crispness, firmness and juiciness intensity. These results indicated that the consumers preferred an apple with higher crispness, firmness and juiciness. The intensity of sweetness and sourness in the apples varied, with WSU5 (Quincy, Pick1) and WSU5 (Brewster, Pick2) being rated lower for sweetness intensity compared to Honeycrisp ($p<0.05$). These two apples were also significantly lower in apple flavor intensity compared to Honeycrisp.

The impact of harvest time on apple sensory properties was also examined. For WSU5 (Brewster), the harvest time (Pick 1 vs. Pick2) had a significant impact on texture attributes, with Pick2 being significantly higher in texture intensity. Based on consumer evaluations, differences in texture acceptance and overall acceptance were observed, with Pick 2 being significantly higher than Pick1. For WSU5 (Quincy), the harvest time had a significant impact on sourness, with Pick 2 being significantly lower in sourness than Pick1. Growing location also influenced the consumer acceptance of several sensory attributes; For WSU5 (Pick1), apples grown in Quincy appeared to have higher acceptance for crispness and firmness compared to those grown in Brewster.

Day 2 Apples

The specific differences between apples are shown in **Table 3**. Based on firmness, WSU7 (Brewster Pick1 and2), Honeycrisp, WSU7 (Quincy Pick2) were the highest while WSU7 (Quincy Pick1) was the least firm. Honeycrisp (Pick2), WSU& (Quincy Pick1 and 2) had the highest juiciness while WSU7 (Brewster Pick1 and 2), and Gala (Pick2) had the lowest juiciness. The apple with the highest sweetness intensity was the Honeycrisp (Pick2), WSU7 (Brewster Pick1) and Gala, with apples of lower sweetness being WSU7 (Brewster Pick2), WSU7 (Quincy Pick1) and WSU7 (Quincy Pick2). Based on sourness, WSU7 (Quincy Pick1 and2), Honeycrisp (Pick2), and WSU7 (Brewster Pick2) were the highest, with Gala being significantly lower. Based on apple flavor intensity, Honeycrisp (Pick2) and WSU7 (Quincy Pick1) were the highest, with WSU7 (Brewster Pick2), WSU7 (Brewster Pick1), WSU7 (Quincy Pick2) and Gala (Pick2) being lower. **Figure 3** shows the separation of these apples based on their sensory properties.

Consumers found apples to vary significantly in all sensory attributes that were examined (**Table 4).** For crispness acceptance, consumers found Honeycrisp and WSU7 (Quincy Pick2) to be the most acceptable and WSU5 (Quincy Pick2), WSU7 (Quincy Pick1) and Gala to be the least acceptable. Based on firmness, Honeycrisp, WSU7 (Quincy Pick2), and WSU5 (Quincy Pick2) were the most acceptable. Juiciness acceptance was highest for Honeycrisp, WSU7 (Quincy Pick2), WSU5 (Quincy Pick2) and lowest for Gala. Based on sweetness, Honeycrisp had the highest acceptance with all the other apples being significantly lower. Sourness acceptance was the lowest for the Gala and WSU7 (Quincy Pick1) and higher for the other apples. Apple flavor intensity was highest Honeycrisp (Pick2), WSU7 (QuincyPick2), and WSU7 (Quincy Pick1). Overall, Honeycrisp was the most accepted, followed by WSU7 (Quincy Pick2), WSU5 (Quincy Pick2) and WSU7 (Quincy Pick 1). The least accepted apple was the Gala. **Figure 4** shows the separation of these apples.

Results from the consumer panel may be put in context of intensity of the sensory attributes as evaluated by the trained panel. The Honeycrisp apple was the highest in all sensory attributes as evaluated by the trained panel, except for mealiness and astringency. While WSU7 (Quincy, Pick1 and 2) were high in juiciness and firmness, they were also higher in mealiness compared to the other

apples. These apples were moderately high in sweetness, high in sourness and high in apple flavor intensity as evaluated by the trained panel. WSU5 (Quincy Pick2) was moderately high in crispness and juiciness, and also in sweetness, sourness and apple flavor intensity. For WSU7 (Quincy), harvest time had a significant impact only on juiciness acceptance, with Pick2 having a higher juiciness acceptance compared to Pick 1.

The analytical measurements of apple quality were also completed on both days. Results showed that the analytical measurement of crispness and °Brix had strong positive correlations with the trained panel evaluation of crispness and sweetness, respectively. Creep had a strong negative correlation with the evaluation of mealiness.

Overall, these results show that apple selections do differ in their sensory properties. These differences are also observed in the consumer acceptance of the apples. Apple texture continues to be very important to consumers and highly influences their acceptance of the apple. Harvest (pick) time does influence the sensory properties of the apples.

Tables and Figures

Table 1. Mean values of sensory attributes for apples evaluated on Day 1 of trained panel. (n=100) as evaluated along a 15-cm line scale. Different letters in the same column indicate significant differences between apples as analyzed by Tukey's HSD ($p \leq 0.05$).

Apple	Crisp-ness	Firm-ness	Juici-ness	Meali-ness	Sweet-ness	Sour-ness	Apple Flavor Intensity	Astringency
Honey Crisp	10.8a	9.2ab	10.4a	1.5b	9.8a	7.6ab	9.9a	2.2b
WSU5 (Brew. Pick2)	10.8a	10.9a	9.7ab	1.9b	7.2b	7.9ab	7.8b	3.6ab
WSU5 (Quincy. Pick1)	10.4ab	9.7ab	9.8ab	2.7ab	6.6b	9.3a	7.5b	3.8a
WSU5 (Brew. Pick1)	9.9ab	9.1b	8.8bc	2.9ab	7.2b	8.6a	8.0b	3.3ab
WSU5 (Quincy. Pick2)	9.4ab	9.2b	9.1abc	2.9ab	7.2b	6.7bc	7.6b	3.3ab
Gala	8.7b	8.2b	8.0c	3.8a	9.7a	4.9c	8.7ab	2.6ab

Figure 1. Separation of the 6 apples as evaluated by the trained panelists on the intensity of their sensory attributes (Day 1).

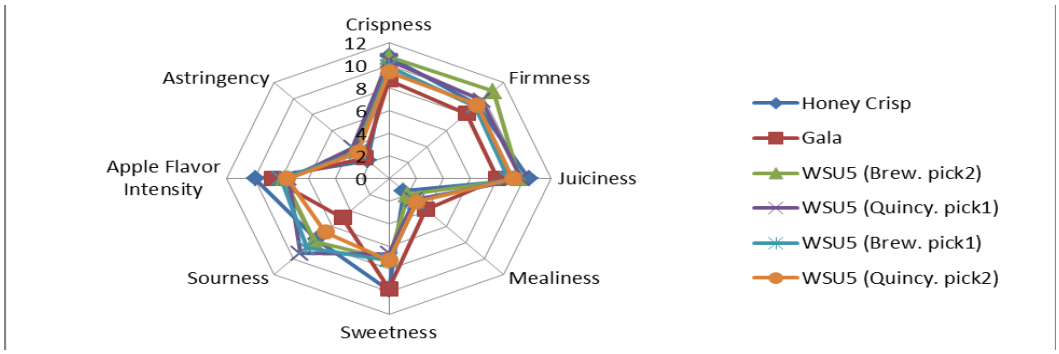


Table 2. Mean values of sensory attributes for apples evaluated on Day 1 of consumer panel as evaluated along a 7-pt scale (1=dislike extremely and 7=like extremely) (n=100) Different letters in the same column indicate significant differences between apples as analyzed by Tukey's HSD ($p \leq 0.05$).

Apple	Crisp-ness	Firm-ness	Juici-ness	Sweet-ness	Sour-ness	Apple Flavor Intensity	Overall Acceptance
Honeycrisp	6.1a	5.8a	6.2a	5.7a	5.5a	5.8a	5.8a
WSU5 (Brew Pick2)	6.1a	5.9a	5.9ab	5.5ab	5.4a	5.3ab	5.6a
WSU5 (Quincy Pick1)	6.1a	5.9a	5.9ab	5.3ab	5.2ab	5.3ab	5.4ab
WSU5 (Brew Pick1)	5.5b	5.3b	5.6bc	5.1ab	4.8bc	4.9bc	4.9bc
Gala	4.9c	4.6c	5.2c	5.3b	4.6c	4.7c	4.7c

Figure 2. Separation of the 6 apples as evaluated by the consumers on their acceptance of the different sensory properties (Day 1).

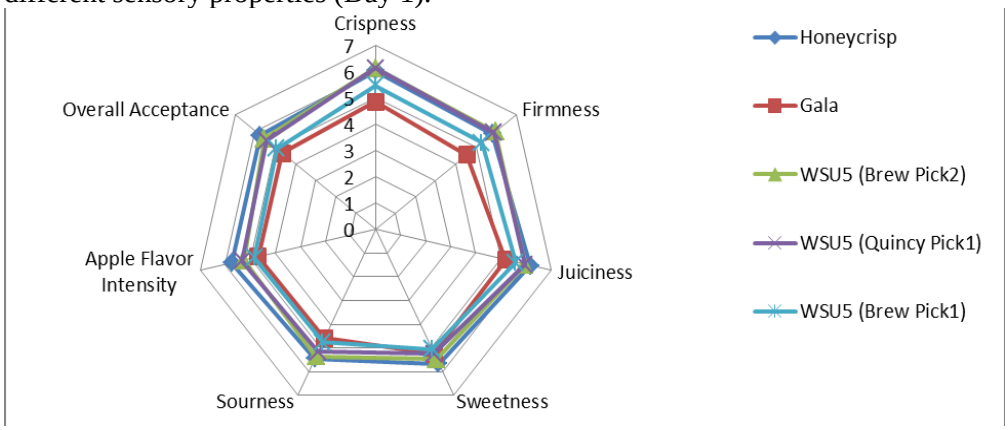


Table 3. Mean values of sensory attributes for apples evaluated on Day 2 of trained panel. (n=10) as evaluated along a 15-cm line scale. Different letters in the same column indicate significant differences between apples as analyzed by Tukey's HSD ($p \leq 0.05$)

Apple	Crisp-ness	Firm-ness	Juici-ness	Meali-ness	Sweet-ness	Sour-ness	Apple Flavor Intensity	Astringency
HoneyCrisp (Pick2)	10.2	8.1ab	10.5a	2.6ab	10.1a	7.1ab	10.1a	2.4
WSU7 (Brew. Pick2)	10.2	9.9a	8.9bc	2.1b	7.7bc	8.6a	7.9b	3.1
WSU7 (Brew. Pick1)	9.3	8.9ab	7.9c	4.1ab	8.7abc	6.2b	8.1b	2.8
WSU7 (Quin. Pick1)	9.7	7.9b	9.2abc	4.4a	8.0bc	7.3ab	8.7ab	2.7
WSU7 (Quin. Pick2)	9.6	8.5ab	9.5ab	3.0ab	7.2c	8.8a	8.5b	2.9
Gala (Pick2)	9.3	8.6ab	7.9bc	2.9ab	9.6ab	3.7c	8.1b	1.6

Figure 3. Separation of the 6 apples as evaluated by the trained panelists on the intensity of their sensory attributes (Day 2).

