APPLE COMMITTEE

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

Project Title: Modeling Washington apple bloom phenology and fruit growth

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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 Achievements:

- 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)

- Fruit diameter measured throughout growing season at 11 Red Delicious, 10 Gala, and 9 Cripps Pink blocks; fruit length also recorded in Red Delicious blocks (Table 1)

- Growth data is being modeled using a 3 parameter Richard's curve using non-linear mixed modeling approach accounting for the repeated observation on the apples (Table 2); the model predicts data well, but seasonal differences (between 2010 and 2011) indicate further refinement would be beneficial; analysis of 2011 data, including temperature data, is ongoing

- Bloom data is modeling using ordinal logit model with each bloom phase as a response. Here we model the phase of growth depending on the days after ½ inch green phase; 2010 data shows promise for this model (Tables 4-7); analysis of 2011,including temperature data, data is ongoing

- Accuracy of bloom phenology models were improved by factoring in degree days relative to two temperature thresholds, 32F and 50F (Tables 4-7)

- New proposal developed for beta-test evaluation and incorporation of models onto WSU AgWeatherNet (AWN) under leadership of Hoogenboom and Salazar

Project Timeline:

2009

- Project team assembled with WSU Extension and WTFRC internal staff
- Jim Olmstead (WSU) and Tory Schmidt (WTFRC) designated as co-PIs
- Field sites secured throughout Central Washington for data collection
- First season of bloom phenology and fruit growth data collected
- Olmstead left WSU; Schmidt assumed project leadership with help from Karen Lewis (WSU)
- Statistician for project (Nairanjana Dasgupta – WSU, Pullman) recruited

2010

- Data collection protocols revised based on recommendations from statisticians
- WTFRC internal staff assumed bloom observation & data collection at all but one site
- Second season of bloom phenology and fruit growth data collected
- Automated field cameras proved inadequate for bloom phenology observations
- Preliminary models built by statisticians

2011

- Third season of bloom phenology and fruit growth data collected
- WSU DAS deferred model validation & beta testing to WSU AWN
- Models updated/improved to incorporate new data, including temperature/degree days
- Hoogenboom and Salazar (WSU AWN) join team to develop new project proposal

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 and 2011 seasons (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; weather data was dumped from the data loggers in June and collated for transfer to the statisticians.

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 with 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. Weather data was gathered from Hobo data loggers after harvest at each site, collated, and submitted to the statisticians for analysis. As with bloom phenology, fruit growth protocols (sample size and intervals) were modified for 2010 and 2011 based on recommendations from statisticians.

Table 1. Roster of sites utilized for apple bloom phenology observations and fruit growth measurements. 2009-2011. (RD = Red Delicious, CP = Cripps Pink, G = Gala)

LOCATION	GROWER	CVs	ELEV (ft)	STAFF	FRUIT GROWTH
Omak[1]	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

[1] Omak site discontinued in 2011 after departure of Suverly from project

Results & Discussion:

Data collection. Despite the losses or revised roles of team members over the course of the project, we successfully collected 3 seasons of solid data to help construct these models. Because we had to initiate the project without the counsel of an experienced statistician or modeler, our 2009 data collection efforts reflected some inefficiencies that were corrected for the 2010 and 2011 seasons. Based on recommendations from Dasgupta and her students, the following changes were adopted:

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

While the 2009 data has value, it is not as complete or robust as 2010 and 2011 data in terms of statistical strength.

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 could 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 in 2010. Unfortunately, the effective focal ranges for these cameras are 5+ feet; images taken of branches inside that range proved too blurry to be useful and branches that were in adequate focus were too far from the camera to discern details of individual buds or flowers.

Modeling. *Fruit growth:* Our pilot data from 2009 and literature survey indicated we use 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):

Where: Y_{ji} represents the growth for apple j at location i,
X_{ji} denotes the time in Julian Days,

β represents the maximal growth reached by the fruit,
δ represents the growth rate
τ represents time when maximum growth occurred.

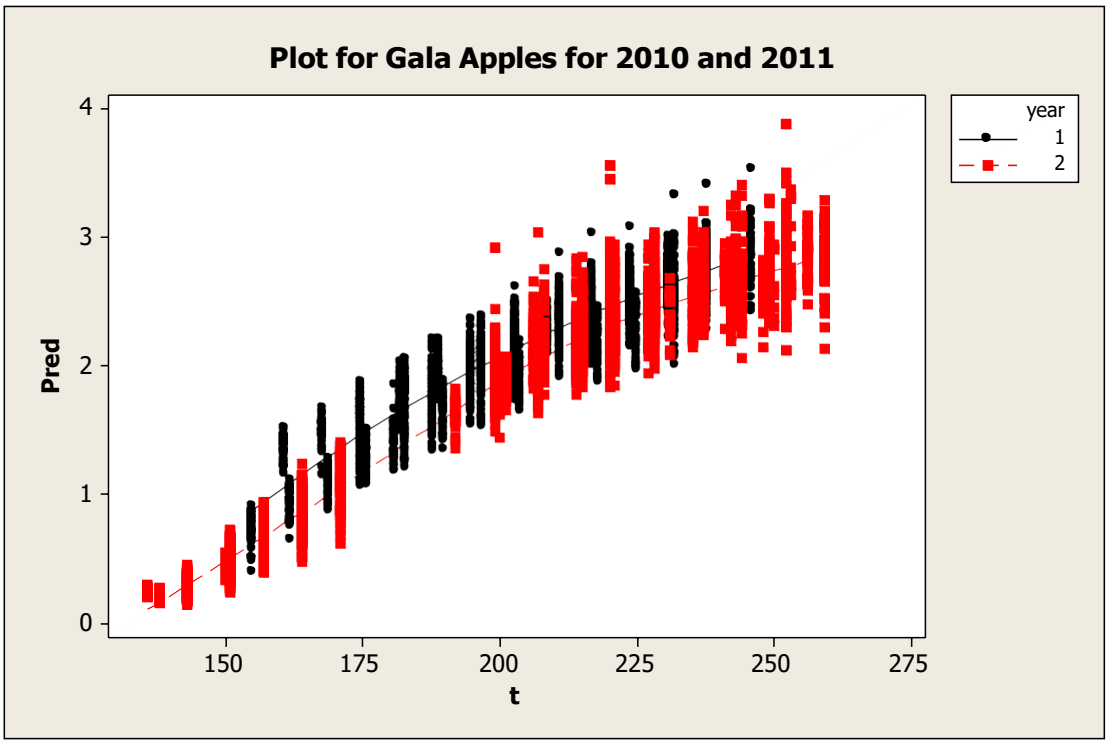
As these parameters all have physical meaning in the context of apples we decided to use this model. Our initial finding showed significant differences across sites. To compare across sites we used a technique that we developed (Many-to-one comparisons for Apple Growth, Dasgupta and Shaffer, submitted to *Journal of Applied Statistics*).

The 2009 growth data was used mainly to get an idea about the scheme for data collection and was used mainly as the pilot study for this project. However data for 2010 and 2011 were used actively for the modeling purpose. In both years we ran the Richard's curve model given earlier. One reason for this choice was the easy interpretability of the parameters as mentioned in the modeling section.

Table 2. Gala data for 2010 and 2011 over all locations

parameter	Estimates from 2010	Estimates from 2011	Estimates combining the two years
β (maximal growth)	3.16	2.68	2.73
δ	179.34	182.38	177.25
τ	.03	.047	.045
Variation due to apple	.093	.1355	.1105
Random variation	.0088	.0034	.06

Table 2 indicates that there were differences in the parameters across both years. In 2010 the estimated maximal size of the apple was predicted bigger than that in 2011. Combining the two years gives us less total variability, but it may not be prudent to combine the data from two different years. The graph below shows the predicted values for Gala for 2010 and 2011 (as year 1 and 2). It can be seen that the Gala apples in 2010 were larger than their 2011 counterparts.



Bloom phenology: We fitted an ordinal logistic regression with the stage as our response variable and put in Julian date, various temperature readings, and location as explanatory variables in the model. This analysis uses a proportional odds model with cumulative logit link and is defined as

_____

where i represents the first 7 categories (bloom stages) which are compared to the 8th stage. The intercept is different for each stage, however the model assumes common slopes between each of the k-1 = 7 regression lines, meaning the cumulative logit comparing the first 7 stages to the 8th stage increases at the same rate across all predictor variables.

We had at first wanted to have a model that incorporated cultivar as an explanatory variable, but we realized that there was interaction between cultivars and Julian date and this would further complicate our model. In Table 3, we see that the "most likely" stage of the cultivar based on Julian Date is different across the cultivars. We computed the "most likely" stage using our observed data over all the locations.

Table 3. "Most likely" stage based on Julian date that was used in the exploratory phase

Cultivar	Red Delicious	Gala	Cripps Pink
Stage			
Green Tip	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

From the table it is clear that between cultivars there is large variation for the days they are in a particular stage, though some of this variability may be due to locational differences. Because growers would be interested in predicting the stages of the cultivars separately, it was decided to obtain a model for each different cultivar. Hence we ran our model for different combinations of temperature, location and Julian date to obtain the simplest model with the best predictions. The purpose was to see if good predictions could be obtained without the location variable as that would make the models more general and more usable by the growers who might not be in a specific location used in our study.

The best models determined by the goodness of fit tests were compared to one another in the context of other measures of model fit. AIC and BIC, which award a model for fitting well but penalize for over overparameterization (BIC penalizes more), R^2 which measures the variability in the data explained by the model, and concordance percent, which is the percent of the stages that were correctly predicted by the logistic model. The results for the best models for the three cultivars are given in tables below:

Table 4. Bloom phenology model performance for Red Delicious

Red Delicious					
Model	Goodness of fit	AIC	BIC	R^2	Concordance (%)
Location, >50, >32, Day	9.317577982	6339.943	6448.135	0.9393	97.2
Location, >50, >32	9.458133537	6339.923	6441.752	0.9393	97.2
Location, >50, Day	9.52692322	6344.385	6446.213	0.9392	97.1
Location, >50	9.52692322	6342.385	6437.849	0.9392	97.1
Day, >50, >32	12.86715709	7234.05	7297.693	0.9207	96
Day, >50	12.59888102	7674.874	7732.126	0.9101	95.7

To see the effect of location we present the following table:

Table 5. Red Delicious predicted stages using "Location, >50, >32" model on the same days across locations

Julian Day	Chelan	Bell	Konnowac Pass	Naches	Olmstead	Smith	Sunrise	Omak
99	B	C	E	B	D	C	C	B
106	C	D	F	D	E	E	D	C
116	E	G	H	F	H	H	G	F

This result is mirrored with the other cultivars with only slight differences between model fits:

Table 6. Bloom phenology model performance for Gala

Gala					
Model	Goodness of fit	AIC	BIC	R^2	Concordance (%)
Location, >50, >32, Day	6.98234159	4892.717	5001.008	0.9567	98.1
Location, >50, >32	6.990776934	4903.697	5005.618	0.9565	98.1
Location, >50, Day	7.605574514	4912.656	5014.578	0.9564	98.1
Location, >50	7.66044118	4914.132	5009.684	0.9563	98.1
Day, >50, >32	12.09768221	6720.874	6784.575	0.9226	96.5
Day, >50	14.78781519	6871.594	6928.925	0.919	96.2

Table 7. Bloom phenology model performance for Cripps Pink

Cripps Pink					
Model	Goodness of fit	AIC	BIC	R^2	Concordance (%)
Location, >50, >32, Day	2.735705285	3749.901	3841.114	0.9533	98
Location, >50, >32	3.030880182	3756.072	3841.204	0.9531	97.9
Location, >50, Day	3.344702934	3854.65	3939.782	0.951	97.8
Location, Day, <32	3.452095538	4271.446	4456.578	0.9388	97.1
Location, >50	3.195721452	3878.671	3978.671	0.9505	97.8
Day, >50, >32	7.802714615	5408.775	5469.584	0.9072	95.4
Day, >50	10.85301005	5911.777	5966.504	0.8878	94.7

The overall finding from the 2010 bloom data is that bloom is best predicted using location, Julian date, temperature over 50 and temperature below 32 as predictors.

Beta testing/web integration. As stated in Objective 3, our original hope was to develop preliminary models that could be evaluated on the WSU Decision Aids System (DAS). While the DAS team has been supportive of developing these two models, they expressed some reservations regarding their lack of experience with validation of horticultural models and suggested that working with Gerrit Hoogenboom and the AgWeatherNet (AWN) team might better serve our needs. In fact, Hoogenboom would have been an obvious choice as a co-PI on this project if he were available to us in 2009. Nonetheless, initial discussions with Hoogenboom and Melba Salazar (AWN) have been productive and we have developed a new proposal for the extension of this project to strengthen and evaluate the models using AWN as a platform for beta testing.

EXECUTIVE SUMMARY

This project was initiated in 2009 as a joint effort between WSU Cooperative Extension and the WTFRC internal program to develop a phenologic model which could help industry improve resource management decisions during spring with better prediction of apple bloom development based on weather forecasts. Three cultivars were selected for study: 1. Cripps Pink (early bloomer) 2. Gala (mid-late bloomer) 3. Red Delicious (historical standard). Observation sites for these three cultivars were also used to collect fruit growth data from June drop until harvest to develop models which can predict the growth curves and ultimate harvest size of these fruit.

Data were collected for both models over three seasons (2009-2011) at 11 nodes across Central Washington. Weather data was recorded by data loggers at most sites, as well as AWN stations near all sites. These data were collated, formatted, and submitted to statisticians at WSU (Pullman) for development of the respective models.