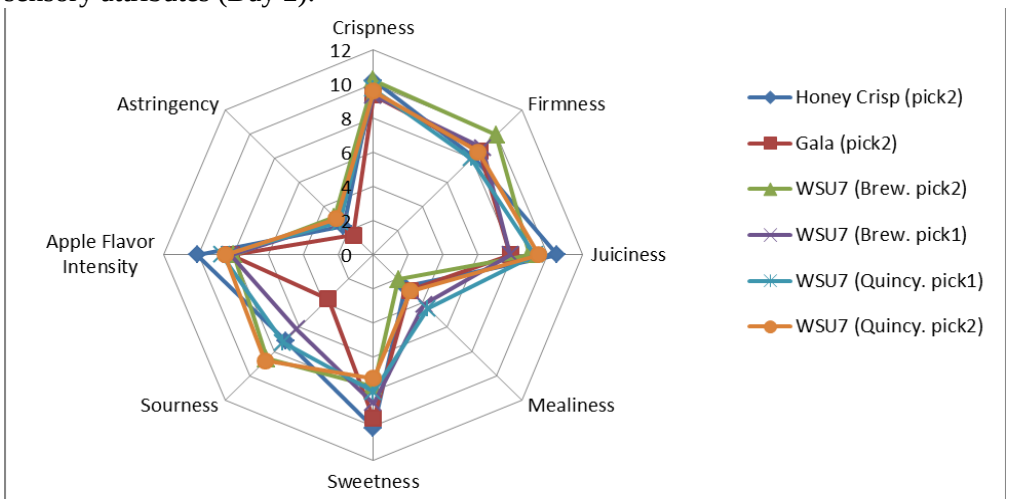
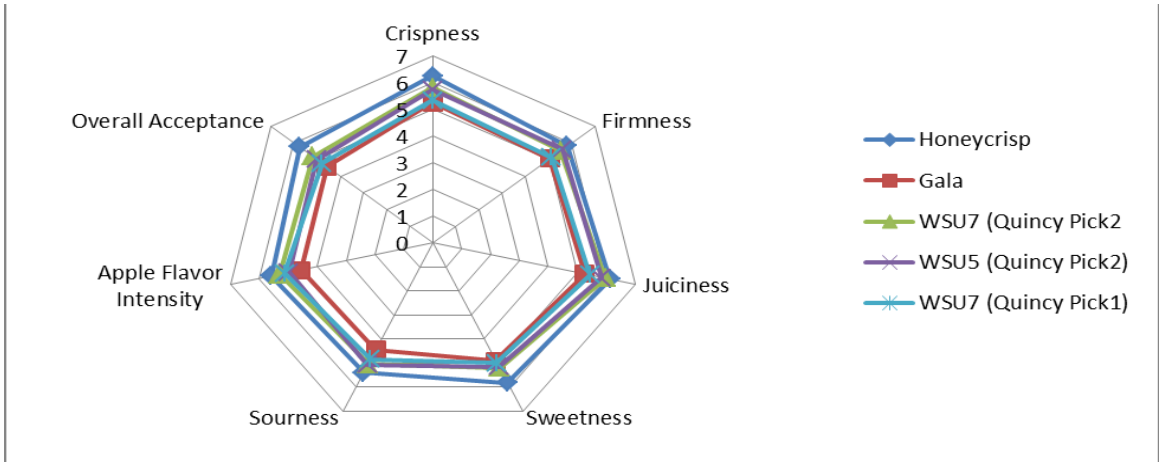


Table 4. Mean values of sensory attributes for apples evaluated on Day 2 of consumer panel as evaluated along a 7-pt scale (1=dislike extremely and 7=like extremely) (n=100). Different letters in the same column indicate significant differences between apples as analyzed by Tukey's HSD ($p \leq 0.05$).

Apple	Crisp-ness	Firm-ness	Juici-ness	Sweet-ness	Sour-ness	Apple Flavor Intensity	Overall Acceptance
Honeycrisp	6.3a	5.8a	6.1a	5.8a	5.4a	5.6a	5.8a
WSU7 (Quincy Pick2	5.8ab	5.5ab	5.9a	5.2b	5.0a	5.3ab	5.2b
WSU5 (Quincy Pick2)	5.7bc	5.6ab	5.8ab	5.2b	5.1a	4.9bc	5.0bc
WSU7 (Quincy Pick1)	5.3bc	5.1b	5.5bc	4.9b	4.9ab	5.1ab	4.8bc
Gala	5.3c	5.1b	5.3c	4.9b	4.5b	4.6c	4.6c

Figure 4. Separation of the 6 apples as evaluated by the consumers on their acceptance of the different sensory properties (Day 2).



CONTINUING PROJECT REPORT **YEAR**: 2010

Project Title: Apple rootstock and scion evaluation

PI: Tom Auvil
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WTFRC Staff cooperators: Felipe Castillo, Tory Schmidt, Jim McFerson, Wenatchee, WA

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

Cooperators: Dave Allan, Rachel Crane, Del Feigal, Ron Wilcox, Dale Goldy, Tim Welsh, Jose Ramirez

Total project funding request: Year 1: 87,102 **Year 2**: 100,725 **Year 3**:94,640

WTFRC Collaborative expenses:

Item	2010	2011	2012
Salaries[2,3]	29,500	30,500	31500
Benefits[2,3]	9,440	9,760	10800
Crew Wages[3]	25,880	26,655	27000
Crew Benefits[3]	8281.6	8529.6	8640
Stemilt RCA room rental	8400	8400	8400
Shipping			
Supplies[4]		10880	1800
Travel[1]	5600	6000	6500
Miscellaneous			
Total	87,102	**100,725**	94640

Footnotes:
[1]Fuel and maintenance
[2]Salaries and benefits for Auvil, Schmidt, and Castillo apportioned to this project.
[3]Rootstock salary and wages are down, Harvest and storage on Phase 3 increased significantly in 2010.
[4]Phase 3 trees for WABP

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

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.

Scion evaluation accomplishments

- 2010 season provided enough fruit for storage and handling trials for several genotypes.
- The low ethylene gene in WA 2 will likely delay shipments until the 2nd or 3rd quarter of the shipping season. Texture of fruit is unchanged after two months of regular storage. Fruit retains 'starchy' harvest flavor two months of Regular Storage. Appearance in December is excellent. The fruit has brightened up considerably and is now bright pinky red on yellow back ground. The fruit is still starchy in December, even after a week of room temperature. Selecting low ethylene genotypes may not develop early season varieties.
- Staggered start can be accomplished by 30 trees in 4 sites or 60-70 trees in two sites.
- Have enough input to doubt the future of 17, 30, 48, and 49.
- 7 and 19 are becoming questionable due to sunburn/appearance and sensitive to bruising respectively.
- WA 5 has a large number of positive traits except its flavor profile tends to be high acid dominate its taste.
- Could advance 38 to P4.
- Completed protocols for crop load, harvest and post harvest treatments. Also standardized harvest and handling protocols for fruit going to the Ross Sensory Evaluation in Pullman.

Significant Rootstock Findings

- Geneva rootstocks continue to show genetic mitigation of replant disorders. Elite selections including G.214, G.41 and G.935 continue to perform well in fumigated and non-fumigated sites. G.890 and CG.4011 are performing well in non-fumigated sites and warrant more evaluation. CG.4814 and CG.4210 are showing virus sensitivity. G.202 does not have competitive crop density and may reduce fruit size a ½ box size.
- Availability of Geneva rootstocks is finally approaching commercial numbers. Demand is significantly greater than supply.
- G.41 and G.11 are smaller than M.9 337.
- G.214 is larger than M.9 337 and was released in 2010.
- G.935 is similar in size to Pajam 2 or M.9 emla. Eastern trials utilizing data from young trees report G.935 is M.26 size. In Washington, G.935 is M.9 class in tree size. G.935 can be vigorous as a newly planted non-bearing tree.
- G.890 is a vigor class larger than M.9. It is very crop efficient and produces good fruit size. Eastern data is overstating its class size, at least for Washington State.
- G.41, G.11, G.214, G.935 are all fireblight resistant.
- In tough replant sites, G.41, G.214, G.890 and G.935 are improving consistency of performance.
- G.11 is not wooly aphid resistant nor replant tolerant. It is reasonably fire blight resistant and per forms M.9 clones in fumigated and unfumigated replant.
- G.16 is available, is fire blight resistant, moderately replant tolerant but is hypersensitive to virus infection. The virus issue is a problem only if grafting with wood of questionable heritage.

Figure 1: 2006 Wapato Gala accumulated yield in bins per acre 2007-2010.

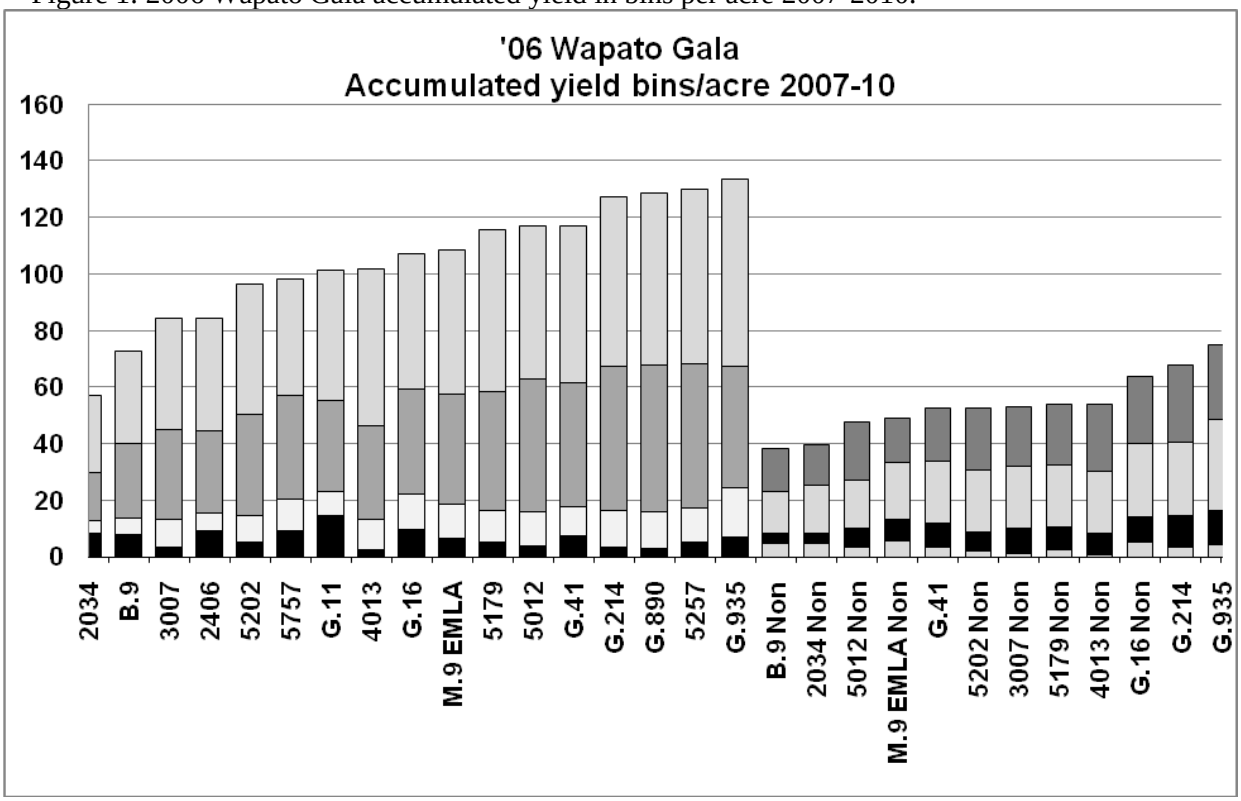


Figure one's data shows that if the new production canopy is short of growth for any reason, it is behind for many years. In this case, in addition to no fumigation the trial had irrigation issues. The nonfumigated plots poor performance in the first two seasons was multiplied by lack of water. Note that G.214, G.935 and even G.41 are recovering from year one and two irrigation problems. Some genotypes such as B.9 and M.26 typically never recover from first year difficulty. This trial demonstrates the importance of genetic resistance to replant disorder. Even though the trees were obviously stressed, the Geneva Elites are recovering and increasing yields annually.

Figure 2: 2006 Vantage Fuji cumulative yield in bins / acre

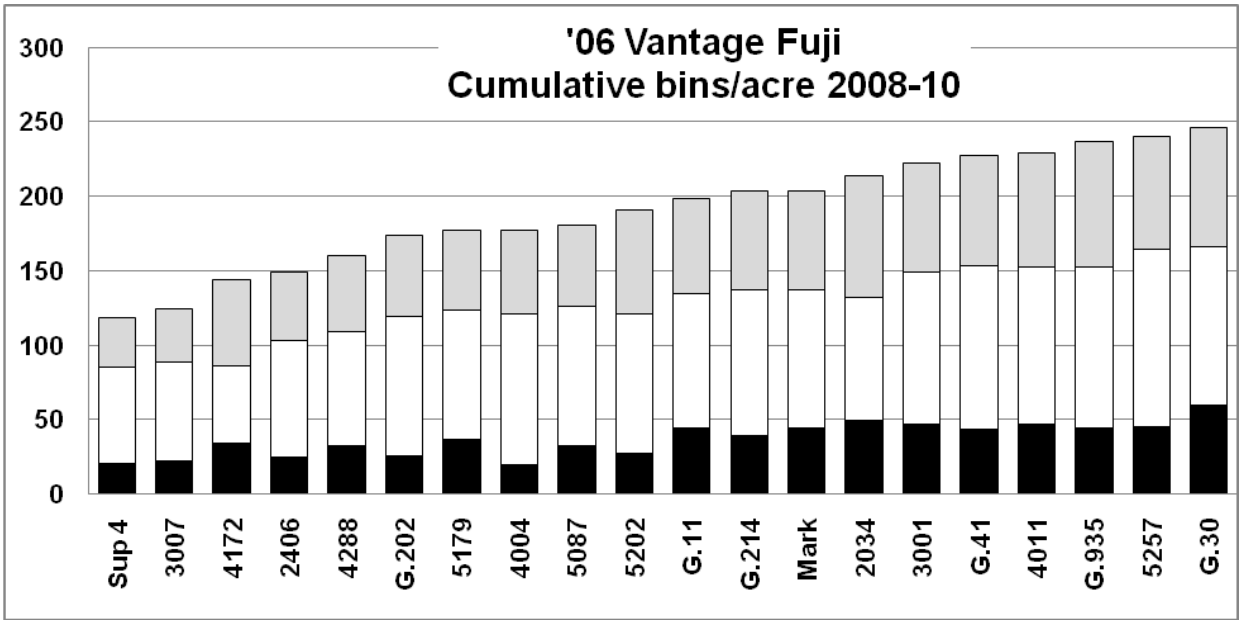
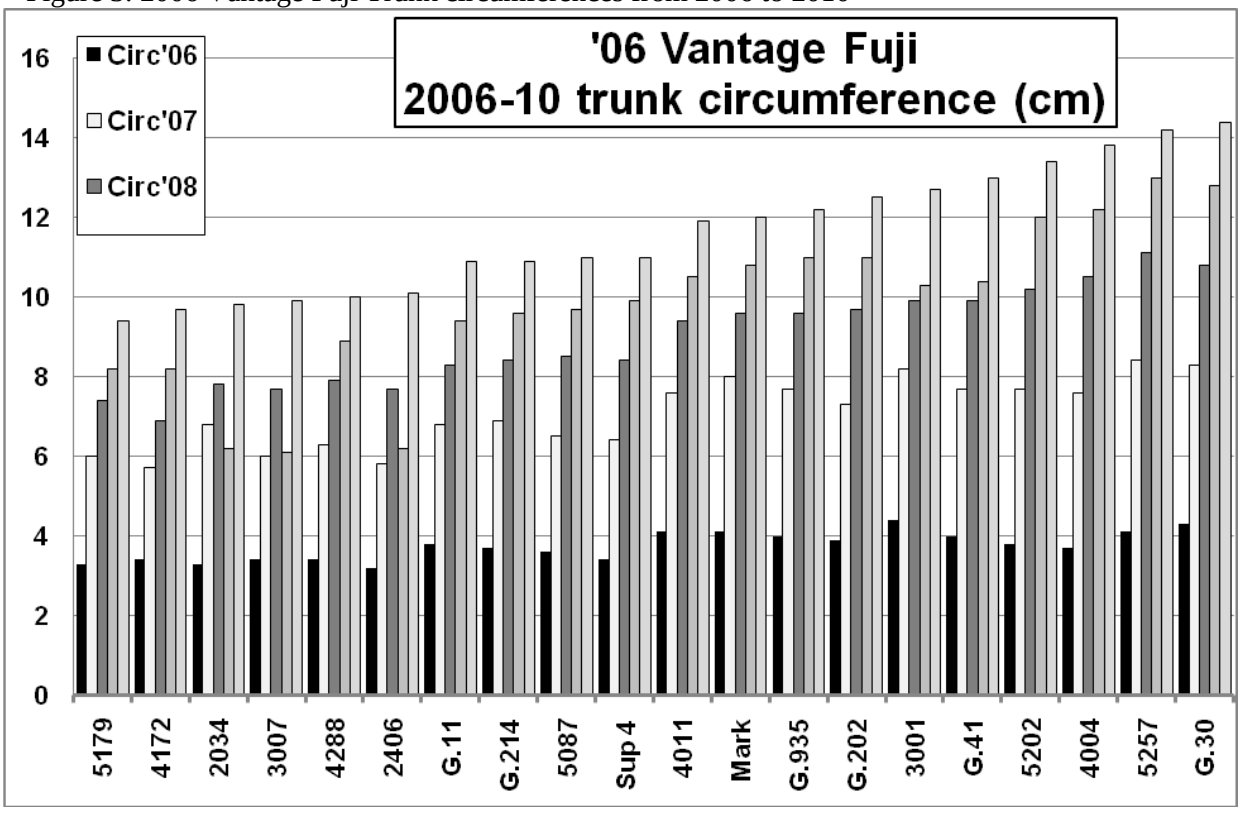


Figure 3: 2006 Vantage Fuji Trunk circumferences from 2006 to 2010



The high performing dwarf rootstocks in the vantage trial have slowed their increase in trunk circumference (figure 3) and have been increasing their annual, thus their cumulative yields (Figure 2). Mark, G.214, G.41 and G.935 have been following similar paths by with increasing yields influencing the reduction of trunk circumference increase. Mark is the standard rootstock in this trial, and the Geneva elites are very comptitive or even better performing for yield than Mark. Some larger, more

vigorous rootstocks such as Supporter 4, have been increasing trunk size but yields are considerably off the pace.

Two vigorous Geneva genotypes, CG5257 and G.30 are demonstrating the precocity of their genetics. Their high yields are in agreement with the hypothesis that the canopy must be developed quickly, then yield follows. The challenge with these two genotypes is they have excessive shoot growth and the cropping efficiency will start to decline. It is too bad that G.30 is such a difficult tree for nurseries to propagate, for its properties make it a high performing, relatively low risk rootstock.

The Vantage and Brewster trials are highlighting another rootstock, CG4011 that is a bit more vigorous than G.214.

CG 4814 has been looking promising especially as a high performing replacement tree, but in 2010 viruses in the fuji blocks laid this rootstock low. CG5046 also appeared to be an excellent choice to replace missing trees in commerical plantings, but it too, succumbed to virus sensitivity.

As of December, 2010, progress in commercial production of Geneva rootstock liners from stool beds and tissue culture is evident. The older selections are available in quantites of 100's of thousands, and the newer elite selections are entering the finished tree production in quanties of 10's of thousands, and rapidly increasing.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP09-903

Project Title: Apple scion breeding

PI:	Kate Evans	**Co-PI (1):**	Cameron Peace
Organization:	WSU Tree Fruit Research and Extension Center	**Organization:**	WSU-Horticulture and Landscape Architecture
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Co-PI(2):	Carolyn Ross	**Co-PI(3):**	Yanmin Zhu
Organization:	WSU-Food Science and Human Nutrition	**Organization:**	USDA-ARS Tree Fruit Research Lab
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City/State/Zip:	Pullman/WA/99164	**City/State/Zip:**	Wenatchee/WA/98801

Cooperators: Bruce H. Barritt, Professor Emeritus, WSU; Amit Dhingra, WSU Pullman; Dorrie Main, WSU Pullman; Tom Auvil, WTFRC; Roger Adams, Willow Drive Nursery, Ephrata.

Total Project Request: Year 1: $169,910 **Year 2:** $239,628 **Year 3: $203,217**

Other funding sources: None

WTFRC Collaborative expenses:

Item	2009	2010	2011
Stemilt RCA room rental	4,200	8,400	8,400
Crew labor	3,500	18,840	19,405
Crew benefits	0	6,029	6,210
Supplies	0	0	10,880
Travel – to plots	5,000	5,600	6,000
WTFRC staff salary+benefits	15,790	23,984	24,856
Total	28,490	62,853	**75,751**

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** ML Bricker and Kevin Larson
Telephone: 509-335-7667, 509- 663-8181 x221 **Email:** mdesros@wsu.edu, kevin_larson@wsu.edu

Item	2009	2010	2011
Salaries[1]	60,540	50,352	55,005
Benefits	23,005	16,616	19,792
Wages[2]	15,500	20,000	20,800
Benefits	2,790	2,960	3,120
Equipment	50,000	0	0
Supplies[3]	0	19,700	17,500
Supplies[4]	0		8,800
Travel	14,200	15,500	16,900
Total	166,035	125,128	**141,917**

Footnotes:
[1] Salaries for Agricultural Research Technologist (Bonnie Konishi@ 1.0FTE) and salary for 3 months for genotyping technician (Terence Rowland @ 0.25FTE)
[2] Wages for time-slip labor for orchard establishment and trait phenotyping.
[3]For orchard establishment supplies
[4]For genotyping supplies for marker-assisted seedling selection

Budget 2
Organization Name: Willow Drive **Contract Administrator:**

Item	2009	2010	2011
Trees	700	6000	0
Phase 2 trees[1]	700	6,000	2,000
Phase 3 trees[2]	0	0	8,300
Seedling propagation[3]	3,175	108,500	51,000
Total	3,875	114,500	**61,300**

Footnotes:
[1] trees for phase 2 at 2 growers sites plus WSU orchard. Five trees of 5 commercial varieties will be planted as standards for each site.
[2] trees for phase 3 at 4 grower sites.
[3] propagation of seedling populations on M.9 rootstock for WSU by Willow Drive Nursery

Objectives:

1. Produce, through traditional breeding methods, promising selections and subsequently elite selections with outstanding eating quality and commercial potential.
2. Use extensive trait phenotyping in combination with genomic tools (phenotype/genotype associations) to develop marker-assisted seedling selection (MASS) for key fruit and tree traits.
3. Use both objective (instrumental) and subjective (sensory) evaluation techniques to identify selections with outstanding eating quality.

Significant findings:

1. Eleven new crosses were made and approximately 21,000 seeds produced in the WSU Apple Breeding Program (WABP). Seedlings from approximately 15,000 seeds from 2009 crosses were grown in the greenhouse, more than 2,500 of which were screened for fire blight resistance; the remaining seedlings were transplanted to Willow Drive nursery.
2. Approximately 5,000 seedlings were screened using DNA markers for long-storage ability and fruit texture; only pre-selected seedlings were then propagated on M.9 for orchard evaluation. More than 7,000 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2011.
3. MASS was also used to pre-select for long-storage ability and texture in more than 2,500 seedlings from 2006 immediately prior to planting in the Phase 1 selection orchard. The final count of new Phase 1 trees planted in 2010 was 12,680.
4. Promising selections in Phase 2 trials (planted in 2004, 2005, 2006, 2007, 2008 and 2009) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
5. Fifteen new promising selections were planted at three evaluation sites in Phase 2 trials in 2010.
6. Seven promising selections made in 2009 were propagated in 2010 for planting in 2012 Phase 2 trials at three diverse sites in Central Washington and one new elite selection was propagated for Phase 3 trials
7. Samples of fruit from two elite selections from different sites and pick dates were profiled by the sensory panel at WSU-Food Science and Human Nutrition under the supervision of Dr. Ross in November 2010.
8. One hundred and thirty-six growers signed up to participate in Phase 4 evaluation of ‘WA 2’ in 2010. Orders have already been taken for growers wishing to move to commercialization. The second release from the program, ‘WA 5’, was released to growers for evaluation at the Washington State Hort Show in December 2010.
9. The analysis of data of fruit from several different breeding progenies with a molecular marker for skin color was published by Dr. Zhu with Drs. Peace and Evans. More than 250 reference cultivars and individuals from breeding progenies based at the Sunrise orchard have been phenotyped in detail and fully genotyped as part of the reference germplasm for the new SCRI RosBREED project, to be combined with similar data from other U.S. breeding programs for powerful analyses.

Methods:

1. Breeding
    a. Use traditional hybridization of parents with desirable traits to produce seed (10,000 to 20,000 per year). Germinate seed and grow seedlings in the nursery and bud each seedling onto M.9 rootstock to produce large trees for the Phase 1 fruit evaluation orchard. From promising seedlings, fruit are evaluated for quality after two months in regular cold storage. Selection is based on appearance (primarily color, uniformity,

freedom from defects) and desirable eating quality (primarily firmness, crispness, juiciness, sugar/acid balance).

b. Promising selections are propagated on M.9 and placed in replicated Phase 2 trials (five trees/selection) at three diverse sites in central Washington. Data on tree health, productivity and fruit quality are collected and outstanding selections are given elite status.
c. Elite selections are propagated on M.9 and planted in multi-tree (25 to 100 trees/selection) Phase 3 grower trials in commercial orchards at four diverse sites in central Washington. Trials are conducted in cooperation with the WTFRC, managed by Tom Auvil. Certified, virus tested, bud wood is produced for elite selections.
d. Outstanding selections are proposed for commercialization.