Preliminary bloom phenology and fruit growth models show promise, but require more data and further statistical refinement to better incorporate weather data and explore variability between cultivars, sites, and years. Data analyses indicate that incorporation of degree days relative to two temperature thresholds (32F and 50F) improve the accuracy of model predictions.

After consultation with DAS staff at WSU-TFREC, it was decided to pursue beta-testing and online model integration with AWN. A new project proposal has been submitted to WTFRC detailing this proposed work.

FINAL PROJECT REPORT

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

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Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA
Dr. Vincent P. Jones, Washington State University TFREC

Other funding sources: None

Total Project Funding: $133,500

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	23,900	27,980	29,213
Benefits	11,467	13,424	14,016
Wages			
Benefits			
Equipment			
Supplies	750	750	750
Travel (to Wash. St. orchard sites)	3000	3000	3000
Plot Fees			
Contractual services & repairs	750	750	750
Miscellaneous			
Total	$39,867	$45,904	$47,729

OBJECTIVES:

Our overall goal for 2009-11 was 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 were:

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.

6) Developing model parameters for Red Delicious and Honeycrisp (2011 – 1st year).

7) Continued collaborative effort to develop computer model program and incorporate into DAS or AgWeathernet.

8) Provide continued training to recognize desirable amount of king bloom open to "start the clock".

9) Expand beta field testing of models for Gala, Fuji, and Golden Delicious (3rd year) to include Cripps Pink (Pink Lady).

SIGNIFICANT FINDINGS 2009 - 2011:

- Added to database of temperature effect on pollen tube growth (high temperature range).
- Refined metrics for the actual time required for fertilization.
- Implemented different calculations in the model based upon cultivar differences in time required for fertilization.
- Refined "triggering method" for start of bloom thinning applications.
- Established style length data base for various apple cultivars and also noted the need for developing a real-time style measurement protocol.
- Developed preliminary pollen tube growth model for beta testing on Cripps Pink (Pink Lady).
- Expanded testing of pollen tube growth modeling program in Washington St. sites.
- Added plantings of Jonagold, Granny Smith, and Cameo for future testing.
- Continued growth chamber studies of pollen germination/tube growth.

RESULTS AND DISCUSSION:

Applying our research findings from past three years and results from previous research projects funded by the Washington Tree Fruit Research Commission (2002-08) we were able to expand on the accumulation of data needed to advance the preliminary models used for predicting pollen tube growth and its relation to applying bloom thinners at the proper time. In doing so we are now ready to proceed with validation and implementation of models for beta-testing on the AgWeathernet site. We (Va Tech and AgWeathernet) are presenting a new 3-year research proposal to the Washington Tree Fruit Research Commission to validate the Golden Delicious, Gala, Fuji and Cripps Pink models which have been used for beta-testing at selected Washington sites. Initial validation of our experimental pollen tube model during the past 3-year project was undertaken by the following grower cooperators: Tom Butler (Washington Fruit & Produce Co.), Kevin Larson (Roche Fruit), Harold Ostenson (Stemilt Fruit), Darin Case (Dovex), Tory Schmidt (Washington Tree Fruit Research Commission), Dena Ybarra (Columbia Basin Nursery), Paul Carter (Stemilt Fruit), and Gary Snyder (C&O Nurseries).

Washington Field Testing – 2009-11

The below figures (Figures 1, 3 & 4) show projected pollen tube growth and bloom thinning application dates with spray timings generated using the models for bloom thinning tests conducted at several Washington sites over the past three years (2009-11).

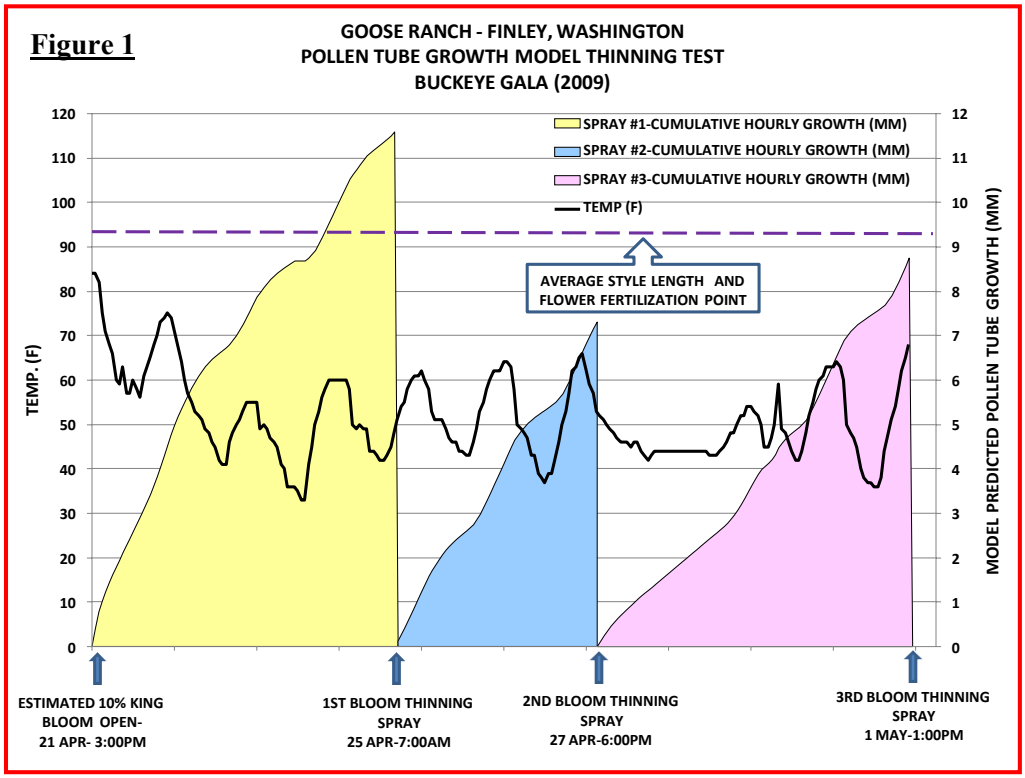
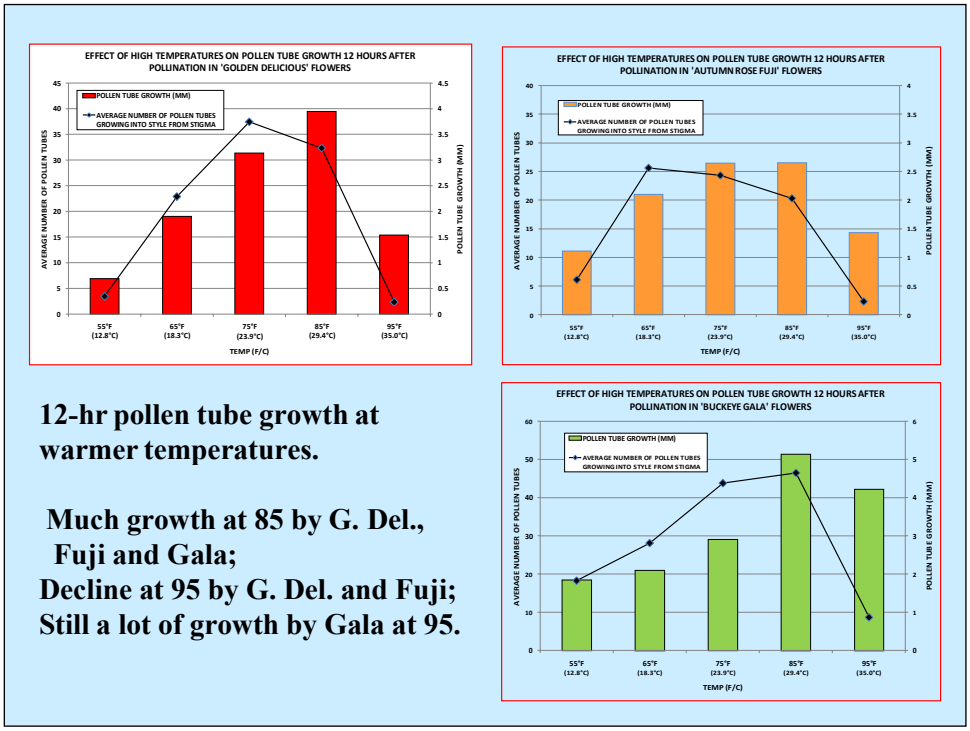


Figure 1 shows predicted rapid pollen tube growth and fertilization of Buckeye Gala king bloom with relatively warm temperatures at Finley in 2009. The first bloom thinning application was intentionally delayed by the grower to allow more set of later bloom in the top half of the trees, knowing that this would result in heavier set of earlier bloom in the bottom.



In 2009, following warm temperatures (85ºF) and rapid petal fall at Finley (Figure 1), we conducted a controlled 12-hr incubation test in the growth chamber (Figure 2), to assess the effects of higher temperatures (75-95ºF) on pollen tube growth. Our results showed significantly greater pollen tube growth at 85ºF by Golden Del. (above, upper left), Fuji (upper right) and Gala (bottom right); There was a decline at 95ºF by Golden Delicious and. and Fuji; but still significant growth by Gala at 95ºF with the apparent optimum at 85º.

Although 2009 was warm as flowers started to open, resulting in fertilization in 55 hr (Figure 3), if bloom had also occurred on April 21 in 2005, the flowers would have been fertilized in only 52 hr.

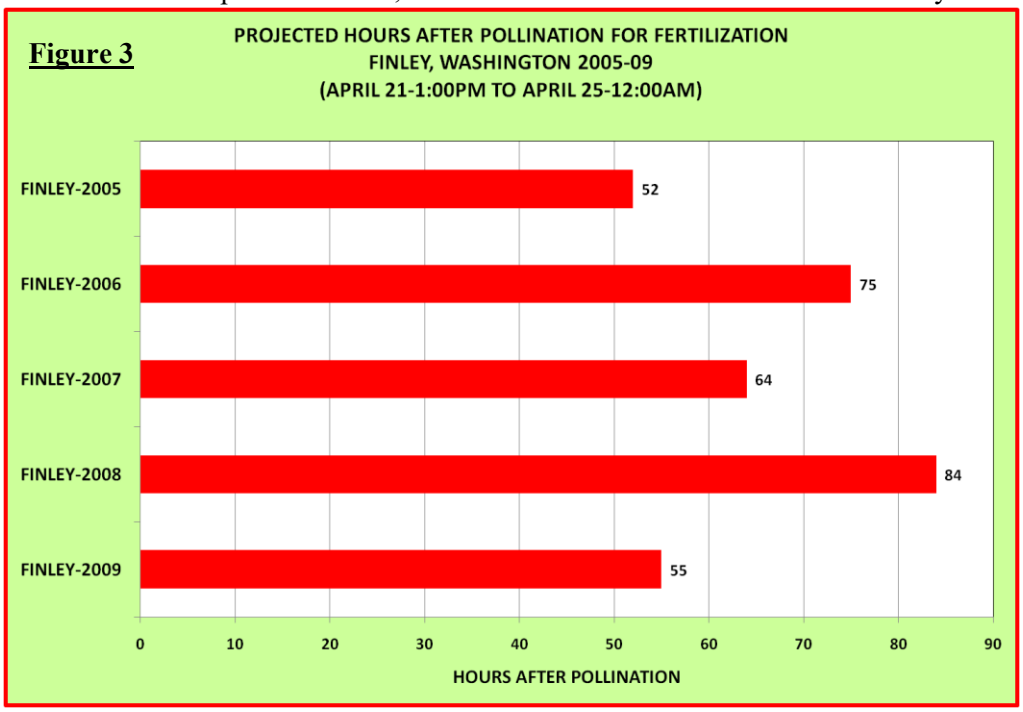


Figure 4 shows the predicted pollen tube growth and timing of bloom thinning sprays April 21-28 at Roche Fruit, Pomona Ranch 2010. The later applications were intentionally timed to prevent set of pollinated blossoms before they were fertilized, thereby reducing the need for hand thinning.