2. Genomics, marker-assisted seedling selection (MASS) and pedigree-based genotyping analysis
    a. In conjunction with the genomics team (Drs. Peace, Dhingra, Main and Zhu), take advantage of new genomics tools to improve breeding efficiency. Develop phenotype/genotype associations for important fruit quality traits including firmness, crispness, juiciness, acidity, soluble solids, fruit color and tree characteristics including precocity, vigor, disease and insect resistance. Use developed markers in MASS, specifically for the ethylene genes *Md-ACS1* and *Md-ACO1*, which influence fruit firmness and storage life, genotype seedlings in populations from parents with different textural characteristics (Honeycrisp, CrimsonCrisp and Cripps Pink) and use these genes as predictive markers to eliminate undesirable seedlings. Use performance data of parent cultivars, selections and breeding populations with the statistical approach of Pedigree-Based Analysis to determine the predictive power of markers.

3. Sensory/Consumer Evaluation
    a. In cooperation with Dr. Carolyn Ross, establish a trained sensory evaluation team. Using established sensory evaluation methods, characterize promising selections for consumer acceptability in terms of fruit texture, flavor and appearance.

Results & Discussion:

Approximately half of the crosses made in 2010 were focused on fire blight resistance; seedlings will be screened in the greenhouse for resistance in 2011. Marker-assisted parent selection was used to determine the best parental combinations of the *Md-ACS1* and *Md-ACO1* ethylene genes and every cross included a WSU selection as one of the parents. More than 21,000 seedlings were produced and are currently being vernalized to induce germination.

2010 was the first year that DNA markers have been used to pre-select seedlings in the apple breeding program. Marker selection was performed on seedlings of different ages in the nursery focusing on the two ethylene production genes *Md-ACS1* and *Md-ACO1*. Seedlings from 2006 crosses which had the least desirable combinations of ethylene genes were discarded prior to planting in the Phase 1 orchard, putting to practical use data that had been generated on these seedlings in an earlier research project. This pre-selection reduced the establishment costs of the planting and will help in the management and selection of that planting. Seedlings from 2008 crosses were screened later in the year (the window predicted to be the most cost-efficient for genetic screening); data was provided to allow culling of undesirable seedlings prior to budding. Nursery charges in 2012 will reflect this reduction in seedlings propagated. The benefit:cost ratio of implementing DNA markers was estimated at more than 5:1. Continuing this practice in the future is, therefore, recommended. We are currently determining the extent to which to increase initial seed number so that cost savings from markers (avoiding later propagation, planting, and evaluation costs) can be reallocated to raising the

genetic bar of the whole program without necessarily reducing the numbers of trees budded and planted each year. However, routine MASS will require securing the part-time services of a full-time genotyping technician/manager. At present, the capable genotyping technician in the PNW Tree Fruit Genotyping Lab in Pullman is full-time only through multiple ad hoc research grants – an unsustainable situation that is not expected to continue from 2011.

Fifteen new selections were planted at the three Phase 2 evaluation sites including WSU's Sunrise Research Orchard. Fruit from earlier Phase 2 plantings were evaluated and one selection, an early season apple, was propagated for Phase 3 grower trials.

Two Industry Advisory Committee (IAC) visits were made to Phase 3 orchards in June and one in September. Phase 3 orchards, with some trees now in their 4th leaf, produced a large crop this season. A harvest and storage regime was developed and managed by Tom Auvil and Ines Hanrahan incorporating regular and CA storage, with and without 1-MCP treatment. Fruit samples for sensory and consumer evaluations by Dr. Ross's team were also factored into the regime. Sensory evaluation training organized by Dr. Ross was made available to the IAC in Wenatchee. All members of the apple breeding team participated in the training and were particularly interested in how the panel was trained to taste sweetness and acidity. The breeding team provided some useful feedback to the panel on the levels of both of these normally found in apples and suggested the standard juice sample provided should be changed. Sub-samples of the fruit tested in November were analyzed in the breeding team lab with our standard set of instrumental measures. Data is presented in Table 1.

Table 1: Instrumental measures of fruit samples assessed by sensory and consumer tests by Dr. Ross's lab.

Day one

ID	pick date	Fruit diam(in)	Fruit Weight(lb)	Firmness *M2(lb)	Creep *C0(in)	Crispness *Cn	°Brix	Mean Starch	Titratable Acidity
Honeycrisp	09/01/2010	3.5	0.8	23.7	0.029	287	15.7	7.6	0.67
WSU 5 (Brew p2)	09/23/2010	3.0	0.6	22.8	0.003	373	12.0	8	0.55
WSU 5 (Quincy p1)	09/15/2010	3.4	0.8	27.6	0.001	328	13.5	6.8	0.70
WSU 5 (Brew p1)	09/16/2010	3.1	0.7	25.1	0.001	292	14.4	7	0.48
WSU 5 (Quincy p2)	09/21/2010	3.1	0.7	22.6	0.006	227	14.0	6.8	0.66
Gala	09/01/2010	3.2	0.7	25.4	0.002	185	14.3	5.8	0.51

Day Two

Honeycrisp	09/07/2010	3.6	1.0	21.2	0.054	242	14.7	7.8	0.63
WSU 7 (Brew p2)	09/23/2010	2.8	0.5	22.5	0.002	206	14.7	7.4	0.55
WSU 7 (Brew p1)	09/16/2010	2.8	0.5	20.9	0.001	188	13.7	7.6	0.51
WSU 7 (Quincy p1)	09/15/2010	3.2	0.7	20.9	0.009	169	13.7	8	0.62
WSU 7 (Quincy p2)	09/21/2010	3.3	0.8	23.0	0.006	202	13.6	8	0.71
Gala	09/08/2010	3.1	0.6	23.7	0.003	168	14.6	6.4	0.56

Note: measures noted * were recorded with a Mohr® DigiTest; creep is a measurement of mealiness (the higher the value, the more mealy the apple) and Cn is correlated to crispness (the higher the number, the crisper the apple).

The IAC met to assess the Phase 3 fruit in November prior to the Variety Showcase tasting at the Hort Show in December. A tasting is planned for the apple review in January plus a final late season tasting possibly to coincide with a Phase 3 blossom walk.

'WA 5' was released into Phase 4 evaluation at the Hort Show in December following the submission of the patent application in September. A two-page article appeared in the October issue of the Good Fruit Grower as well as an article in Capital Press.

Publicity events for 'WA 2' continued this year with a presentation to the Pomology Club in Yakima. Field days were hosted in September and October at two Phase 3 trial sites (Quincy and Brewster) where growers had the opportunity to see fruit of 'WA 2' on the tree as well as sample some earlier harvested 'WA 5'. Last year's sign-up period for 'WA 2' resulted in 136 growers signing up for Phase 4 evaluation. Commercialization licenses have been developed should any Phase 4 participants wish to move into Phase 5. Certified virus tested material of 'WA 2', 'WA 5' and 'WSU 7' was made available to PNW nurseries to establish further mother tree blocks from the National Clean Plant Network-Fruit Trees in Prosser in September 2010. Virus testing of six further elite selections is underway.

The first stage of establishing the bar-coding system in the orchard is complete; all Phase 2 trees and all Phase 1 trees planted in 2010 have bar-coded labels. Field measurements are entered into a hand-held data-logger which also scans in the bar-coded tree label. Fruit samples can be bar-coded in the orchard before moving into the analysis lab reducing the possibility of errors and increasing efficiency. Bar-coded sample names can be scanned into the analysis equipment; data output via Excel spreadsheets can be directly uploaded into the new Breeders toolbox database.

WABP breeding parents and seedling populations form approximately a third of the larger apple germplasm set for the SCRI RosBREED project. A detailed phenotyping protocol was designed in collaboration with the other RosBREED apple breeders (Jim Luby, Univ. Minnesota, and Susan Brown, Cornell Univ.) and more than 250 trees from the Sunrise Research Orchard's Apple Germplasm Library were comprehensively phenotyped for fruit quality in the 2010 season. Samples out of storage continue to be evaluated. The bar-coding system outlined above is also being used for the RosBREED samples. Leaf samples of all germplasm individuals were sent to USDA-ARS, Corvallis for DNA extraction and genotyping.

Breeding progenies and elite/advanced selections continue to be an important source of genetic material for genetic dissection of various fruit quality characters. Drs. Zhu, Evans and Peace published the results of screening several progenies with a DNA marker (*MdMYB1*) associated with fruit skin color. Advanced and elite selections are currently being analyzed in Dr. Dhingra's lab to further elucidate the molecular control of bitterpit.

Further analysis of several years' phenotypic data and new genotypic data in the Peace lab has produced some early indications of additional DNA markers linked to key quality characteristics. Some of these results are being used within RosBREED to establish the predictive power and breeding utility of these additional markers.

CONTINUING PROJECT REPORT **YEAR:** 1 of 3

Project Title: Fruit metabolic responses to controlled atmosphere O_2 and CO_2 stress

PI:	Jim Mattheis	**Co-PI**:	Dave Rudell
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Cooperators: Chris Watkins, Department of Horticulture, Cornell University
Michael Young, Stemilt Growers

Total Project Request: **Year 1**: $64,225 **Year 2**: **$65,509** **Year 3**: $66,819

Other funding sources

Agency Name: USDA-NIFA (SCRI)
Amount requested (Federal + non-Federal): $2.4 million
Notes: D. Rudell is Project Director, J. Mattheis is a Co-PI. The Standard Research and Extension Project, "A diagnostic toolbox for integrated management of postharvest apple necrotic disorders" was submitted (01/12/10) for the current funding cycle. WTFRC and AgroFresh, Inc. are co-sponsors.

Budget

Organization:USDA, ARS	**Contract Administrator:** Chuck Myers
Telephone: (510)559-5769	**Email:** Chuck.Myers@ARS.USDA.GOV

Item	**2010**	**2011**	**2012**
Salaries	43,278	44,176	45,093
Benefits	18,457	18,933	19,326
Wages			
Benefits			
Equipment			
Supplies	2400	2400	2400
Travel			
Miscellaneous			
Total	$64,225	**$65,509**	$66,819

Footnotes:salary and benefits for GS-9 technician

Objectives:

1. Identify volatile compounds that accumulate during CA storage.
2. Characterize volatile compound dynamics during storage in atmospheres that induce low O_2 and/or high CO_2 injury.
3. Determine if recognition of changes in volatile compound production during low O_2 or high CO_2 stress has utility for CA system management.
4. Develop sampling protocols to enable detection of biomarkers for scald and other disorders.

The proposed research is consistent with the WTFRC 2010 Apple High Priority CA storage and storage regimes: low oxygen regimes with and without SmartFresh, and subsequent impacts on physiologic disorders; determine methods for mitigation of soft scald, bitterpit, internal brown, ethylene reception and activity etc.; continued improvement in scald control methods, prediction and post-harvest components.

The goal of this research is to develop an active fruit monitoring system that alerts storage operators to undesirable CA conditions in real or near-real time. This system would incorporate some if not all existing technologies for storage room monitoring while providing additional measures to identify abnormal fruit metabolism. While the bulk of the proposed research will utilize GC-MS as the analytical system, we anticipate commercialization of this concept could utilize existing instruments and/or expertise already in place at some warehouses, consulting businesses, and ag chemical supply companies. Successful system development and implementation would reduce storage disorder risk that results from CA gas concentrations outside the range tolerable by apple fruit.

Significant Findings

1. CA chamber volatile content differed with cultivar ('Delicious', 'Fuji', 'Granny Smith'), chamber O_2 and CO_2 concentration, and storage duration.

2. Ethyl acetate and ethyl propanoate accumulation increased with decreased O_2 content in CA chambers containing 'Delicious' apples, however, no ethanol accumulation was detected.

3. Butyrate and hexanoate ester accumulation increased with increased CO_2 content in CA chambers containing 'Fuji' apples.

4. Through 2 months storage, no scald-related volatile compounds were detected in chambers holding 'Granny Smith' apples.

5. Volatile compounds in commercial CA rooms containing 'Delicious', 'Fuji', and 'Granny Smith' apples were similar in identity but not quantity to those detected in research CA chambers.

Methods:

Fruit from commercial orchards were used for all experiments. Laboratory scale experiments used 'Delicious' for low oxygen injury, 'Fuji' for high CO_2 injury, and 'Granny Smith' to assess accumulation of volatile compounds produced prior to scald symptom development. Some experiments will utilize SmartFresh to allow the role of ethylene perception in CA response to be evaluated. All fruit will be stored utilizing existing cold storage and controlled atmosphere facilities in our laboratory. Fruit quality analyses (color, firmness, texture, soluble solids content, titratable acidity) will be conducted using established methods and existing equipment. Fruit firmness/texture

assessment will be conducted using a recording penetrometer. Ethylene and CO_2 production will be measured using gas chromatography with flame ionization detection, and other volatiles will be analyzed by gas chromatography with mass selective detection. Nitric oxide is measured using a chemiluminescent detector. Sampling of other volatile compounds (aldehydes, alcohols, esters, others) produced by fruit during storage and present in trace amounts will utilize solid sorbent traps to concentrate volatile compounds to detectable amounts. Peel fluorescence of fruit stored in low (less than 1%) O_2 will be monitored using existing HarvestWatch sensors.

Year 1: *Objectives 1and 2*: Develop and validate volatile compound sampling protocol utilizing our existing CA chambers. This work will be conducted during July-August prior to harvest of the 2010 crop. Fruit from the 2009 crop year will be used to optimize gas sampling procedures including retrofitting CA chambers with gas sampling ports and optimization of gas sample volume to concentrate volatile compounds above the GC-MS detection limits. Protocols for fall 2010 experiments will proceed as follows. Harvested fruit will be cooled to 33 ^{o}F over 2-4 days. SmartFresh will be applied during this period as appropriate. Fruit will then be placed into CA chambers and CA conditions established over 24-36 hours. Low oxygen injury experiments will establish O_2 concentrations of 1.5 -2% (normal), 0.1% (abnormal), and a third concentration determined using chlorophyll fluorescence monitoring (O_2 concentration at which fluorescence changes +0.2%). CO_2 in these experiments will not exceed 1%. Carbon dioxide injury experiments will establish 0.5, 1.5, or 5% CO_2 with 1.5-2% O_2. Volatile samples will be collected on alternate days for 14 days, then at 7-14 day intervals through 9 months. Fruit removed from CA after 2 weeks and 1, 2, 3, 6, and 9 months will be assessed for disorders and quality. The volatile sampling and fruit assessment schedule is designed to determine if volatiles characteristic of physiological disorders are detectable prior to symptom development.
Objective 4: 'Granny Smith' studies will include monitoring for volatile compounds previously identified by Dave Rudell to be possible biomarkers for predicting superficial scald development.

Year 2: *Objectives1,2,4:* Repeat and revise year 1 studies to evaluate seasonality and validate the initial results. Begin commercial room monitoring study to characterize identity and concentrations of volatile compounds accumulating under warehouse conditions. Previous work in commercial rooms indicated multiple volatile compounds from bins and other non-fruit sources can be detected.

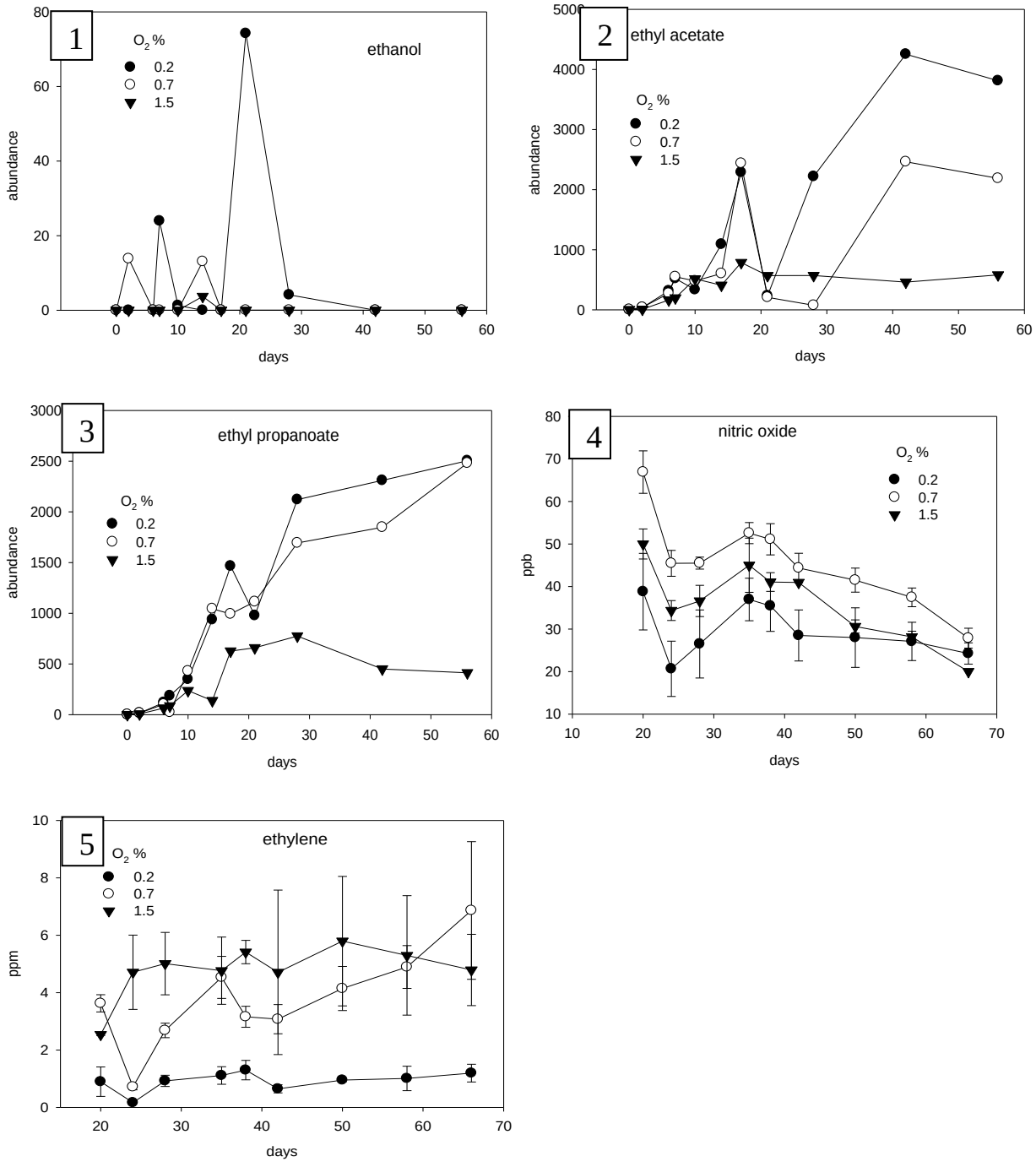
Year 3: *Objective 3:* Validate results from years 1 and 2 using multiple lots for a subset of cultivars based on strength of the first two year's results. Initiate studies to evaluate system capacity to mitigate injury development by relieving CA low O_2 or high CO_2 stress after stress- related volatile biomarkers are detected.

Results will be communicated to industry by presentations at industry meetings, and to extension and research personnel by publications in peer reviewed journals.

Results and Discussion

Volatile accumulation from 'Delicious' apples stored in low O_2. Ethanol did not accumulate over the first 2 months of CA (Figure 1). Ethyl-acetate and ethyl propanoate accumulated in CA with amounts increasing as O_2 content decreased (Figures 2 and 3). Although CA was established within 2 days of harvest, accumulation of ethyl esters related to O_2 concentration was not apparent until 2 to 3 weeks after harvest. The accumulation of ethyl esters but not ethanol in chambers may indicate chamber O_2 is sufficient to allow ethanol conversion to ethyl esters to occur. Nitric oxide (NO) and ethylene content were also related to chamber O_2 concentration (Figures 4 and 5). NO and ethylene were both lowest for most of the first 60 days in chambers held at 0.2% O_2. Fruit stored at 1.5% O_2 had a

pineapple-off flavor at removal from CA. This off-flavor was not present after fruit were held 7 days at 70 ^{o}F.

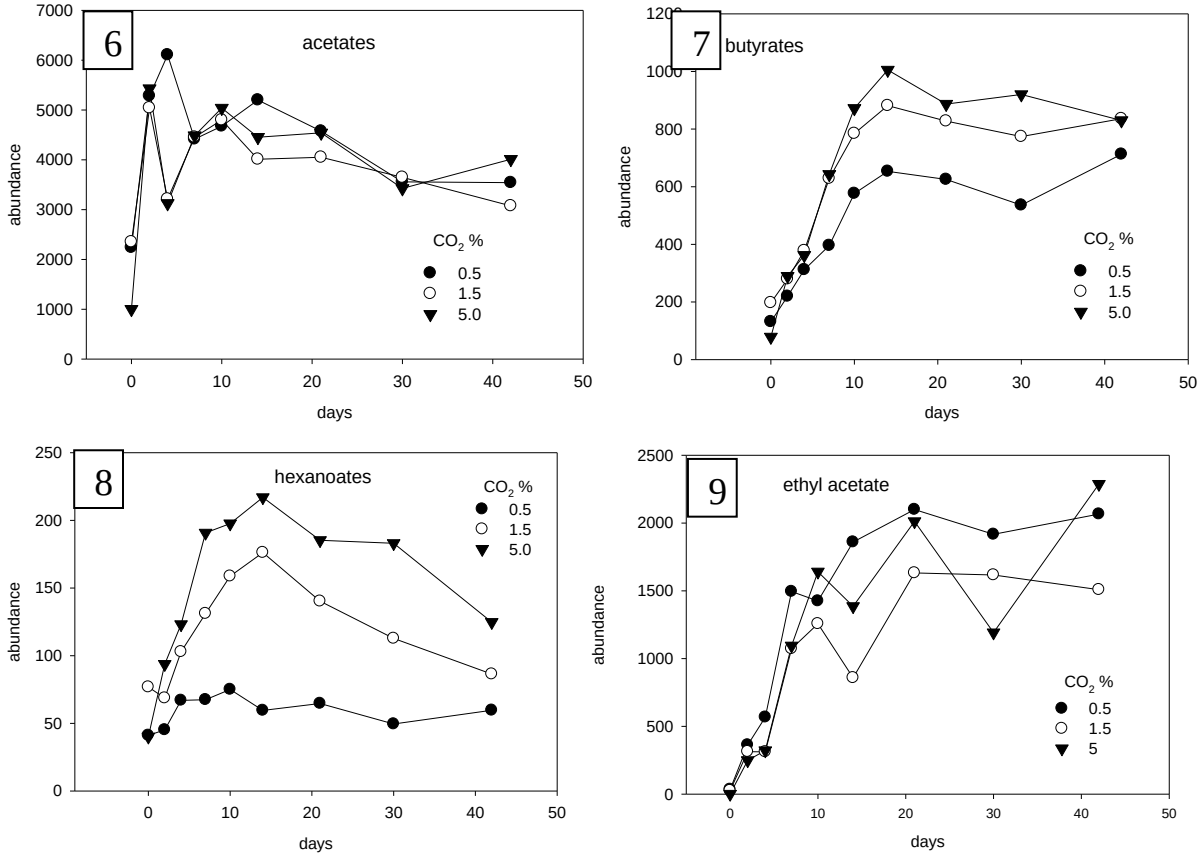


Figures 1-5. Volatile accumulation in CA chambers containing 'Delicious' apples. Fruit were stored at 0.2, 0.7, or 1.5% O_2 with 1% CO_2. A change in chlorophyll fluorescence was detected at 0.2% O_2. Gas samples were collected from CA chambers onto solid sorbent traps or into Tedlar^{TM} bags.

Volatile accumulation from 'Fuji' apples stored in high CO_2. Acetate esters were the largest component of chamber volatiles collected (Figure 6) and no accumulation related to chamber CO_2

content was detected. Butanoate and hexanoate ester content increased with increased CO_2 concentration (Figures 7 and 8). Ethyl acetate accumulated over time in storage but no pattern related to CO_2 content was apparent (Figure 9).

Small amounts of ethylene were detected for 38 and 12 days in chambers held at 0.5% and 1.5% CO_2, respectively (data not shown), however, no ethylene was detected in chambers held at 5% CO_2. Fruit stored in 5% CO_2 developed core browning (28%) and cavities (11%) during the first 60 days in CA. An off-odor present in CA chambers was most pronounced at 5% CO_2 and was observed after 1 and 2 months storage. The odor may be the result of butyrate and hexanoate accumulation.



Figures 6-9. 'Fuji' volatile accumulation during CA storage. Fruit were held in 1% O_2 with 0.5, 1.5, or 5% CO_2.

Volatiles accumulating during 'Granny Smith' storage. Fruit were stored in air or CA (1% O_2, 1% CO_2) with or without pre-storage DPA (2000 ppm) treatment. Through 2 months storage, volatile compounds previously related to scald development have not been detected from fruit stored in air or CA. Accumulation of some esters including butyl butyrate, 2-methylbutyl acetate, and butyl 2-methylbutyrate was greater in chambers held at 1/1 O_2/CO_2 containing DPA-treated fruit. Contrary to 'Delicious's and 'Fuji', ester accumulation increased throughout the first 8 weeks CA storage of 'Granny Smith'.