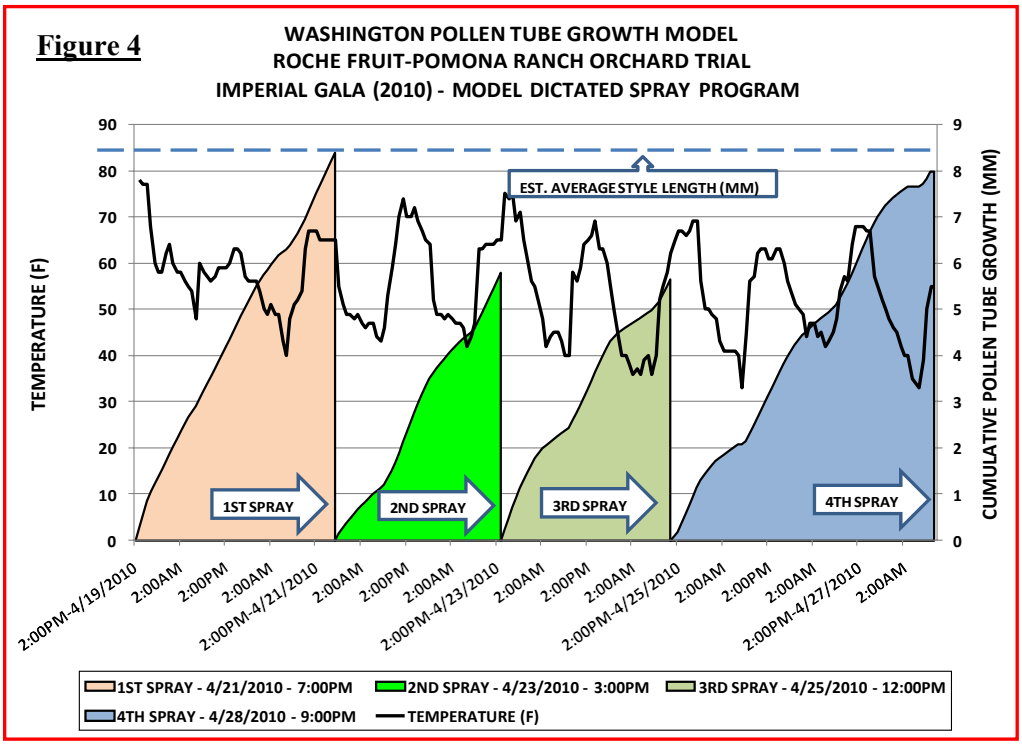
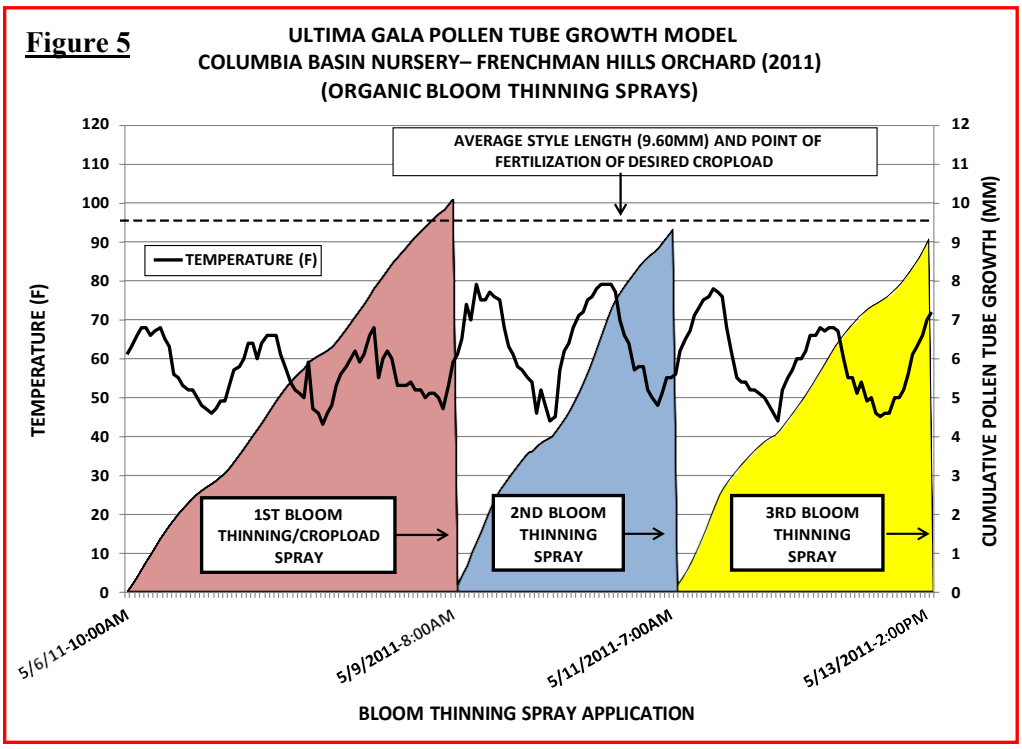


Figure 5



Differences in average length of styles among Gala strains (Figure 6) and Pink Lady (Figure 7) demonstrate the importance of field tests with on-site style length measurements at different locations.

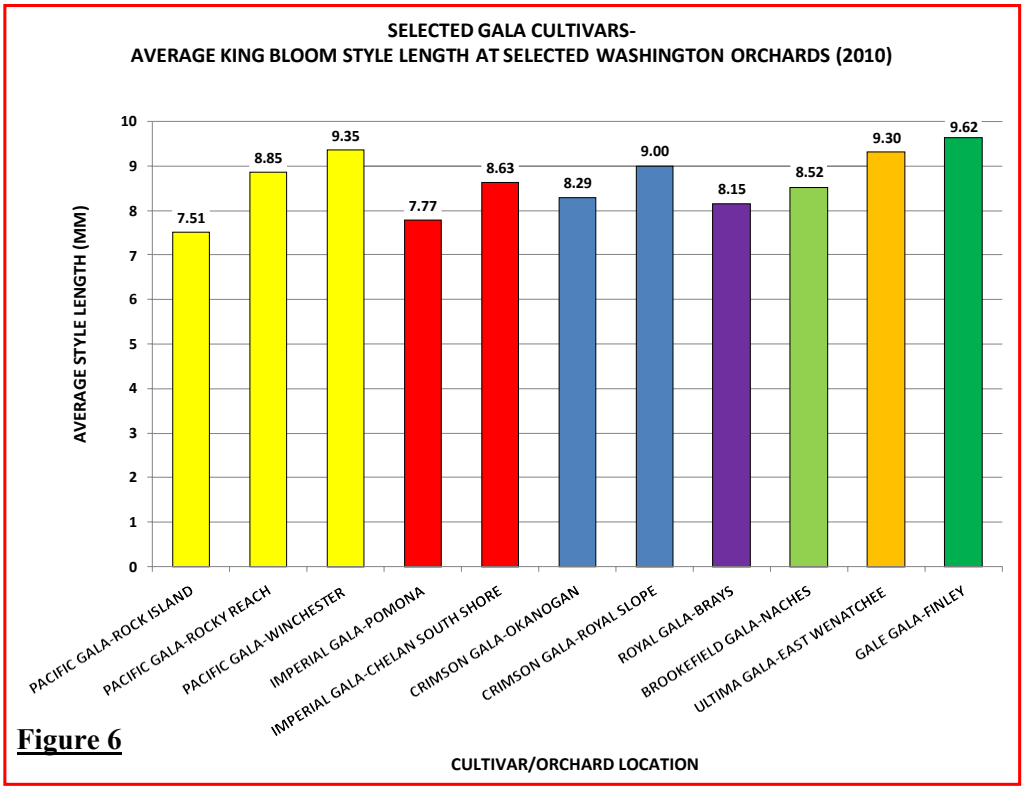


Figure 6

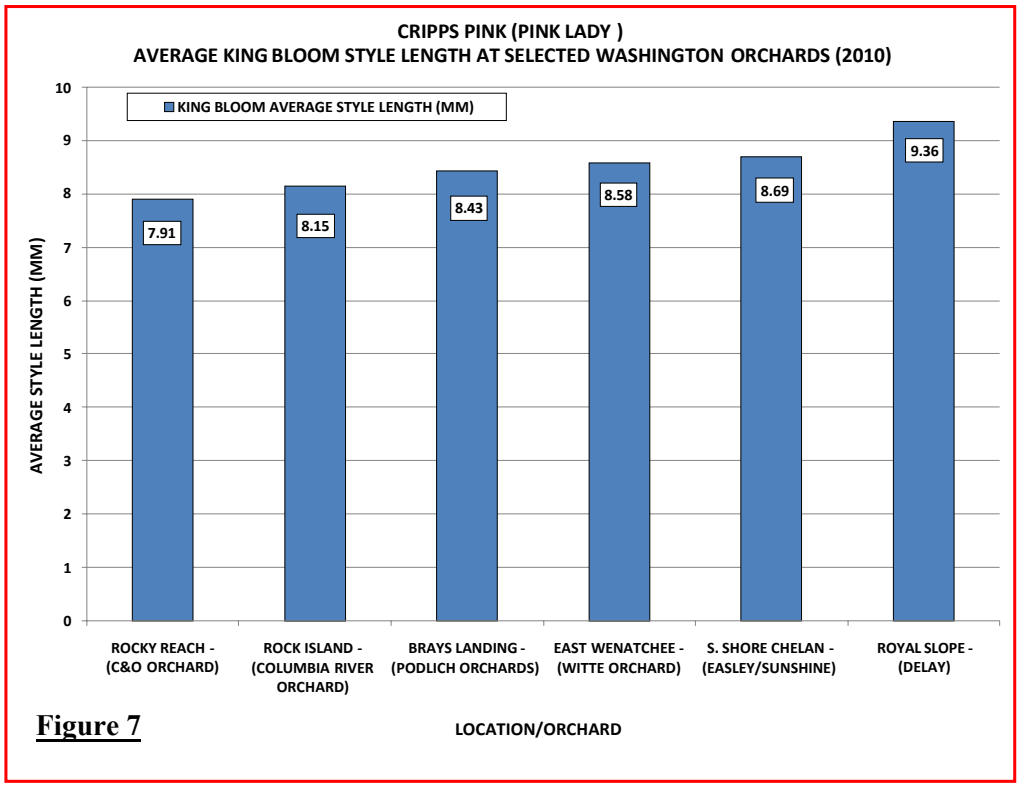


Figure 7

Figures 8 and 9 show the percent of sampled king bloom flowers that had been fertilized and later side bloom that were not yet fertilized after timing thinning sprays using the pollen tube growth model in two test blocks in 2011. Non-organic block (Figure 8) and organic block (Figure 9).

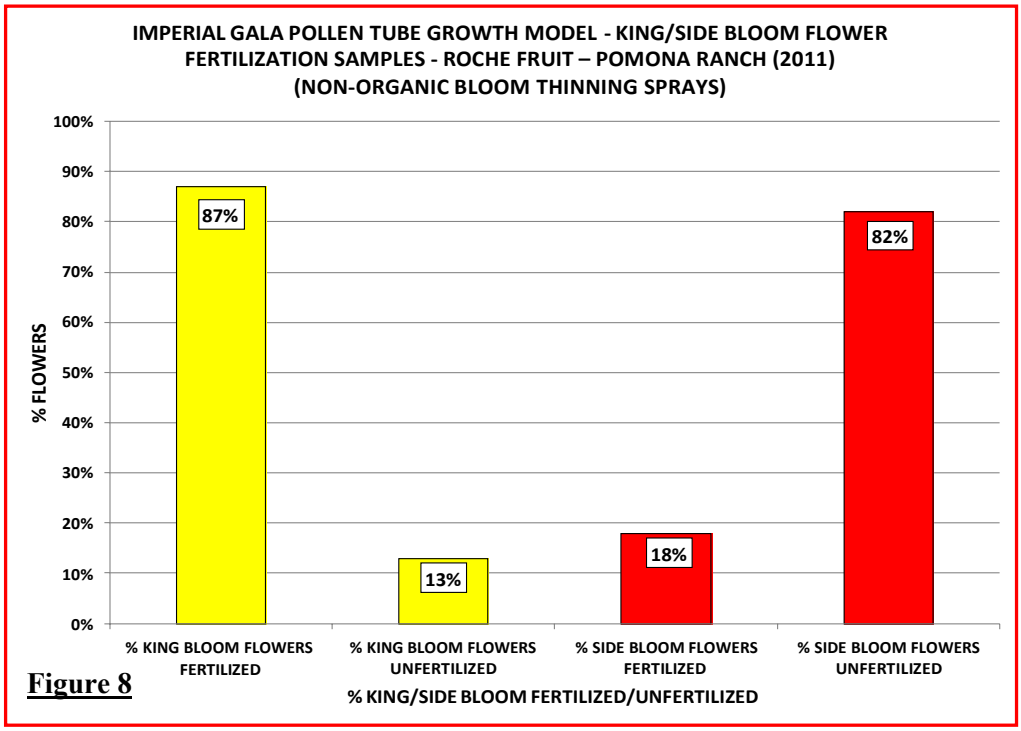


Figure 8

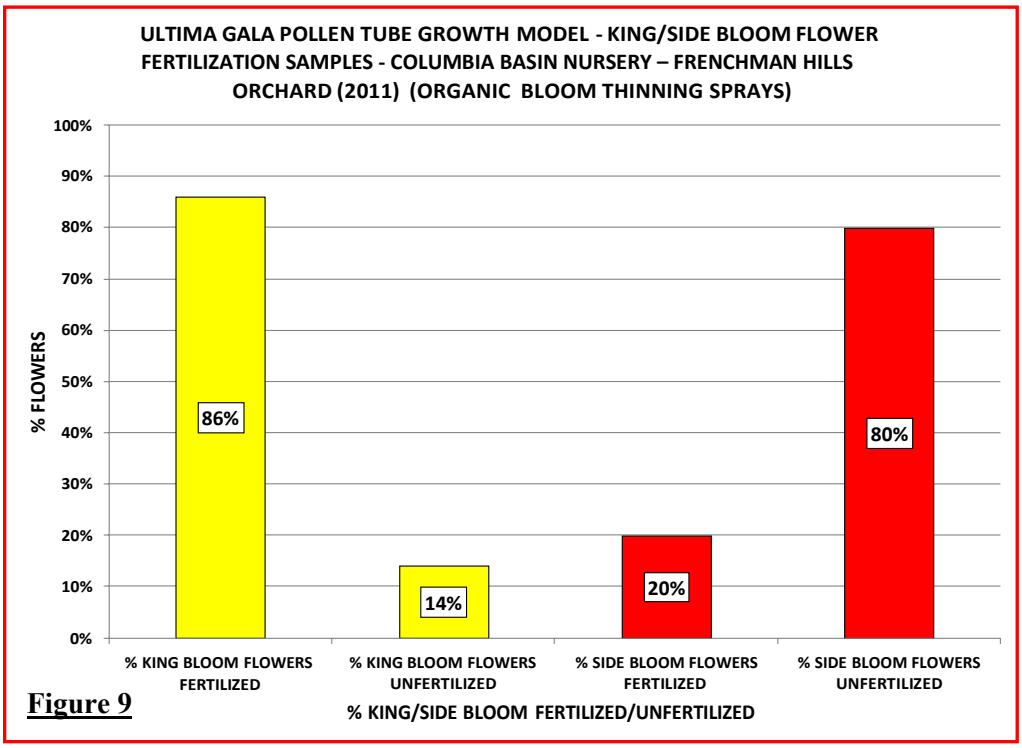

ULTIMA GALA POLLEN TUBE GROWTH MODEL - KING/SIDE BLOOM FLOWER FERTILIZATION SAMPLES - COLUMBIA BASIN NURSERY – FRENCHMAN HILLS ORCHARD (2011) (ORGANIC BLOOM THINNING SPRAYS)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% FLOWERS
86%
14%
20%
80%
% KING BLOOM FLOWERS FERTILIZED
% KING BLOOM FLOWERS UNFERTILIZED
% SIDE BLOOM FLOWERS FERTILIZED
% SIDE BLOOM FLOWERS UNFERTILIZED
% KING/SIDE BLOOM FERTILIZED/UNFERTILIZED

Figure 9

EXECUTIVE SUMMARY

The primary goal of the pollen tube growth model for 2009-11 was to continue studies of pollen germination/tube growth under natural field temperature and light conditions in Washington test sites as compared to laboratory and field experiments in 2005-08. With the help of the listed beta-testers we expanded our testing to include many apple producing regions in Washington. Limited testing has also been conducted in Chile. By using commercial growers located in a wide range of growing conditions, we further validated the effectiveness of the model under numerous field conditions. The cooperating growers were able to assess the model in the field and provided useful feedback on the effectiveness and usability of the model.

Our grower cooperators have provided us with the following feedback:

> "The big value of using the model may be in taking out the guess work on the application timing. Our current method of timing for Gala is to look for 40% and then 90% open flowers. Your model could take a lot of that guess work out if you were planning to treat 2 times or 3 times. It also is a big stress reliever for the managers".
> **Kevin Larson, Roche Fruit**.

> "We have learned a lot from working with you and have managed to incorporate some of what we've learned into our chemical thinning program".
> **Dan Plath, Washington Fruit & Produce**.

> "These Models are great tools; people just have to figure out how to use them".
> **Darin Case, Dovex Fruit Company.**

> "The model work well for us this year". **Dena Ybarra, Columbia Basin Nursery**.

> "Bottom, bottom line is "we are on the right track". I see the future of the model as an important aid to managing crop load and reduction of biennial bearing throughout the industry. I have a strong opinion that the model can, is, and will be an important contributor to increase bloom thinning effectiveness and consistency for our growers".
> **Harold Ostenson, Harold Ostenson Consulting**.

With the positive feedback from our beta-testers, we are now ready to integrate the model on the AgWeathernet site, which will integrate weather data into the model software and provide real time model updates. To accomplish this goal, we have submitted a new 3-year collaborating proposal with Dr. Gerrit Hoogenboom, Director of AgWeatherNet (AgWeathernet). In addition to this goal, we also will continue to add models for more cultivars, such as Honeycrisp, Red Delicious, and Granny Smith. These will be added to the models already in use and being field beta-tested: Gala, Fuji, Golden Delicious, and Cripps Pink (Pink Lady). Additional models can be added over time as deemed relevant by the Washington apple industry.

NEW PROJECT PROPOSAL **PROPOSED DURATION**: 1 year

Project Title: Consulting for the Washington apple breeding project

PI: Fredrick A. Bliss
Telephone: (530) 756-5154
Email: fbliss@dcn.org
Address: 214 Inca Pl.
City/State/Zip: Davis, CA 95616

Cooperators: Jim McFerson, Kate Evans, Cameron Peace, Amit Dhingra, YanMin Zhu, Gennaro Fazio

Total Project Request: **Year 1:** $7,500.

Other funding sources: None

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

Item	**2012**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel	$2,000		
Miscellaneous	5,500		
Plot Fees			
Total	**$7,500**		

Footnotes:

Original objectives:

- Coordinate and lead monthly conference calls among members of the apple team to facilitate discussion of important issues related to apple breeding, genetics and genomics.

- Support the program breeder, Kate Evans and other collaborating scientists through presentation of ideas, evaluation of strategies and plans, assuring focus on commercially-oriented objectives and measurement of progress against project goals.

- Provide analysis and critique of proposals for competitive funding of research and development related to apple breeding.

- Identify other programs, breeders, and scientists in the public and private sectors that can provide collaborative support to the breeding program.

SIGNIFICANT ACTIVITIES AND FINDINGS

- Coordinated conference calls with members of the apple team.
    - Eight conference calls during the year to discuss issues relevant to the apple team activities. Participants included Jim McFerson, Kate Evans, Cameron Peace, Amit Dhingra, YanMin Zhu, Gennaro Fazio.

- Reviewed and critiqued research proposals for apple team members
    - Individual submissions to the NRI competitive grants program
    - Pre-proposals from individuals prior to submission.
    - Funding proposals for apple research to the WTFRC

- Facilitated integration of MAS into the Washington apple breeding program.
    - Worked with Cameron and Kate to critique integration of MAB applications.
    - Reviewed procedures to use molecular markers for selection for important traits in the apple breeding program

- Participated in the workshop on "Improving decision confidence in Apple cultivar development and adoption"
    - Utilized teleconferencing for various sessions, Dec. 5-9, 2011.

- Participated as member of the RosBREED Scientific Advisory Panel, January 2011.

- Provided references to apple team members.

- Participated in the 2010 Apple Research review.

- Submitted invoices for expenditures on a quarterly basis.

RESULTS & DISCUSSION

The monthly conference calls provide a forum for apple team members to bring perspectives from different points of view on important issues and opportunities through group input During each call

there is primary focus on scion and rootstock breeding progress from Kate and Gennaro, respectively along with discussion of activities by other members that impact breeding. Dorrie Main's participation provides updates on her research program at WSU and progress on developing the breeders' toolbox and activities associated with the Genome Database for Rosaceae. YanMin's participation provides a link with his research as well as continuity with activities of the ARS/USDA scientists in Washington State. Cameron is the leader for critical issues related to all aspects of marker assisted breeding. Jim provides information from the WTFRC, W.S.U. and U.S. National Program activities. The collaborative efforts of Amit Dhingra and other research programs world wide have been important to achieve the sequencing the apple genome. The emerging information is being used by W.S.U. and ARS scientists for various projects that contribute to success of the breeding program and other research projects.

The WABP lead by Kate Evans is well managed and new methods, tools and materials are being incorporated to enhance standard breeding practices. There are a large number of promising elite selections in the pipeline that are being evaluated for commercial potential. MAB is being implemented for parental selection and seedling selection with initial use of markers that affect fruit firmness and storability via the ethylene pathways. The program is continuing to make use of the new genomics and genetics resources to enhance selection efficiency and effective evaluation for release of new materials to benefit the Washington apple industry.

It is important to have in place a streamlined procedure for commercializing newly released clones from the breeding program. The many elite selections at stages 2, 3 and 4 will require thorough evaluation for commercial potential. I have helped with exploring how to develop an efficient plan of action to utilize objective phenotypic data from field trials, molecular information and subjective grower evaluations and consumer opinions about important traits and performance for new clone evaluation. The information from the project "Improving decision confidence in Apple cultivar development and adoption" and the workshop held in Wenatchee, Dec. 5-9, 2011 will provide guidance for more effective ways to identify elite selections with high commercial potential.

Work is underway toward implementing a suitable data base (e.g., the Breeder Information Management System (BIMS) concept being developed in RosBreed)for collecting, storing and using information about parents and breeding populations, and identifying market-leading cultivars in target markets for use as standards or checks for comparison.

WSU faculty and ARS scientists continue to play key roles in the RosBREED project. This will help derive value from the activities supported by that project for cultivar development and to characterize and evaluate genotypes of current cultivars important to Washington Apple production. Members of the GGB team continue to garner funding for competitive programs which extends the value of the support from the WTFRC. I serve on the Scientific Advisory Panel for RosBreed, which meets annually and review activities as requested throughout the year.

I continue to work with Cameron Peace and his lab to critique ideas about effective use of DNA-based information for marker-assisted breeding and application of the MAS Decision Support spreadsheet tool for assessing the value of MAS and different approaches for integrated trait development. New markers developed through the RosBREED project are becoming available to breeders so there must now be decisions about which ones have value in the WABP. There is considerable work on identifying the most important target traits for breeder selection using combined phenotypic evaluation and MAS so the breeding program can integrate marker-locus-trait targets into selection protocols for cultivar development. The information gained from industry personnel about the important traits for commercial success will be useful in setting goals for selection and evaluation of elite selections for commercial potential.

EXECUTIVE SUMMARY
Title: Consulting for the Washington apple Breeding Project
PI: Fredrick A. Bliss
WTFRC Funding: $7,500.

My project objectives were to: 1) Coordinate and lead monthly conference calls to facilitate discussion of important issues related to apple breeding, genetics and genomics, 2) support the program breeder and other collaborating scientists, 3) Provide analysis and critique of proposals for competitive funding of research and development, and 4) Identify other programs, breeders, and scientists in the public and private sectors that can provide collaborative support.

I provided consultation to the Washington apple breeding program which is focused on developing new cultivars that will improve the global competitiveness of the Washington apple industry. I read and critiqued competitive grant requests to various funding groups at the regional and national levels and to evaluate proposals to the WTFRC for funding. Monthly conference calls were held to facilitate sharing ideas, concerns and opportunities among team members about scion and rootstock breeding and research applicable to apple improvement. In addition to scientists from the PNW, participation of Dr. Fazio from Geneva, N.Y. provides information and feedback about apple rootstock development activities to complement the scion breeding work underway in Washington.

The breeding program is expanding use of DNA-based information to complement classical breeding methods for identifying parents, identifying outstanding selections for further evaluation, testing advanced elite selection for commercial value and identifying clonal materials. Integration of marker assisted selection is progressing, with extensive input from supporting scientists at WSU and members of the RosBREED project. Work by the GGB team to evaluate traits for developing molecular markers continues with re-evaluation and new priority setting to assure relevance and cost effectiveness for the WABP. The MASS decision tool developed by Peace helps focus priority for DNA technology development and application to apple breeding.

The Washington Apple Breeding Program is functioning smoothly. New selection populations from crosses among new parents and prior selections from the program are being evaluated each year to provide genetic variability for traits important to target markets in Washington. Numerous elite selections are being evaluated in stage 2, 3 and 4 trials at on station-plots and in growers' fields to identify putative new commercial cultivars. A continuing flow of new selections each year will require a well-coordinated evaluation plan using numerical data, molecular information, grower evaluations and consumer input for deciding which should be released. In order to identify new selections with high predictability of commercial utility as efficiently and quickly as possible, molecular tools and information contributing to marker-assisted breeding (MAB) should be combined with classical breeding methods. Information from the workshop on "Improving decision confidence in Apple cultivar development and adoption" coordinated by Cameron will provide useful information for helping decide which elite selections may have commercial potential.

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

Project Title: Apple scion breeding

PI: Kate Evans
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Co-PI (1): Cameron Peace
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Co-PI(2): Carolyn Ross
Organization: WSU-Food Science and Human Nutrition
Telephone: 509-335-2438
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City/State/Zip: Pullman/WA/99164

Co-PI(3): Yanmin Zhu
Organization: USDA-ARS Tree Fruit Research Lab
Telephone: 509-664-2280
email: zhu@tfrl.ars.usda.gov
Address: 1104 N. Western Ave
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 Funding: **Year 1**:$169,910 **Year 2:**$239,628 **Year 3:**$203,217

Other funding sources: None

Budget 1History:
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
Plot Fees	0	0	0
Total	166,035	125,128	141,917

Budget 2 History:
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
Plot Fees	0	0	0
Total	3,875	114,500	61,300

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 DNA-informed seedling selection 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. An Industry Advisory Council (IAC) for the WSU apple breeding program (WABP) was established in 2009. IAC visits have included the Phase 1 seedling evaluation orchards and a Phase 2 planting as well as the Phase 3 grower trials. The IAC actively participated in developing evaluation and commercialization guidelines for WSU apple releases together with WSU and the WTFRC.
2. Thirty-six new crosses were made and approximately 57,000 seeds were produced in the three years of this project. Seedlings from approximately 52,000 germinated seeds from 2008-2010 crosses were grown in the greenhouse, more than 11,000 of which were screened for fire blight resistance.
3. Approximately 12,500 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 27,000 seedling/M.9 trees were subsequently produced in the nursery for planting in Phase 1 seedling orchards within the three years of this project.
4. DNA-informed seedling selection 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 in 2010.
5. Promising selections in Phase 2 trials (planted in 2004, 2005, 2006, 2007, 2008 and 2009) at three trial sites in Central Washington were evaluated for productivity and fruit quality.
6. Forty-six new promising selections were planted in Phase 2 trials at three trial sites within the three years of this project (2009, 2010 and 2011).
7. Bar-coding technology has been incorporated into the fruit laboratory and orchards (Phases 1 and 2) to facilitate sample tracking and reduce possible transcription errors.
8. One new elite selection was propagated for Phase 3 trials in 2010.
9. Samples of fruit from eleven Phase 2 and 3 selections from various sites and pick dates were profiled by the sensory panel in Pullman and rated for consumer acceptance within the three years of this project.
10. Four members of the TFREC breeding team received sensory panel training at WSU-Food Science and Human Nutrition under the supervision of Dr. Ross.
11. The first two new apple varieties have been released from the WABP following guidelines developed by the IAC. ‘WA 2’ was released in 2009 (U.S. patent # PP21,710 P3) and ‘WA 5’ in 2010 (patent-pending). A third release has been approved by WSU’s Cultivar Release Committee, collection of patent data is complete and trees began large-scale propagation in 2011.
12. DNA marker development has progressed well. The WABP became one of 12 “demonstration breeding programs” with the start of the multi-million dollar SCRI-funded project, RosBREED. A reference germplasm set for identifying and refining new DNA tests was established that includes more than 250 cultivars and seedlings at the Sunrise Research Orchard. Since 2010, this Sunrise germplasm has been comprehensively phenotyped and genotyped in the RosBREED project, to be combined with similar data from other U.S. apple breeding programs for more powerful analyses. A DNA marker for skin color was published by Dr. Zhu with Drs. Peace and Evans.