Volatiles accumulating during commercial CA storage. Gas samples from one room each of 'Delicious', 'Fuji', and 'Granny Smith' apples were collected in mid-December from a Stemilt facility in East Wenatchee. O_2 and CO_2 content (%) in the rooms at the time of sampling were:

	O_2	CO_2
'Delicious'	1.2	1.5
'Fuji'	4.0	0.5
'Granny Smith'	0.7	0.7

Amounts of volatiles detected in all room samples were low compared to samples collected from CA chambers. 2-methyl-1-butanol was the most abundant volatile collected in all three rooms, and ester production was low in all rooms. As similar gas volumes were collected from research CA chambers and the commercial rooms, volatile production or accumulation in commercial rooms may be less compared to CA chambers, or headspace volume in rooms versus chambers may be different. Additional sampling from rooms is needed to determine optimum sampling conditions.

CONTINUING PROJECT REPORT

Project Title: Programs to increase packouts of apples

PI: Ines Hanrahan
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Address: 104 N 1st St., Suite 204
City/State/Zip: Yakima, WA, 98901

Cooperators: Tory Schmidt, Felipe Castillo, Tom Auvil, Jim McFerson, WTFRC, Wenatchee, WA

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

Item	2009	2010	2011
Salaries	19,860	16,000	16,000
Benefits	9,346	5,120	5,120
Wages	6,740	7,000	7,000
Benefits	1,170	1,200	1,200
Equipment+supplies	2,190	2,200	2,200
RCA rental	18,270	12,600	12,600
USDA rental	750	750	750
Travel	4,000	4,000	4,000
Total gross costs	63,326	48,870	48,870
Reimbursements	(29,000)	(14,000)	(14,000)
Total net costs	**33,326**	**34,870**	**34,870**

Footnotes:

Salaries:	include proportional time spent on outlined projects for Hanrahan, Castillo, Schmidt, Auvil
Wages:	covers timeslip expenses
RCA rental:	numbers based on fiscal year (@ approx. $6,300/room/year)
USDA rental:	access to packingline and storage space for equipment
Travel:	fuel costs to travel to and from trial sites and vehicle maintenance
Reimbursements:	monetary contributions by chemical suppliers
Other:	all chemicals were donated by industry suppliers

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

OBJECTIVES

1. Compare sunburn protectant efficacy in apple.
2. Determine effect of Raynox on development of delayed sunburn in storage.
3. Determine long term storage regimen for Honeycrisp apples.
4. Devise methods to minimize disorder development when storing Honeycrisp apples.
5. Determine maturity indicators that best correlate to long term storability of Honeycrisp apples.

SIGNIFICANT FINDINGS

1. Sunburn protectants tested in 2010 did not increase the percentage of sunburn-free fruit.

2. After six months of storage, up to 50% of fruit showed delayed sunburn; Raynox treated apples exhibited 30% less delayed sunburn.

3. Honeycrisp apples were stored sucessfully in CA for up to 8 months.

4. Disorder development was minimized when storing slightly immature Honeycrisp at 50F for one week, followed by CA storage (0.5% CO_2, 1.5% O_2).

5. Under long-term storage conditions, the best performing Honeycrisp apples display the following traits: high titratable acidity, light green background color, sufficient red color, and some residual starch levels.

METHODS

Sunburn suppression: Three contract sunburn trials subsidized by private chemical companies were established in 2010 (Granny Smith in Prosser and Wapato, Golden Delicious in Manson) testing 3 sunburn protectants (Raynox, experimental Pace, experimental Orcal). All materials were applied five times starting on June 23, according to each product's respective labeled rate. At harvest, individual fruit was graded for sunburn according to the Schrader/McFerson system (0 = clean, 6 = necrosis). Trials were set up to test the onset of delayed sunburn in storage as influenced by Raynox in 2009 (Granny Smith, Manson) and 2010 (Granny Smith, Wapato).

Honeycrisp storage: In 2009, we sampled fruit from 5 mature, annually bearing Honeycrisp orchards in Washington. Harvest timings were one week prior to anticipated first pick (early pick) and first pick (best-storing pick, referred to as standard pick). Fruit was held for 1-3 weeks at 50F before being stored at 36F in regular atmosphere (RA), controlled atmosphere (CA: 0.5% CO_2, 1.5% O_2), or dynamic controlled atmosphere (DCA: 0.5% CO_2, 0.7% O_2) for up to 8 months (Oct. – May). Subsamples of fruit were pulled to evaluate storage performance.

2010 samples are currently being stored. We selected 3 mature, annually bearing Honeycrisp orchards under similar horticultural management. In each location, we marked trees based on crop load: low = 2-3frt./TCSA, medium = 5-6 frt./TCSA, high = 8-9 frt./TCSA and sequentially harvested the trees (up to 3 picks). Fruit is being stored for 4 or 8 months in CA or RA (with or without 1-MCP) under conditions described above.

RESULTS & DISCUSSION

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 in 2007 and repeated the trials in 2008 and 2009 as several new products were introduced to the market (Table 1).

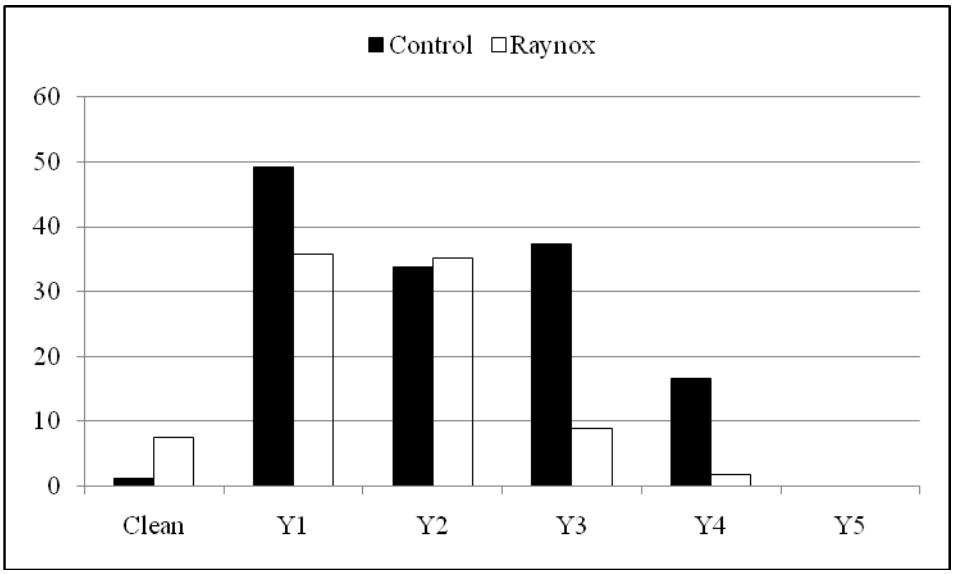
Table 1. Sunburn protectants used in field trials. WTFRC 2007-2010.

Active Ingredient	Commercial product(s)
Plant Wax	Raynox Plus
Kaolin	Surround WP, Cocoon
Talc	Invelop
Calcium carbonate	SunGuard, Eclipse, SunShade
Calcium carboxylic acids	Fruit Shield

In 2010, we scaled back our efforts and did product evaluation for two companies on a contract basis (data not shown). The field trials had varying degrees of sunburn pressure (48 – 91% clean fruit in untreated controls). Low sunburn incidences as observed in both of our Granny Smith trials make it difficult to observe any treatment effects. The Golden Delicious trial had high sunburn pressure. No treatment increased the number of clean fruit in this trial, but all materials reduced the number of tan fruit. No effect on harvest maturity or defects was observed.

In 2009, we followed a set of Granny Smith apples though RA storage to determine if sunburn suppressants applied during the growing season, such as Raynox, would influence the development of delayed sunburn. After six month of storage up to 50% of fruit in each sunburn class showed delayed sunburn (Figure 1). If fruit was clean at harvest, little change happened over time. Raynox treated apples exhibited 30% less delayed sunburn in class Y1, which would likely influence packouts of lower grade fruit.

Figure1: Percent change of fruit in each sunburn class after six months of RA storage as compared to at harvest values. Granny Smith/2009.



Honeycrisp storage: High demand and premium pricing has led to rapid increases in Honeycrisp acreage in Washington State. Most fruit is packed by December and sold by January. However, with rapidly increasing product demand, the marketing window for this cultivar needs to be extended. Successful fruit storage is complicated by several fruit quality problems in storage, including bitter pit and fruit sensitivity to chilling. In 2009 we repeated the basic experiments started in 2008 with particular focus on harvest timing and started to investigate the influence of crop load.

Ranges of several fruit quality parameters for fruit from one orchard, at harvest and after 8 months of CA storage, are shown in Table 2. Honeycrisp is known to retain firmness well; trial fruit lost 0.5 to 0.9 pounds over the 8 month storage period. Titratable acidity decreased significantly over time (see also Figure 4), independent of storage treatment. Most of the loss occurred within the first four months of storage. Some internal browning (4-5% of fruit) developed over time. Other quality parameters measured (SSC, greasiness, soft scald, bitterpit) did not change significantly, indicating that the CA conditions utilized in this experiment were adequate to deal with Honeycrisp's inherent challenges. However, we did observe differences in flavor when eating the apples, with earlier picked fruit tasting better than fruit picked at the 1st commercial pick. Common quality measurements did not pick up flavor differences.

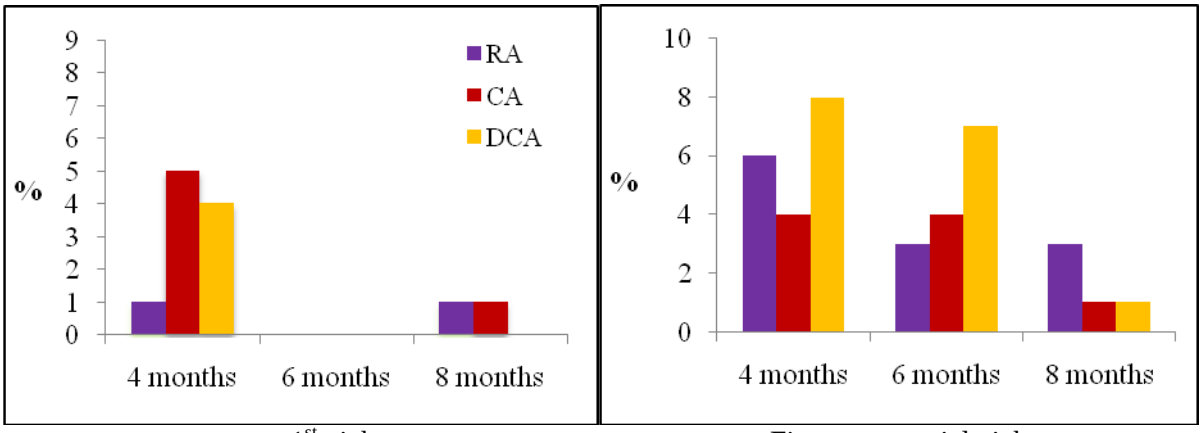
Table 2: Comparison of storage performance of Honeycrisp apples picked at 2 different dates and stored for 8 months in CA storage. WTFRC 2009.

	1st pick	1st commercial pick
Firmness (lbs.)		
at harvest	16.7	15.2
after storage	16.2	15.1
TA (% malic acid)		
at harvest	0.62	0.59
after storage	0.25	0.24
SSC (% Brix)		
at harvest	12.4	12.8
after storage	12.2	12.7
Greasiness (%)		
at harvest	0	0
after storage	0	0
Soft scald (%)		
at harvest	0	0
after storage	1	1
Bitterpit (%		
at harvest	0	14
after storage	1	1
Internal browning (%)		
at harvest	0	0
after storage	4	5

We continued to track disorder development over time and as influenced by storage regimen in 2009 (Figure 2, 3). Soft scald development in the present experiment was influenced by harvest timing.

Earlier harvest lead to less disorder development over time. Generally, after four months in storage, soft scald symptoms were fully expressed, and no significant change over time and/or storage regimen was observed.

Figure 2: Comparison of softscald development of Honeycrisp apples picked at 2 different dates. WTFRC 2009.



1st pick First commercial pick

Internal browning often occurs as soggy breakdown, when fruit is stored too cold or as browning with or without cavity formation, when fruit is held at unsuitable carbon dioxide concentrations. We have not distinguished between the different types of browning in 2009, as we generally had a low disorder incidence. Similar to soft scald, most of the internal browning observed was expressed in fruit from later picks (Figure 3).

Figure 3: Comparison of internal browning development of Honeycrisp apples picked at 2 different dates and stored for 8 months. WTFRC 2009.