RESULTS & DISCUSSION

The aim of the WABP is to produce a portfolio of new improved unique varieties, especially selected for the growing environment of central Washington. The reporting years of this project have seen significant leaps forward in achieving this aim: 1) the release of Washington's first apple varieties bred and selected specifically for its growing conditions; and 2) the first use of routine marker-assisted selection for fruit quality not only for this program but also any woody perennial fruit crop worldwide.

Apple Releases

The efficiency of the program compared to other apple breeding programs was highlighted by the release of the first variety only 15 years from the cross (a more typical timeframe being 18+ years).

'WA 2' was released in 2009 from an open-pollinated 'Splendour' seedlot created in 2004 ('Gala' was later identified via DNA markers as being the father). This first WABP release is a compact, precocious tree with no reported problems of sunburn, bitter pit or mildew. Fruit harvest is 'Red Delicious' season after which fruit quality is improved by a few months of storage. Storage tests to date have shown it has excellent potential for long term storage (9+ months) without the need for 1-MCP.

'WA 5' was released in 2010 from a 'Splendour' x 'Coop15' cross also made in 1994. It has a similar tree habit to 'WA 2' but is earlier in season ('Golden Delicious' season) and eats well straight off the tree. 'WA 5' may benefit from 1-MCP application although storage tests are still underway.

A U.S. Plant patent was approved for 'WA 2' in 2011; the patent for 'WA 5' is pending. The WTFRC is the licensee for both varieties and the release strategy was developed in collaboration with the WSU Research Foundation, with guidance from the apple Industry Advisory Council (IAC) chaired by Brent Milne. Release of both varieties in the U.S. is restricted to Washington State growers.

Marker-assisted breeding

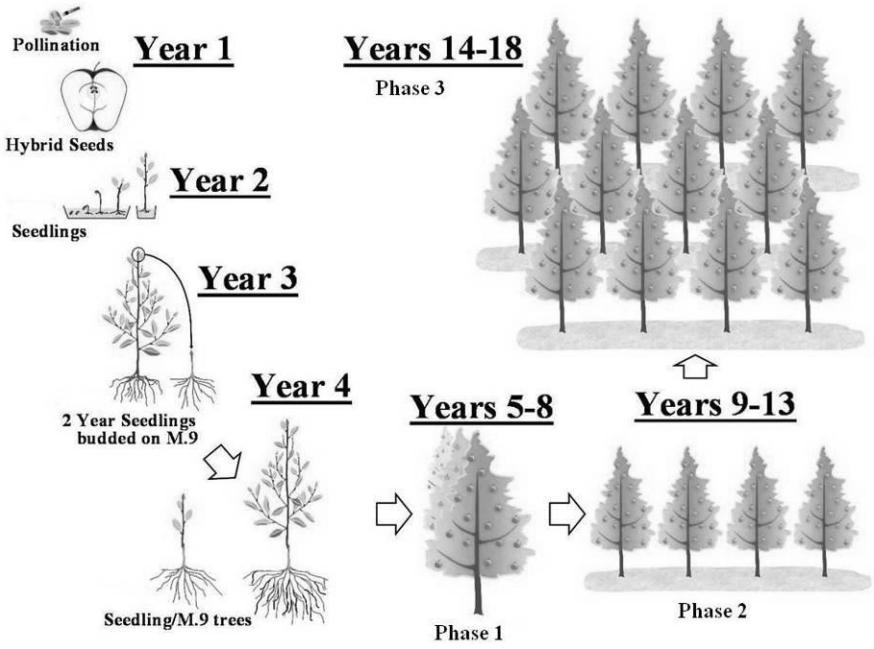
In 2010, the WABP was the first apple breeding program in the world to implement DNA markers to routinely pre-screen seedlings for fruit quality. Seedlings in the nursery were screened with allelic tests for two genes involved in ethylene production (*Md-ACS1* and *Md-ACO1*) that indicate genetic potential for storability. Seedlings with the least favorable allelic combinations (genotypes) were culled before planting in Phase 1 orchards. Nursery charges for 2012 will reflect this reduction in seedlings propagated and maintained. The benefit:cost ratio of using DNA markers was estimated at more than 5:1.

In 2011, a third genetic test was added to the pre-screening, for the *Ma* locus associated with acidity, crispness and juiciness. The test was used to screen seedlings at the earliest stage in early spring in the greenhouse. To facilitate timely execution of genetic screening, we secured the part-time services of a full-time genetic screening technician in the Peace laboratory, Terry Rowland.

Ethylene gene predictive genotypes for potential parental varieties and selections have been used in the WABP for several years to determine optimal cross combinations. The 'pool' of parental germplasm was routinely tested with all available predictive markers as they were developed. Progenies from the breeding program were also used to develop a new apple skin color marker by Dr. Zhu.

General project update

The WSU apple breeding program has continued to generate new progenies either by inter-crossing the best of its selections or by crossing these selections with new parental germplasm. DNA information now plays a significant part in choice of cross combination, and this information is continually updated for common parents and selections as new markers are developed. The addition of this type of data to the breeding program database is a powerful tool, enabling the breeder to search for superior genetic potential when developing a crossing scheme or selecting seedling germplasm to move forward.



Washington Apple Breeding Program schematic

In 2010 and 2011, several newly germinated seedling progenies were inoculated with fire blight to identify resistant individuals. All other progenies not being marker-screened received a basic screen for resistance to mildew. A new genetic marker for fire blight resistance was developed for WABP germplasm in late 2011 and will be incorporated into parent selection decisions from 2012.

Considerable effort has been made towards improving the logistics of sampling seedlings for genetic screening. Spring 2012 will see the first large-scale test of our new '96-pot' format that mimics the '96-well' molecular laboratory format. This change should reduce opportunities for sampling error and increase confidence in accurately connecting laboratory results to the correct seedlings whilst minimizing labeling.

Phase 1

More than 27,000 trees were propagated onto M.9 rootstock and planted into Phase 1 orchards within the timeframe of this project. Two key changes to standard protocol were initiated during this time: all plots were fumigated prior to planting and trees were given bar-coded labels to facilitate sample tracking in the fruit analysis laboratory. The use of a hand-held datalogger and mini-printer enables fruit samples to be given bar-coded labels in the orchard; these labels stay with the samples throughout storage and laboratory evaluations. Juice samples for soluble solid concentration and titratable acidity are also bar-coded. The fruit analysis laboratory was partially equipped using funding from this project. The Mohr® DigiTest, the digital refractometer and the autotitrator all accept scanned bar-codes as samples IDs and, once measurements are completed, output data in an easily accessible format that is readily transferred into Excel spreadsheets formatted for streamlined data entry to the new Breeders Toolbox database. This system greatly reduces the possibility of sample mixing and data entry errors as well as saving a considerable amount of data entry time.

New selection fruit samples were harvested as close to Cornell starch stage 3 as possible, to standardize "commercial maturity", and stored for two months in regular atmosphere 34^{o}F before being evaluated. 'Keepers' (selections that rated well in earlier years) were harvested up to four times in weekly intervals (crop-load permitting), with at least one harvest being before Cornell starch stage

3, and each sample was tested both at harvest and after two months in regular atmosphere at 34ºF. 'Keepers' that continue to rate well are propagated for Phase 2 trials. In 2011, G41 was used for propagation rather than M.9 to reduce the need for fumigation of future plantings.

Phase 2
During the course of this project, two new Phase 2 sites have been established. The WSU Columbia View orchard was replaced by one at Sunrise and the Basin City orchard was replaced by a new orchard adjacent to the Phase 3 block in Prosser. The bar-code labels have also been adopted in the Phase 2 orchards, easing sample tracking through the laboratory. Fruit samples were harvested similarly to the Phase 1 'keepers' but, as more fruit is available, samples were also taken for extended storage (four months in regular atmosphere at 34ºF). The dataloggers are also now linked to calipers for trunk diameter measurements to reduce another source of transcription error.

Phase 2 fruit was tasted and rated by industry members at each of the three annual Washington State Horticultural Association annual conferences within the duration of this project. Four Phase 2 selections were tested by Dr. Ross's trained sensory panel and rated by consumers in 2009. Of these, one has now been propagated for Phase 3 trials.

Phase 3(managed in collaboration with the WTFRC [Auvil & Hanrahan])
Advice and regular visits from the IAC has led to development of thinning recommendations for crop-load management in Phase 3 trials. Harvest dates were determined by the WTFRC using a combination of starch level and color break with a typical three to four picks per selection per site (crop-load permitting). Small samples of each have been evaluated through the fruit evaluation laboratory. As the volume of fruit increased, more detailed storage testing were undertaken by the WTFRC, with the protocol in 2011 including post-harvest drenching, 1-MCP treating of some fruit and both regular and controlled atmosphere treatments for up to eight months. Some samples have also been run over a packing line including a wax application.

Two Field Days were hosted in 2010 (Quincy and Brewster) and one in 2011 (Quincy) to give growers the opportunity to see the Phase 3 plantings, particularly 'WA 2' fruit on the tree.
Four Phase 3 selections were tested by Dr. Ross's trained sensory panel and rated by consumers in 2009 with a more detailed analysis of two of them (pick dates and sites) in 2010. This was followed in January 2011 by development of a sensory profile of five standard varieties to help establish a baseline and identify gaps in the current market selection of varieties.

One new selection was added to the Phase 3 plantings in 2009, bringing the total up to ten. Of these, the IAC recommended discontinuing the evaluation of three selections (WSU 7, 48 and 49) as potential licensable cultivars by the WTFRC or by its soon-to-be-established Management Entity. A further selection was found to be virus-infected so was withdrawn from Phase 3. A decision was made in 2011 by the WTFRC to disengage from 'WA 5'.

It became apparent in 2011 with the increased volume of fruit now present that detailed evaluations of all remaining Phase 3 selections each year would be unmanageable. Priorities were agreed upon and the team focused on a manageable number of elite selections in 2011, taking only a smaller sample of lower priority selections. A new site was planted with one selection in Mattawa in 2011. Due to a change of ownership of the Brewster site, a new northern site is being sourced. One new selection has been propagated for planting in Phase 3 in 2012, an early-season apple of 'Honeycrisp' parentage. One elite selection from the 2008 plantings was propagated in 2011 with a view to commercial Phase 4 release in winter 2012. A successful application was made to WSU's Cultivar Release Committee in 2011. Patent information was collected and an application is in preparation.

EXECUTIVE SUMMARY

The aim of the Washington State University Apple Breeding Program is to produce a portfolio of new improved unique varieties, especially selected for the growing environment of central Washington. The reporting years of this project have seen significant leaps forward in achieving this aim: 1) the release of Washington's first apple varieties bred and selected specifically for its growing conditions and 2) the first use of routine marker-assisted selection for fruit quality not only for this program but also any woody perennial fruit crop worldwide.

Washington State growers are now able to grow 'WA 2' commercially; a number of growers applied for licenses at the first opportunity in 2010. When originally planned, it was expected that the full cycle of Phases 1 to 3 of the breeding program would take 18 years (considerably shorter than other apple breeding programs) which would mean that the first release would be expected in 2012. Not only was this timing reduced, but the development and application of marker-assisted breeding has greatly enhanced this program's ability to develop further superior varieties in the future.

Within the timeframe of this project, the WABP has set up a new fruit analysis laboratory, sourcing equipment (partially funded by this grant) that enables efficient analysis and reduced data entry effort and error. Sample tracking fruit from the orchard to the laboratory has been streamlined with a bar-coding system that is compatible with fruit evaluation equipment. Significant progress has also been made in streamlining the logistics of routine marker-assisted seedling selection; an area frequently over-looked but potentially a huge source of error. Seedlings will be planted using a new 96-pot system in January 2012.

Deliverables:

1. Two new apple cultivars released to the Washington industry.

2. One further apple cultivar propagated for release in 2012.

3. World-first for integrating a potentially revolutionary new technology (marker-assisted breeding) into routine breeding operations – an outcome of the Technology Roadmap.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: TR-10-109

Project Title: Breeding in the 21st century: technology platform for fast breeding

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City/State/Zip:	Pullman/WA/99164	**City/State/Zip**:	Pullman/WA/99164

PI:	Kate Evans	**PI**:	Nnadozie Oraguzie
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City/State/Zip:	Wenatchee, WA	**City/State/Zip**:	Prosser, WA

Cooperators: Ralph Scorza and ARS team at the Ag Station in Virginia, Derick Jiwan, Fred Bliss and Jim McFerson

Total Project Request: **Year 1:** $89,668 **Year 2: 82,575**

Other funding sources: None
WTFRC Collaborative Expenses: None

Item	2011	**2012**	
Salaries[a]	36,000	37,440	
Benefits	10,368	10,783	
Wages[b]	12,000	12,480	
Benefits	1,800	1872	
Equipment	9,500		
Shipping			
Supplies[c]	5,000	5000	
Travel	3,000	3000	
Plot Fees[d]		2000	
Miscellaneous[e]	2,000	10,000	
Plant screen/transgenics	5,000		
Breeding/Crossing	5,000		
Total	$89,668	**$82,575**	

Footnotes:
a. Post-doc salary and wages. The post-doc will work with all PIs.
b. Technical help – Time slip employee for plant handling and assisting in crosses, data collection etc.
c. Tissue culture supplies and reagents
d. Greenhouse/tissue culture facility use
e. Plant screening/transgenics and breeding/grafting program costs

OBJECTIVES

NOTE: The commissioners voted to have this project moved to apple review from technology review. The focus will be only on apple during the remaining duration of the project. These techniques could be obviously applied to Sweet Cherry and other crops once we have standardized the procedures in apple.

Our primary objective is to *reduce the generation cycle* in breeding so that time and resources can be saved in combining multiple traits in lesser time for the varieties desired by the farmers and the consumers. Results from the three conditions tested in this project can be used alone or in combination to obtain pollen from selected seedlings within a year to accelerate combining of multiple traits in the final varieties to be release by the WABP. In this project, we are physically comparing and testing three alternative means of reducing the generation time primarily in apple breeding with direct implications for cherry breeding.

The three alternative methods of reducing generation cycle being tested are:

1. Environmental and horticultural approach

For this approach, progenies were selected from crosses that had already been made for the WABP including a reasonable level of diversity including seasonality (e.g. Akane and Fuji) whilst not using Cripps Pink. These are baseline comparison progenies which are not expected to be precocious.

a. Physiological manipulation of seedling growth and development
b. Grafting onto precocious rootstocks

2. Genetic approach

For this approach, parental overlap was intentionally maintained with genotypes used in objective 1 for a direct comparison. Cripps Pink (Elite cultivar) and M.zumi (Wild donor) were used as the precocious parent.

a. Standard breeding practice (no precocity)
b. Elite cultivar as precocity donor (Cripps Pink)
c. Wild relative donor of precocity (M. zumi)

3. Transgenic intermediate approach

a. Incorporating quick flowering gene from silver birch and apple in 3 advanced WABP selections (WSU 38, 48 and 34)
b. Incorporating quick flowering gene from silver birch and apple in 7 M. sieversii accessions

SIGNIFICANT FINDINGS

Significant findings for objective 1

- A clear impact of genetic background under modified environmental conditions on the growth rate is visible based on number of nodes generated per month.

- Knowledge regarding the growth rate and the time when the seedlings flower will help future crosses with similar parental material. The information will help in predicting flowering time; enable planning for pollen collection and subsequent crossing in the WABP. This experiment will help test the concept that 120 nodes are required to break juvenility.

- Progenies from WABP cross 1006 (Fuji x WSU34) are most vigorous with most number of nodes generated in 8 months (7.25 nodes/month) compared to cross 1009 (HoneyCrisp x WSU 34) with only 3.4 nodes generated per month on an average.

- Progenies from WABP Cross 1017 (WSU 5 x Akane) show intermediate vigor with 5.75 nodes per month.

Significant findings for objective 2

- Inclusion of Cripps Pink as a parent in different crosses results in 18 to 47% of progeny producing flowers in year 2.

Significant findings for objective 3

- Elite WSU selections and M. sieversii accessions carried a large pathogen load making it difficult for them to be established in tissue culture after repeated efforts. This led to development of modified sanitizing and operating protocols for these accessions.

- Tissue culture media for micropropagation of WSU 34, 38 and 48 has been established which will be used for subsequent experiments in this project. The micropropagation methods will enable generation of large amount of plant material rapidly that will ensure availability of the desired variety for different phases of variety commercialization.

METHODS

Details below target apple, as concepts and resources are further advanced from the outset. Opportunities for improving the generation time of sweet cherry are also being explored and could be targeted in the future using concepts developed in this project. *There are no apparent sources of precocity for sweet cherry breeding. During the course of this project, we will seek out worldwide sources of precocity for use as pollen parents crossed onto elite cultivars to develop breeding lines which we can later assess for precocity.*

1. Environmental and horticultural approach

a. Physiological manipulation of seedling growth and development

A sub-sample of seedlings from each of five crosses among standard cultivars (excluding 'Cripps Pink' and selections with that cultivar in their parentage) was retained in the greenhouse and forced into rapid growth by artificial season cycling to determine whether genetic background affects the rate at which juvenility is broken. Physiological conditions prescribed by Aldwinckle (1975) are being used to obtain 120 nodes to break juvenility.

b. Grafting onto precocious rootstocks

To evaluate the impact of precocious rootstocks, seedlings will be grafted on to them and data on time to flowering generated for comparison purposes.

2. Genetic approach

a. Standard breeding practice (No precocity)

Years to flowering was previously recorded for families of many crosses among parents of the WSU apple breeding program (WABP) that have no precocity. Data from these crosses will be used as a baseline comparison with other methods for reducing generation time being tested in this project.

b. Elite cultivar as precocity donor (Cripps Pink)

'Cripps Pink' has been used frequently as a parent in the WABP and short juvenility times of resulting seedlings previously observed. If necessary, additional observations will be collated and compared with alternative methods to confirm the value of 'Cripps Pink' in transferring precocity to subsequent generations. Furthermore, controlled crosses have been made using

'Cripps Pink' as a parent and seeds collected. The seedlings will be raised in the greenhouse for rapid growth.

c. Wild relative donor of precocity (M. zumi)

Controlled crosses were made using *M. zumi* as a parent in combination with several standard cultivars and seeds collected. Seedlings will be raised and budded onto 'M9' rootstocks following the typical WABP timeline. A sub-sample of seedlings will be transferred to the greenhouse for rapid growth.

3. Transgenic intermediate approach

a. Incorporating quick flowering gene from silver birch and apple in 3 diverse advanced WABP selections (WSU 38, 48 and 34)

b. Incorporating quick flowering gene from silver birch and apple in 7 M. sieversii accessions

The quick-flowering gene from silver birch is being introduced into three WABP selections and two wild sources that impart valuable traits such as powdery mildew resistance. The transgene is being introduced into apple and cherry hosts using standard Agrobacterium and/or particle gun methods. Transgenic plants obtained at the end of the process will be maintained in the greenhouse on the Pullman campus. Plants will be screened using molecular tools to ascertain their transgenic status. Pollen will be collected and stored for use in subsequent crosses.

RESULTS & DISCUSSION

1. Environmental and horticultural approach

a. *Physiological manipulation of seedling growth and development*: On average 17 seedlings from each of the crosses made with elite selections were placed in the greenhouse at WSU Pullman where they were forced into rapid growth (to attain 120 nodes) by growing them under controlled temperature and light. The initial growth (for four months March – June) was at a temperature range 62^0F – 70^0F (17-21°C) with 16 hour photoperiod. Later, the temperature was increased to 72^0F – 79^0F (22-26 ^{0}C) with 16 hour photoperiod as suggested by Aldwinckle (1975). Seedlings were placed in the greenhouse on 3/11/11. The average number of nodes was calculated for each of the crosses on 11/7/11 and based on that time to flowering was calculated (Figure 1). This calculation is based on the long-held belief that juvenility is broken when 120 node stage is reached (Aldwinckle, 1975). *In this experiment we will be able to test this concept rigorously*. It was observed that the plants in the

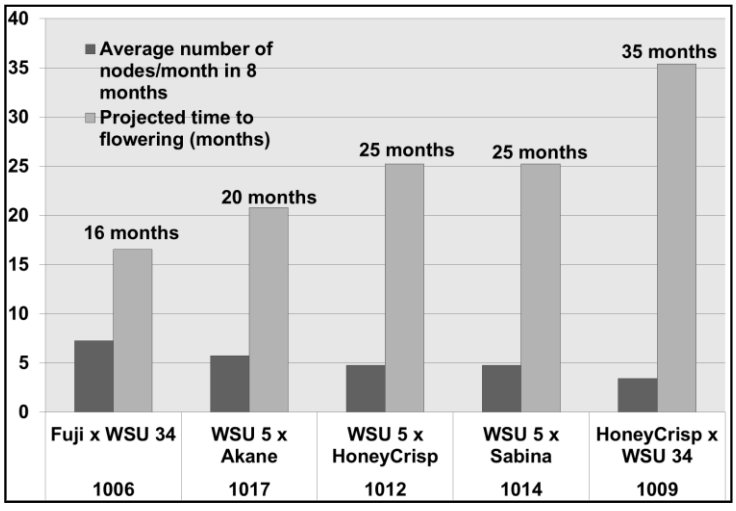

Figure 1: Physiological manipulation to induce break of juvenility. An average of 17 seedlings from each of the five crosses was grown under modified environmental conditions. Data was collected 8 months after the initiation of the experiment. Average number of nodes generated per month was calculated for each progeny set (represented as dark bars) and total time required to reach 120 nodes was predicted (break of juvenility) is shown as gray bars. For some seedlings, it can take up to 3 years. Numbers 1006, 1017, 1012, 1014 and 1009 represent cross number.

greenhouse were in a ragged state. This could be due to the modified physiological conditions with higher disease pressure that the plants experience in a greenhouse. *Out of these crosses, WSU5 has been discontinued as a future variety by the WA industry however it will continue to be used as a parent in the WABP and the results obtained from this experiment will be of direct use in the breeding program.*

2. Genetic approach

a. *Standard breeding practice* (no precocity): This data on flowering serves as baseline data to show the time taken to flowering in seedlings that harbor no precocity. As shown in Figure 2, the percentage of progeny flowering in year 2 is minimal to none in one of the crosses. These results are similar to what was observed in physiological manipulation of seedling growth.

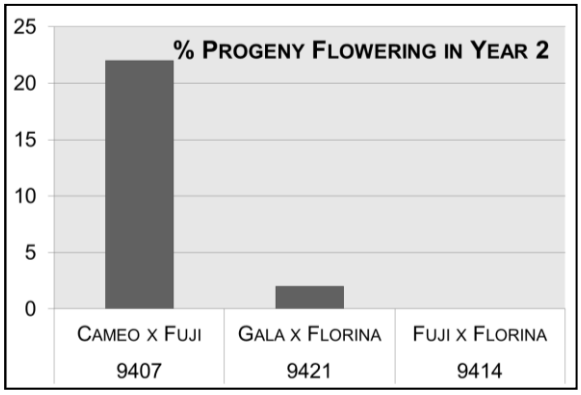

Figure 2: Standard breeding practice: Without any source of precocity in the parents, seedlings require upwards of 2 years to break juvenility.

b. *Elite cultivar as precocity donor* (Cripps Pink): When Cripps Pink was used as one of the parent; most seedlings were precocious as expected. Figure 3 shows the percentage of progeny breaking juvenility and flowering in year 2. In addition, crosses were made with advanced WABP selections and other elite cultivars in 2011 and seeds collected from each of the crosses listed in Table 1. A maximum of 100 seeds from these crosses have been germinated and will be forced into rapid growth with change in temperature and light in the greenhouse.

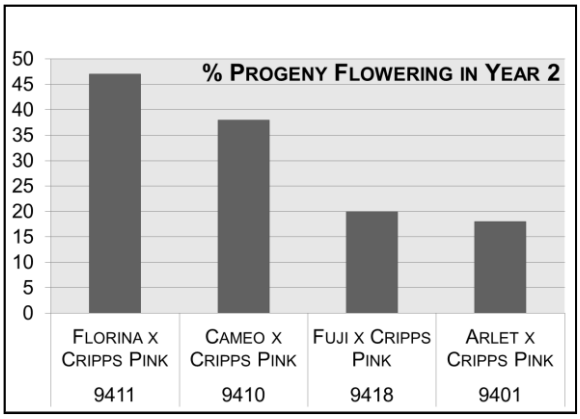

Figure 3: Elite cultivar as precocity donor. Over 15% of progenies in all crosses produced flower in year 2.

Table 1: Crosses made in 2011to introgress cultivar source of precocity (only 100 seeds per cross will be used for further work)

Parents	Number of seeds
Cripps Pink x Honeycrisp	105
Cripps Pink x WA5	1968
Cripps Pink x Sabina	159
Cripps Pink x Fuji	0

c. *Wild relative donor of precocity* (M. zumi): These crosses were made in 2011 and seeds collected for each of the crosses in Table 2. Seedlings from these crosses will be grafted onto M9 rootstock and flowering dates will be recorded. A subset of these grafted plants will also be forced into rapid growth in the greenhouse.