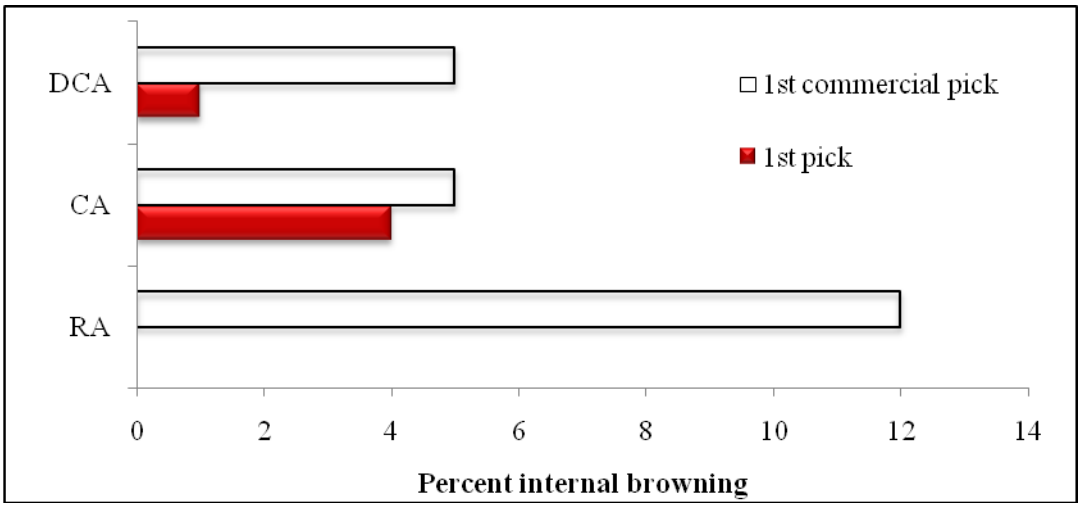
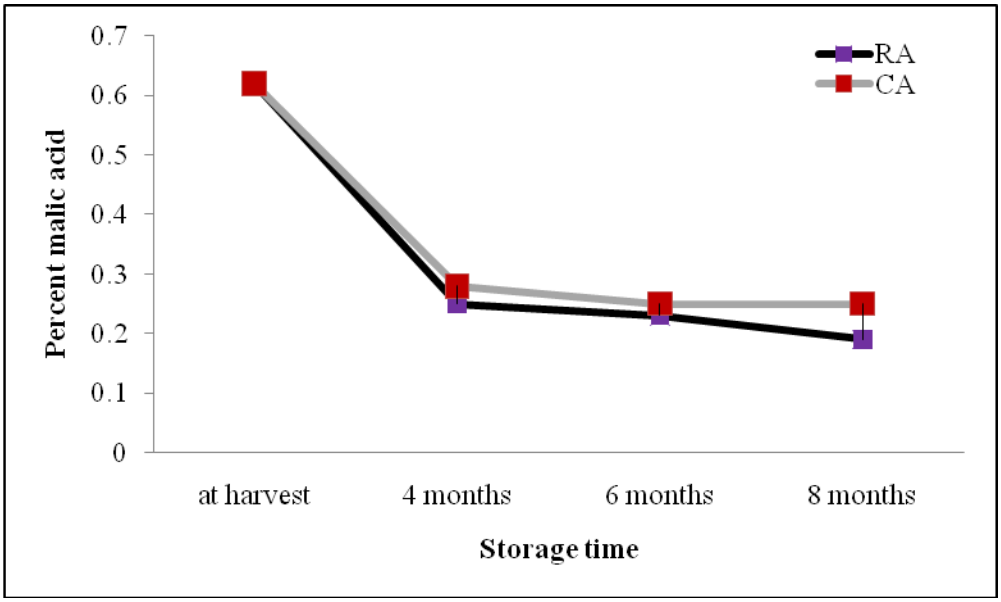


Figure 4: Development of titratable acidity levels in Honeycrisp apples. WTFRC 2009.
1st pick TA 2009



In summary, these results suggest that Honeycrisp apples may be stored for longer periods of time without compromising fruit quality when utilizing specific controlled atmosphere conditions. Under long-term storage conditions, the best performing Honeycrisp apples display the following traits: high titratable acidity levels at harvest (0.600% malic acid), light green background color, sufficient red color development to facilitate early harvests, and at least some remaining starch (5, when using a 1-6 scale). Factors not highly correlated to storage performance are: soluble solids concentration and firmness at harvest. It remains a challenge to relate superior eating quality of fruit from certain lots to any commonly used fruit maturity parameters.

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

Project Title: Validation of fresh apple packing food safety interventions

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City/State/Zip:	Pullman, WA 99164-6376	**City/State/Zip**:	Pullman, WA 99164-6376

Cooperators:

Deborah Carter, Technical Issues Manager of the Northwest Horticultural Council. Contact information: (509) 453-3193, carter@nwhort.org

Several chemical suppliers have donated supplies, other resources and time to discuss aspects of this project and ensure relevancy to the apple packing industry.

Several apple packing facilities have agreed to or expressed interest for participating in packing plant studies to validate interventions to ensure that laboratory results relate to large scale production treatments.

Total Project Request: Year 1: $50,990 **Year 2:** $53,030 **Year 3: 55,152**

Other funding sources

Agency Name: Washington State USDA Specialty Crop Block Grant Program
Amt. awarded: $55,868
Notes: This project supported a literature review, initial laboratory experiments to select methodology for apple inoculation and an educational meeting with the tree fruit industry. The literature review provided information on the state of knowledge regarding antimicrobial interventions for whole, fresh apples, which was found to be relatively limited. Additionally, the review of literature indicated that methodology used in evaluating antimicrobial interventions for apples varied significantly between studies. Therefore, microbial studies were conducted to assist in selection of methods for apple inoculation, such as preparation of apples prior to inoculation, inoculation methods and media and drying time. These experiments developed a foundation for methodology that was utilized in Years 1 and 2 and the proposed work of Year 3 in order to provide the industry with scientific information using standardized methods that will allow for comparison of results. An educational food safety meeting, "Safety of Northwest Produce" was conducted with 100 participants, primarily from the Washington tree fruit industry, and provided important opportunities to discuss research needs with industry representatives.

An equipment donation to WSU with estimated value of $10,000 is expected to arrive in January 2011.

WTFRC Collaborative expenses: None

Budget 1

Organization Name: WSU **Contract Administrator:** Mary Lou Bricker

Telephone: (509) 335-7667 **Email address:** mdesros@wsu.edu

Item	2009	2010	2011
Salaries	22,014	22,895	23,811
Benefits	1,560	1,622	1,688
Wages	5,100	5,304	5,516
Benefits	816	849	883
Equipment	0	0	0
Supplies	16,500	17,160	17,846
Travel	5,000	5,200	5,408
Total	$50,990	$53,030	**$55,152**

Footnotes:

Objectives:

1) Perform laboratory validation studies to examine foodborne pathogen and indicator organism reduction by antimicrobial treatments currently used in the apple packing industry

2) Validate antimicrobial interventions under industrial packing line conditions using indicator organisms

3) Conduct appropriate food safety extension outreach with the apple packing industry

Significant Findings:

- Our study to date represents the most robust examination of the effectiveness of peroxyacetic acid (PAA) on whole, fresh apples using industry-relevant concentrations and exposure durations as indicated by currently available scientific literature.

- In Year 2, laboratory experiments focused on peroxyacetic acid (PAA). To meet industry needs, the study design was altered to examine PAA concentrations (between 40-80 ppm) using a direct PAA application time of 5 seconds followed by 4 exposure times (10, 25, 40 or 60 seconds) to represent time of exposure to PAA after the spray bar until fan drying begins.

- Laboratory results of Year 2 indicate that concentrations and exposure durations of PAA currently used in the industry can result in at least a 0.5 – 1.3 $\log_{10}$ reduction of *E. coli* O157:H7, a pathogenic strain.

    - At 40 ppm PAA, reduction in *E. coli* O157:H7 ranged between 0.5-1 $\log_{10}$ and a 0.8-1.3 $\log_{10}$ reduction in generic *E. coli* was observed.

    - At 60 ppm PAA, reduction in *E. coli* O157:H7 ranged between 0.8-1.2 $\log_{10}$ and a 0.9-1.6 $\log_{10}$ reduction in generic *E. coli* was observed.

    - At 80 ppm PAA, a 1.2-1.3 $\log_{10}$ reduction was observed in *E. coli* O157:H7 and a 1.3-1.6 $\log_{10}$ reduction in generic *E. coli* was observed.

    - Duration of exposure after direct application in the laboratory did not appear to significantly affect microbial reduction. Laboratory data would indicate that exposure time during conveyance after PAA direct application does not result in further microbial reduction. However, use of brushes in an industry setting may result in differences between laboratory and packing line data, emphasizing the need for experiments that reflect typical packing conditions.

- Laboratory results of Year 1 indicate that increasing direct PAA application time from 5 seconds to 30, 60 or 120 seconds would increase reduction of *E. coli* O157:H7 to 1.5 – 2 $\log_{10}$. Many food safety experts utilize a 2-3 log reduction as a benchmark when evaluating the effectiveness of a single antimicrobial intervention in a given process.

Methods:

Laboratory Validation Studies. In Year 2, to maximize industry-relevant results, further examination of peroxyacetic acid was performed. Industry input during the Year 1 report indicated that application times examined in Year 1 were longer than typical applications on industry packing lines. Therefore, application times were adjusted in Year 2, based on industry input and plant visits to observe typical application times for peroxyacetic acid application at the spray bar. Input and

observational plant data indicated that the shortest application time directly under the spray bar was approximately 5-10 seconds, followed by continued exposure to PAA for 20-60 seconds on a conveyance system followed by fan drying. Therefore, in the Year 2 study design, apples were placed directly in PAA for 5 seconds followed by removal and 4 exposure times (10, 25, 40 or 60 seconds) to mimic time on a conveyance system. In Year 1, apples were placed directly in PAA for 30, 60 and 120 seconds.

A study was designed to predict microbial reductions at concentrations and exposure durations throughout the range of those specifically tested (40-80 ppm and 10-60 seconds of exposure after 5 seconds of application). In each experiment, 16 treatment combinations were examined with 5 apples in each of the 16 treatment combinations. Both *E. coli* O157:H7 and generic *E. coli* were examined using this design. Three replications were performed. Preliminary statistical analysis was conducted, but further analysis is necessary.

In Year 2, an agreement with a supplier was negotiated for WSU to receive equipment necessary for studies with chlorine and chlorine dioxide, with delivery expected in January 2011. Relationships with chemical suppliers were established to provide supplies. In Year 3, chlorine and chlorine dioxide will be investigated, aligning with recommended manufacturer concentrations, standard industry practices and published literature (Rodgers et al., 2004; Suslow, 2004).

A series of experiments focusing on oxidation reduction potential (ORP) of chlorine and chlorine dioxide will be performed. Sufficient quantities of chlorine or chlorine dioxide will be added to adjust the ORP levels of the aqueous solution to 665, 750 and 850. To mimic dump tank application times, apples will be directly exposed to chlorine or chlorine dioxide for 2, 3.5 and 5 minutes. Although apples may be exposed to longer application times in the dump tank, emphasis will be placed on the shortest application times to address minimum microbial reductions achieved for treated apples. To mimic spray bar application times, a design similar to the PAA Year 2 study will be utilized. If sufficient time and resources are available, experiments focusing on specific chemical concentrations will be performed. Chlorine will be examined at 25, 50, and 75 ppm free residual chlorine with efforts to maintain a stable pH, and chlorine dioxide will be examined at concentrations of 3, 5, and 7 ppm with efforts to maintain a stable pH.

Studies will utilize pathogenic and generic *E. coli* using a similar experimental design as described above with at least twelve treatment combinations being examined. Inoculated apples will be exposed to at least three selected concentrations of the antimicrobial treatment and three selected exposure durations. After antimicrobial treatment, apples will be rinsed and serial dilutions will be performed. Appropriate dilutions will be plated on sorbitol MacConkey (SMAC) agar for enumeration of *E. coli* O157:H7 and violet red bile agar (VRBA) for enumeration of generic *E. coli*. Plates will be incubated at 95˚F and enumerated manually or by an automated Q-Count system.

Packing Line Studies. It is critical to validate the effectiveness of antimicrobial treatments using conditions that reflect industrial packing systems. Wang et al. (2007) demonstrated that flow velocity and agitation contributed to microbial reductions, and these conditions are difficult to accurately simulate in the lab. Several apple packers have either committed to or have expressed interest in participating in industrial scale line studies using generic *E. coli* to validate individual intervention steps or an entire packing system. The project team will communicate with industry partners and chemical suppliers to determine appropriate study design for packing line studies. It is anticipated that individual antimicrobial intervention steps will be examined first, such as PAA at the spray bar or chlorine in the dump tank, to establish the contribution of each antimicrobial intervention separately. If time and resources allow, examination of antimicrobial reductions achieved by a whole packing process including washing, rinsing, antimicrobial applications and drying will also be pursued.

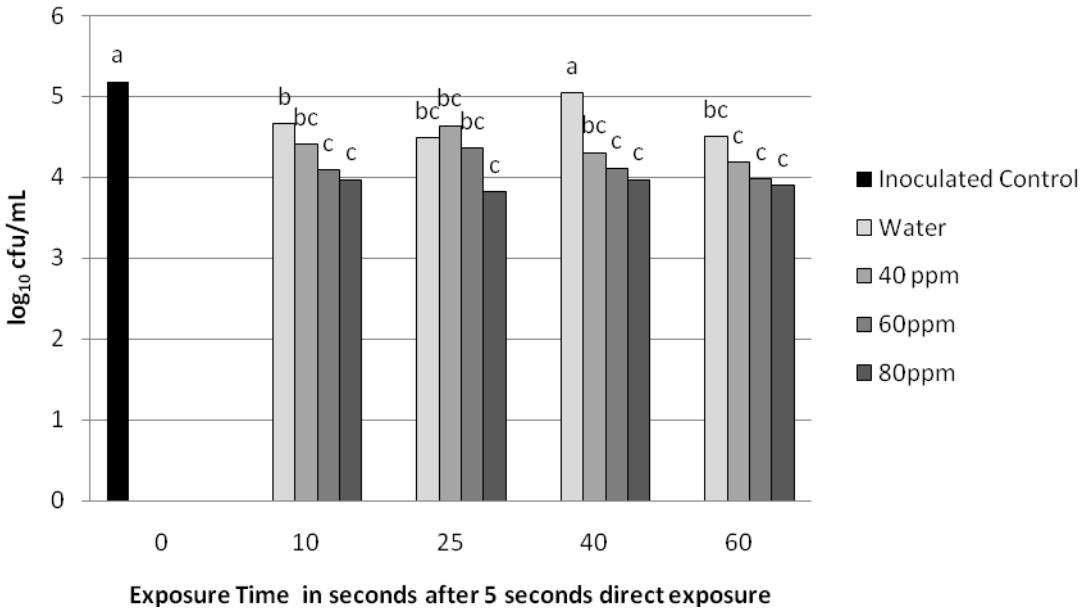
Food Safety Outreach and Education. Presentations at relevant industry meetings will be given priority. Additionally, potential extension publications will be discussed to provide producers and packers with relevant information.

Results and Discussion:

Validation Methodology. Initial microbial studies on appropriate methodology for antimicrobial validation indicated that inoculated apples should be unwaxed and rinsed in water prior to inoculation. The most effective method of microbial inoculation was determined to be immersing and massaging the apples in an inoculation solution for 10 minutes rather than a spot inoculation at the stem end. Optimal drying time for bacterial attachment was determined to be 1 hour. The observed inoculum level on the apple of 100,000 cells is sufficient to demonstrate up to a 4 $\log_{10}$ reduction achieved by antimicrobial treatments without exceeding the detection limit for the method used for microbial enumeration.

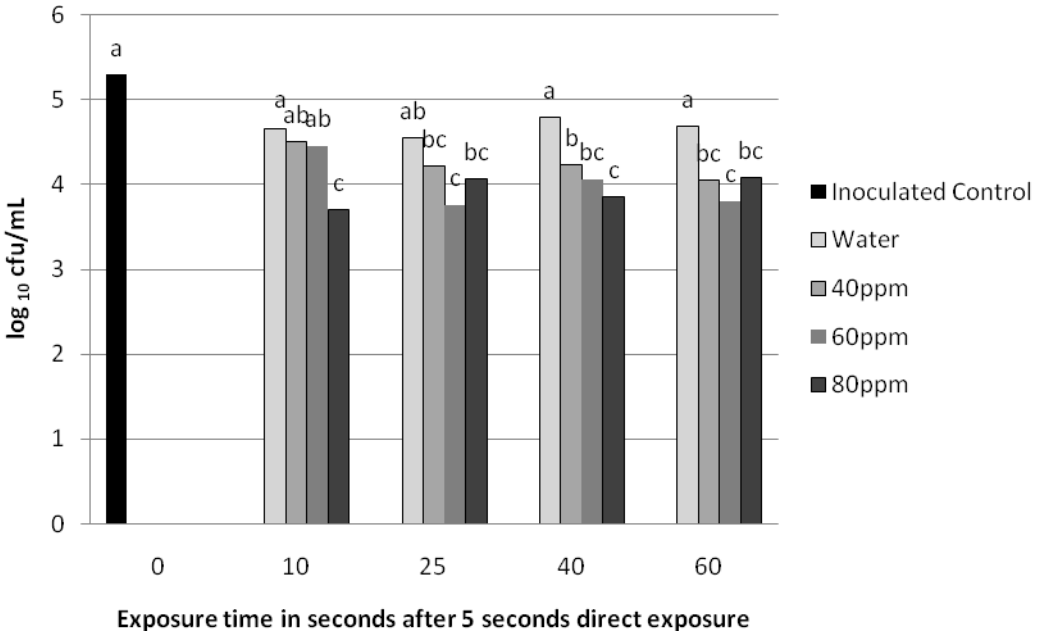
Peroxyacetic Acid (PAA) Validation Laboratory Study. The laboratory results of Year 2 indicate at concentrations and exposure durations currently used in the industry, PAA treatment can result in at least a 0.5 – 1.3 $\log_{10}$ reduction of *E. coli* O157:H7 (Figure 1). At two levels of exposure time (25 and 60 seconds) the reductions achieved by PAA treatments were not significantly different than the reductions achieved by water. In Figure 2, slightly greater reductions were observed for generic *E. coli* (0.8-1.6 $\log_{10}$ reduction). Generic *E. coli* appeared to be slightly more sensitive to PAA treatments than the pathogenic *E. coli* O157:H7 strains. Comparison of pathogenic and generic *E. coli* response allows for the opportunity to examine microbial reductions under packing line conditions where only generic *E. coli* can be utilized. Therefore, data collected in plant validation studies using generic *E. coli* will be adjusted to acknowledge that reduction of pathogenic strains may be slightly less (approximately 0.3-0.6 $\log_{10}$) than observed with generic *E. coli*.

Figure 1. Average *E. coli* O157:H7 levels on apples after inoculation, direct application of PAA (concentrations 40, 60 and 80 ppm) for 5 seconds and exposure times of 10, 25, 40 and 60 seconds. Values reported in $\log_{10}$ colony forming units (cfu)/ml scale (5=100,000 cfu/ml, 4=10,000 cfu/ml).



[a-c] Values that do not share a common superscript differ significantly ($p<0.05$)

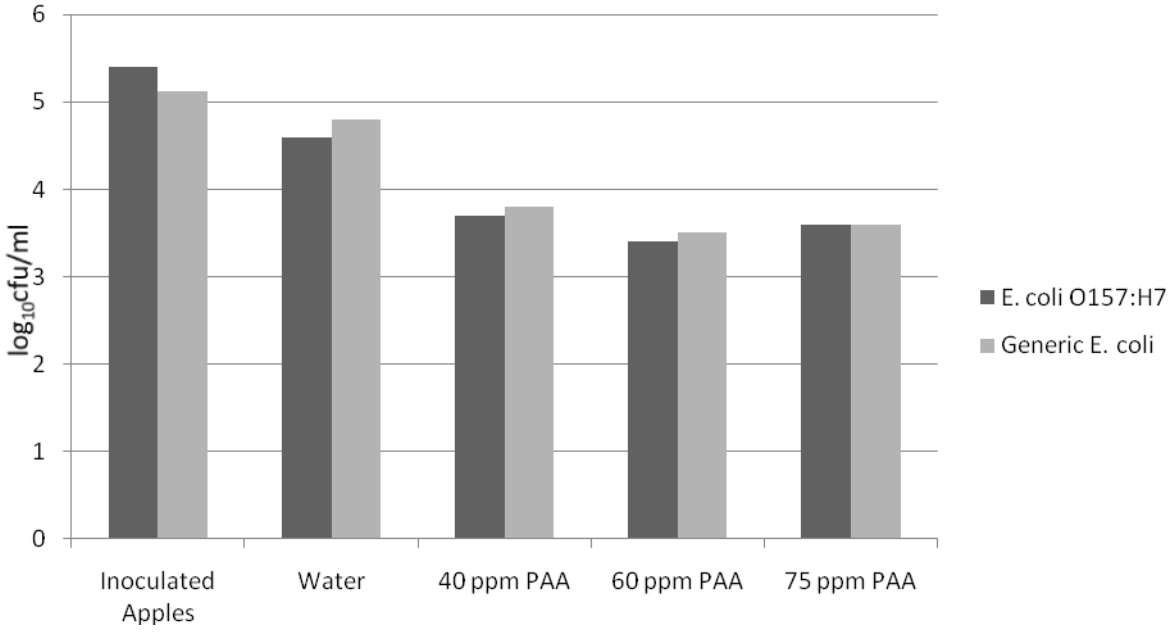
Figure 2. Average generic *E. coli* levels on apples after inoculation, direct application of PAA (concentrations 40, 60 and 80 ppm) for 5 seconds and exposure times of 10, 25, 40 and 60 seconds. Values reported $\log_{10}$ colony forming units (cfu)/ml scale (5=100,000 cfu/ml, 4=10,000 ml).



[a-c] Values that do not share a common superscript differ significantly ($p<0.05$)

The laboratory results of Year 1 (Figure 3) which utilized longer direct application (30-120 seconds) of peroxyacetic acid demonstrated a 1.5 – 2 $\log_{10}$ reduction of *E. coli* O157:H7 (Figure 3). Moreover, the PAA treatments examined in Year 1 appeared to be more effective than treatment with water, whereas the treatments examined in Year 2 were similar to water in some instances. Due to the logarithmic scale of the data, the magnitude of the 1 $\log_{10}$ reductions observed in Year 2 (equivalent to a 90% reduction) compared to the Year 1 reductions of up to 2 $\log_{10}$ (99% reduction) is an important difference. Most food safety experts consider a 2-3 $\log_{10}$ reduction an effective antimicrobial intervention. Comparison of data from Years 1 and 2 indicates that longer direct application of peroxyacetic acid would increase microbial reductions and reduce pathogen risk.

Figure 3. Average *E. coli* O157:H7 and generic *E. coli* levels (log_{10} colony forming units/ml, cfu/ml) on apples after microbial inoculation and treatment with 40, 60 and 75 ppm peroxyacetic acid (PAA). Values are averaged over application times of 30, 60 and 120 seconds. Values are reported in log_{10} scale (5=100,000 cfu/ml, 3=1000
cfu/ml).



Peroxyacetic Acid Plant Validation Study.

Plans for an in-plant validation study were made; however, due to a late harvest season, the timeline for in-plant studies was adjusted. Experiments can begin early in 2011 as soon as arrangements with participating plants are finalized. A discussion guide regarding approaches for in-plant studies was developed for distribution to interested industry partners. Several additional industry partners have been identified for study participation. Conversations to develop in-plant validation studies are ongoing.

Summary.

These results provide valuable data to packers who are asked to demonstrate the effectiveness of food safety interventions through validation studies to third-party auditors, customers and regulators. The ability to document the effectiveness of current antimicrobial treatments as a food safety intervention for apple packing is limited due to a lack of scientifically reviewed data for use as supporting documentation. Most data currently available has not been performed at concentrations or durations of exposure relevant to the fresh, whole apple packing industry (Wang et al., 2007; Rodgers et al., 2004; Wisnieskey et al., 2000) or has been performed on other produce items. Economic benefits include the ability to demonstrate to customers that food safety interventions address potential food safety risks. Furthermore, trade associations can utilize the data to demonstrate control of food safety hazards and promote whole, fresh apples as a low risk produce commodity. Finally, the economic benefit to preventing an outbreak in whole, fresh apples is tremendous as this type of event would result in devastating economic losses for the entire industry.

A network between research and extension faculty, research commission and trade association representatives, several apple packing industry representatives and chemical suppliers continues to strengthen research and extension efforts. This network proved instrumental to

determining study design, chemical application levels and relevant industry practices for project implementation.

References:

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