Table 2: Crosses made in 2011 to introgress wild source of precocity

Parents	Number of seeds
M.zumi x Honeycrisp	132
M.zumi x WA5	1025
M.zumi x Sabina	159
M.zumi x Fuji	47

3. Transgenic intermediate approach

a. Incorporating quick flowering gene from silver birch and apple in 3 advanced WABP selections (WSU 38, 48 and 34)

b. Incorporating quick flowering gene from silver birch and apple in 7 M. sieversii accessions

In order to genetically modify the advanced selections and M. sieversii accessions, it was first required to establish the selections in tissue culture to obtain clean material. However, the plant material had a large pathogen load resulting in development of aggressive sanitization strategies. This delayed our proposed timeline by 3-4 months. Now, WSU 34, 38 and 48 material is available in tissue culture (Figure 4) and is currently being multiplied via micropropagation to initiate the transformation experiments in January 2012. Royal Gala is routinely transformed in the laboratory. The genes that have proposed to engineer in advanced WSU selections are also being tested in Royal Gala while the experimental procedure are worked out in the advanced selections. This experiment provides a control to compare the transgenic experiments with the advanced selections. The quick flowering gene from silver birch has been used for three transformation experiments with Royal Gala using Agrobacterium as well as gene gun-mediated transformation. The first two experiments did not yield any transgenics. The last transformation experiment performed on 9/10/11 is expected to provide transgenics with the quick flowering gene. Over the last few months, all methods have been streamlined to be applied towards transformation of advanced selections. Due to heavy pathogen load in the advanced selections, their establishment in tissue culture was delayed. However, now we have overcome this problem and have the plant material currently under micropropagation for subsequent experiments.

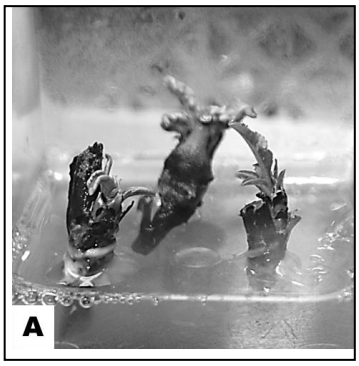

Figure 4: WA-38 an elite WSU cultivar in early stages of micropropagation.

Inclusion of a transgenic intermediate in the plant breeding process currently remains under review at USDA APHIS. However, we were informed by Ralph Scorza, USDA-ARS Research Leader that there is a general agreement on using “null segregant” lines (lines that no longer contain the transgene for early flowering).

Comparison between different approaches: Figure 5 represents a comparison of time to flowering between the three scenarios of not having any precocity in the parents, having precocious parents (Cripps Pink and M. zumi) and the quick flowering transgenic approach. Inclusion of precocity (genetic and transgenic) can reduce the generation cycle by 1 to 4 years.

In summary, our aim is to identify the best environmental, genetic and

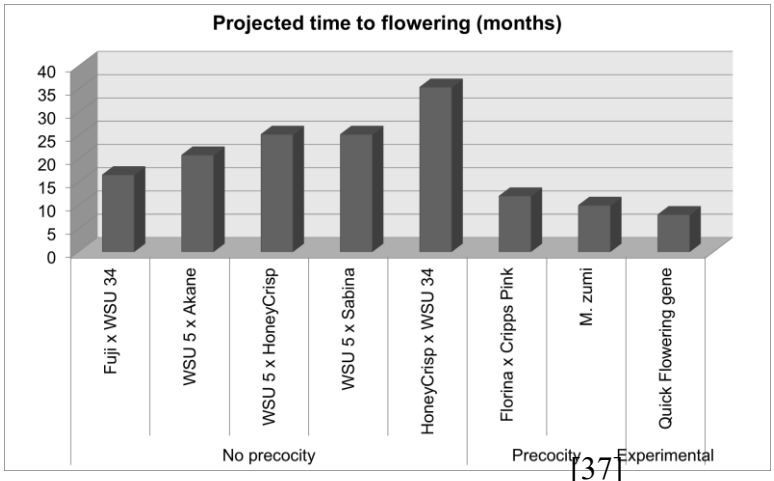

Figure 5: A comparative analysis of time to flowering once the seedlings are planted in soil in the greenhouse.

transgenic intermediate approaches to reduce generation cycle time to accelerate incorporation of multiple traits in the final variety using traditional and gene assisted breeding where possible.

Timeline and completed/expected milestones as indicators of success in the project

Time Frame	Activities	Milestones
Mar – Dec 2011	• Amplification, cloning, sequencing and development of plant transformation vectors with quick flowering gene from silver birch and apple	Completed
	• Establish plant material from advanced selections in tissue culture for micropropagation	Completed
	• Establish seedlings from non-precocious crosses and manipulate growth using modified physiological conditions	Completed
June 2011 – June 2012	• Transformation of Royal Gala as a control experiment with quick flowering gene	Ongoing April 2012 – Confirmed transgenic in the lab June 2012 - Transgenic Royal Gala in the greenhouse
Oct 2011 – Oct 2012	• Standardize transformation and leaf-based regeneration of advanced selections and M. sieversii	Ongoing July 2012 – Confirmed transgenic in the lab Oct 2012 - Transgenic advanced selections with quick flowering in the green house
Mar 2011 – Aug 2012	• Maintain non-precocious seedlings in greenhouse till they flower at 120 node stage and record data	Ongoing Aug 2012 – Flowering progeny from cross 1006 Dec 2012 – Flowering progeny from cross 1017 May 2013 – Flowering progeny from cross 1012 and 1014 May 2014 – Flowering progeny from cross 1009
Feb – Aug 2012	• Raise seedlings from seeds collected in 2011 (M. zumi and Cripps Pink as one of the parent)	Ongoing Aug 2012 – Established plants grafted on M9 in the greenhouse

FINAL PROJECT REPORT

Project Title: Further development of an online toolbox for tree fruit breeding

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Cooperators: Amy Iezzoni (MSU), Gennaro Fazio (USDA-ARS), Deven See (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:

Budget History:

Item	2011	Year 2:	Year 3:
Salaries[1]	31,180		
Benefits	11,069		
Wages	0		
Benefits	0		
Equipment	0		
Supplies[2]	1500		
Travel[3]	1500		
Plot Fees	0		
Miscellaneous	0		
Total	**45,249**		

*[1]20% salary for Sook Jung and 40% salary for Chun-Huai Cheng
[2]$1500 toward data backup costs and IT facilities rental costs
[3]$1500 for travel to Prosser, Wenatchee and Pullman for face-to-face group meetings

RECAP ORIGINAL OBJECTIVES

1. Develop a computational pipeline to process genotyping data from the WA apple and cherry breeding programs
2. Integrate marker/genotyping data with the breeders toolbox
3. Develop standard and customizable breeder reporting tools for each breeder within their toolbox.

SIGNIFICANT FINDINGS

Objective 1: Develop a computational pipeline to process genotyping data from the WA apple and cherry breeding programs

A computational pipeline has been developed to process and extract the genotyping data from the excel template. This now completes all the computational scripts needed for a programmer to upload all genetic and breeding data for the WA apple and cherry breeding programs. All available phenotypic and genotypic data for the apple breeding program have been uploaded and integrated in the database. The cherry breeding team are in the process of adding their data to the excel templates. The tools are in place to quickly create the online toolbox for the cherry team as soon as the data is made available.

Objective 2: Integrate marker/genotyping data with the breeders toolbox

All private marker and genotyping data have been uploaded to the database for the WA apple breeding program and collection of the same data for the cherry breeding program is in hand and will be complete by the end of March, ensuring the proposal goals are met. By the end of March, the private and public RosBREED marker data for the Apple and Cherry Crop Reference and Breeding Pedigree Sets will be also be available for our breeding programs**.** Apple and cherry QTL data have been collected from the literature and standardized nomenclature assigned to each trait. A total of 485 QTLs for 93 traits are now available for searching on GDR for apple and while yet integrated with markers and the Breeders Toolboxes, this will be included in the GDR makeover we are currently working on as part of the tfGDR SCRI project.

Objective 3: Develop standard and customizable breeder reporting tools for each breeder within their toolbox

Interfaces have been developed with links from the home page (see Figure 1) that allow the Apple breeding team to search and compare both their private and public (RosBREED) evaluation data by variety, trait, marker, allele, parentage, year, and site location. The results are customizable and downloadable as excel files or as a file for input into the Pedimap analysis program. The same customizable features will be made available to the Cherry breeding team after the data has been provided and uploaded to the database. We anticipate this will be available by the end of March and fulfill all the objectives of this proposal.

Publications and Presentations

Following development of a Natural Diversify module to expand the generic database schema, Chado, to accommodate large scale phenotyping and genotyping data, a paper entitled “The Chado Natural Diversity module: a new generic database schema for large-scale phenotyping and genotyping data” was published in the peer-reviewed Databases journal (Jung *et al*, 2011). This expansion to Chado has generated considerable interest within the database and bioinformatics community and is anticipated to significantly expand the use of Chado in the development of integrated genomics, genetics and breeding databases.

The apple toolbox was highlighted at several presentations during the course of the year. These included:

(1) The 2011 Plant and Animal Genome Conference ("GDR" computer demonstration by Dr. Jung, and the "Cacao Genome Database" presentation at the Cacao Workshop by Dr. Zheng),

(2) The 2011 National Citrus Genomics Conference ("Citrus Genome Database" presentation by Dr. Jung)

(3) The 2011 University Industry Consortium Meeting ("Knowledge Warehouses for Fruit Farms of the Future" presentation by Dr. Main),

(4) The 2011 American Society of Horticultural Science Annual Conference ("RosBREED: Enabling marker-assisted breeding in Rosaceae" presentation by Dr. Peace and a poster " RosBREED enables marker-assisted breeding for apple" by Dr Peace *et al*.)

(5) The 2011 American Society of Agronomy, Crop Science Society of America, and the Soil Science Society of America International Annual Meeting ("Building Database Resources For Translational Research in Crop Science" presentation by Dr. Jung)

(6) The 2011 Eucarpia Fruit Breeding and Genetics Meeting ("Apple breeding in the Pacific Northwest" by Dr. Evans)

(7) The 2011 Clemson Field Day Peach Growers Meeting ("GDR: A Community Resource for Rosaceae Genomics, Genetics and Breeding Research" presentation by Dr. Main)

(8) The NSF Future of Plant Genome Sequencing and Analysis Workshop (Plant Community Databases: The Stewards of Knowledge" presentaion by Dr. Main

During the course of the year, the whole team met in person to go over data and interface development and the apple team had several online meetings to further refine the functionality needs of the Toolbox.

RESULTS AND DISCUSSION

We are on track to meet all our objectives (end of March 2012). 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 and genotyping data are available. We have also developed data templates and a data uploading system that is flexible for various breeding programs. Another major work is that we identified the format of browse/query/download web interfaces that will be most useful for breeders using the WA apple and cherry breeding programs as models (Figure 2). The development of these web interfaces is completed, as are all the uploading scripts, although the breeding teams still need access to a programmer for the scripts to be run. 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 which has been integrated with private and public data.

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 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 are utilizing DNA markers to test the genotype of their breeding selections. When the RosBREED genotyping data become fully 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 WA Apple Breeding Program Toolbox Home Page

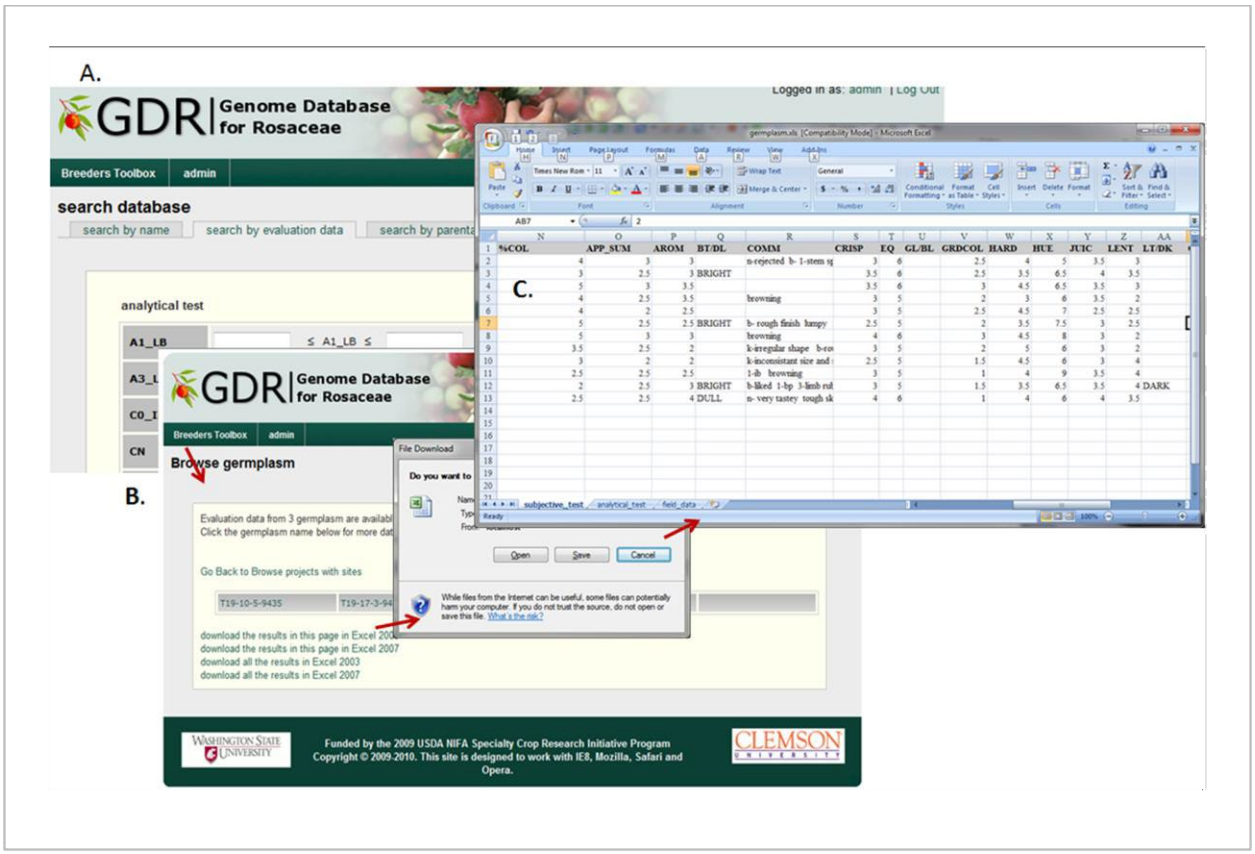

Figure 2. Screen Shots of the web interfaces of breeders' toolbox. A. Users can browse by projects (not shown) or search by name, evaluation data, or parentage. B. Users get a result page with list of germplasm names corresponding to various queries or browse selections made along with the options to 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 Cherry Breeding program (PNWCBP) 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 (published in a peer reviewed journal in 2011) facilitating a more comprehensive genomics, genetics and breeding data structure.

Having modeled the database structure and created an excel template for breeders to store and update their breeding data, scripts were written to complete upload of all genetics and breeding data into the database by a programmer. Interfaces have been developed that enable the breeding teams to easily and quickly search and compare evaluation data by variety/germplasm, trait, marker, allele, parentage, year, and site location. The Toolbox seamlessly integrates private breeding data with the publicly available Crop Reference Set data provided by the RosBREED project, allowing the breeder to use all the available data in decision-making. This significantly enhances the breeder's ability to manage and track selection data and make more informed crossing and selection decisions, thereby facilitating the creation of new cultivars which meet consumers' needs and sustainable agricultural practices.

Further work is still needed to make the database more breeder autonomous and useful. To this end a number of features have been identified by the breeders and the development team. These include the ability for the breeding teams to directly edit germplasm and evaluation data and upload new data themselves, eliminating the need for a programmer, as is currently the case. We would also like to include more automated error checking of data to highlight where entered values are outside a specified threshold, thus allowing data to be easily user-edited. In addition, we would like to have results of searches returned to the screen (particularly useful when only a small output of data is expected), as well as be downloadable in excel and as input files for analysis in other programs that breeders routinely use, such as FlexQTL. Finally, the ability to save, update and compare searches would be a most useful and time-saving feature for the breeding teams.

We are confident that the interdisciplinary, team-based approach to creating this database and toolbox will continue to 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: Increasing decision confidence in cultivar development and adoption

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Cooperators: Fred Bliss (Davis, California), Tom Auvil and Jim McFerson (WTFRC), Eric van de Weg (Plant Research International, Netherlands), Yingzhu Guan (PhD student, WSU Wenatchee), Sujeet Verma (PhD student, WSU Pullman), Industry Advisory Committee of the Washington Apple Breeding Program

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: Cameron Peace. Co-PIs and major collaborators: include Evans, van de Weg, McFerson. Supplied valuable information on cultivar genetic potential provided by key ethylene and related texture markers.

Agency Name: WTFRC Apple Review
Amount awarded: $635,201 (2009-2011)
Notes: "Apple Scion Breeding" PI: Kate Evans. Co-PIs include Peace. The foundation program on which this project was built. Develops new cultivars and engages the IAC.

Agency Name: USDA-CSREES, Specialty Crop Research Initiative
Amount awarded: $7,200,000 + same matched by universities and industry (Sep 2009 – Aug 2013)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Amy Iezzoni. Co-PIs include Peace, Evans, Main, Bink, and van de Weg. Useful outputs included *Ma* locus haplotype categorization and socio-economic knowledge on trait values.

Total Project Funding:

Budget History:

Item	2011		
Salaries			
Benefits			
Wages	$ 4200		
Benefits	$ 630		
Equipment			
Supplies	$ 5000		
Travel	$ 4000		
Plot Fees	$10,000		
Miscellaneous	$73,000[1]		
Total	**$96,830**		

[1] $48,000 for co-PI Craig Hardner (40% time, $1000 computer supplies, $7000 for 2 x Aus-WA), $10,000 for co-PI Marco Bink (50 hours time), and $15,000 for project-culminating workshop in Wenatchee.

RECAP ORIGINAL OBJECTIVES

Overall goal: Improve release and adoption decisions about the WA apple breeding program's new cultivars by revealing and communicating genetic potential for commercial performance.

Specific objectives:

4. Improve efficiency and predictability of advanced/elite selection trials by optimizing field experimental design.
5. Put rapidly accumulating DNA information to immediate use for WA apple growers by describing genetic potential of new cultivars in the context of commercial production.
6. Demonstrate the efficacy of a combined field-design and DNA-based approach and opportunities to the WA apple industry.

SIGNIFICANT FINDINGS

- The most important genetic-based drivers of grower adoption of new cultivars are consumer appeal, biotic stress resistance, yield, packout, and timing and length of the harvest and market windows.

- The WABP's current P2 trial design efficiently identifies genetic potential in selections for most fruit quality traits, but we have identified opportunities for improvement.

- Differences in performance among selections are relatively stable across P2 locations, and so the WABP can confidently predict orchard performance, but P3 data on postharvest performance is essential.

- Trial location, season, tree age, and storage each affect most fruit quality traits by increasing or decreasing the observed mean, but rarely change the relative ranking among varieties.

- Genetic potential for most fruit quality traits can readily be determined in few years, and selections that fail to perform well in early years of a trial can be safely discarded early.

- Effects of trial location, season, tree age, and storage must be calculated and adjusted for before genetic potential of a selection can be determined from a dataset that contains multiple levels of one or more of these factors.

- For certain traits that are highly unpredictably influenced by environmental conditions, such as sweetness, enhanced replication is required to obtain robust estimates of genetic potential.

- Available DNA tests currently used in the WABP can help predict performance for texture traits within and across WA growing regions.

- Pre-screening of parents and seedlings with predictive DNA tests for any traits currently evaluated in P1-P3 field trials is especially valuable for improving breeding efficiency.

- Opportunities exist to incorporate more DNA information for increasing decision confidence in new cultivar development.

- Multiple avenues of information delivery to growers and feedback to the WABP on new cultivar genetic potential for commercial success are important to maintain.

RESULTS & DISCUSSION

Choice of scion-rootstock combinations is the single most profit-influencing decision that a grower can make. While planting standard scion cultivars is an option to mitigate risk, new cultivars from breeding efforts provide sustained genetic solutions to flaws in standard cultivars or to address market opportunities. Successful genetic improvement of apples for the Washington industry does not end with the release of superior new regionally adapted cultivars from the Washington apple breeding program (WABP). To have industry impact, a cultivar must first be adopted by growers and shippers and then managed effectively to maximize its genetic potential. For long-standing cultivars, genetic potential has mostly revealed itself to the industry over a multitude of orchards and seasons, and hopefully this practical information base has been complemented by objective cultivar-specific research and effective outreach. However, to make planting and management decisions on *new* cultivars, limited commercial experience with these cultivars means decisions rely on other sources of information to predict genetic potential: orchard and storage performance in multi-year breeding trials in representative locations with commercially relevant horticultural management, pedigree knowledge, and confidence in the breeder's rigor in selection. Strong experimental design of evaluation trials in breeding programs enables the genetic potential of selection under commercial conditions to be predicted with optimal time- and resource-efficiency. Also, the accuracy of evaluation trials, determination of pedigree, and selection may each be enhanced with genetic information at the DNA level – a more direct predictor of genetic potential. Finally, information gained on genetic potential from multiple sources needs to be appropriately compiled and delivered to the industry to allow sufficiently informed cultivar planting and management decisions that maximize the probability that profitable production outcomes can be achieved.

The original proposal posed the following:

> **The key question underlying selection decisions made by breeders and growers is:**
> ***How well do different sources of information predict cultivar performance in commercial orchards?***

Many approaches were taken in 2011 to answer this question, and a project-culminating Workshop was held in December to discuss and finalize our understanding. The following describes how well each of 20 sources of information predict cultivar performance in commercial orchards. More detailed reports developed in 2011 on some of these topics are available on request.

1. Information from grower feedback

Grower, including IAC, feedback on selection performance and potential for commercial release provides a valuable source of advice for the WABP and for other growers. To date, this information has been provided subjectively, and would benefit from a formal, systematic method of obtainment allowing objective incorporation with other information sources to improve confidence in breeding decisions. The "comfortable redundancy" in number of trial locations (point 7 below), especially in Phase 3 (P3), has the added benefit of enhancing opportunities to engage growers locally in selection evaluation. P4 evaluations are another critical information source on optimal tree management, fruit handling, and market targeting of new WABP cultivars. Currently, existing WSU and WTFRC personnel are stretched thin across this bottleneck in the system. A dedicated WSU expert in technology transfer focusing on elite selection and new cultivar evaluation for the Washington production environment is a strategic necessity to the WABP-industry's continuum of new cultivar development, adoption, management, and superior product delivery.

2. **Information from P3 trials**

For the few elite selections showing outstanding performance in P2, P3 trials use heavy replication with the aim to efficiently obtain estimates of genetic potential for harvest window, yield, storability, and packout. With effective preceding P2 trials, P3 can emphasize those traits that critically contribute to field operations, market performance, and return on investment but are highly sensitive to environmental conditions, very expensive or laborious to evaluate for each selection, and/or for which minor differences have large effects on the bottom line. Selection rejections in P3 to date appear to have mostly been for reasons that have later revealed themselves to be important to the current industry (e.g., a flavor profile that doesn't fit the industry's expectation of a Washington apple) rather than for poor performance that was not detected in traits evaluated in P1 and P2. Replication of each elite selection involves at least fifty of trees over at least four locations and with at least three years of fruiting trait evaluation. For practical reasons, fruit samples for a selection within a season at a location are bulked over trees, comparison to standard cultivars is ad hoc and depends on what is already growing in adjacent orchards, and management regimes are different across locations – all of which may limit conclusions that can be made on estimates of genetic potential. A rigorous quantitative genetics statistical examination has yet to be undertaken on the power of the current P3 trial design to predict commercial genetic potential of elite selections for P3-evaluated traits.

	Gala/Vantage	WA 2/Quincy			
	Aug. 27	1st pick Sept. 29	2nd pick Oct. 7	3rd pick Oct. 13	4th pick Oct. 20
Firmness (pounds)	20.1	20.3	19.6	19.4	19.5
Diameter (inches)	3.2	3.3	3.4	3.5	3.4
Weight (grams)	247.0	263.0	289.0	317.0	287.0
Color (1-5)	5.0	3.3	3.3	3.4	3.4
Starch (1-5)	3.0	2.4	2.8	2.6	3.9
Sugar (Brix)	11.4	12.7	11.7	13.0	12.3
Acid (percent malic acid)	0.370	0.558	0.546	0.537	0.538

Figure 1: Example of performance information provided on new WABP cultivars from P3 trials Excerpt is from article in Good Fruit Grower*, Aug 2011: "Apple selections evaluated for postharvest performance" by Ines Hanrahan, Tom Auvil, and Kate Evans.*

3. **Information from P2 trials**

For dozens of promising selections to date, P2 uses some replication with the aim to efficiently obtain estimates of genetic potential for productivity, tree health, fruit quality, and susceptibility to storage disorders. Of these, only fruit quality is also previously evaluated in P1. The empirical observation that P3 rejections are for reasons other than poor performance for fruit quality traits evaluated in P1 and P2 gives confidence that P2 is effective. In other words, relative performance of selections observed from P2 provides a good estimate of genetic potential for commercial production environments for those traits evaluated in P2.

In addition to providing decision confidence for selection advancement decisions, P2 trials provide extensive, deeper performance data for fruit quality and productivity traits that influence P3decisions of selection advancement to commercial release. P2 replicated trials are currently conducted with annual plantings at three locations of several new selections randomized with up to five "check" cultivars, with five trees of each variety (selection or check cultivar), and evaluation of fruiting traits for at least three years. Analytical efforts in 2011 on relative magnitudes of sources of variation in WABP trials (points 6-12 below) focused on P2 as there are fewer varieties in P3 trials and no replication in P1 trials.

4. **Information from P1 trials**

P1 single-tree trials on thousands of new seedlings per year provide an opportunity to eliminate genetically inferior seedlings prior to significant resource investments (in P2 and beyond) that are required to gain information on performance for certain traits. P1 evaluations are therefore for those that are cheap and simple to conduct for each seedling, are little influenced by non-genetic factors, and/or are critical but only a low proportion of seedlings meet the designated threshold. Because the reasons for selection rejection in P2 include poor performance for traits that are or could be evaluated in P1, P1 can be considered to be somewhat "leaky" and some adjustments may be possible to improve overall efficiency. Pre-screening with predictive DNA tests for any traits currently evaluated in P1-P3 field trials would be especially valuable – thus the investment in establishment of marker-assisted breeding capability for the WABP over the last five years.

5. **Information from bulked sampling in P2**

A design element of P2 trials to date limits conclusions that can be made about genetic performance differences at specific locations. Bulked sampling is used, where the single samples obtained for a variety from each pick date are pooled across five trees to evaluate fruit quality traits. While it allows streamlined fruit handling and evaluation, this bulked sampling method does not allow the critical determination of differences among varieties at a location to be made because there is no independent replication of a variety at each location. Differences among varieties for the single observation of a trait at a single location are confounded by variation in the trait within a variety among its trees and among fruit on a single tree.

This sampling design also does not provide sufficient information to allow alternative sampling designs to be evaluated. The estimates of repeatability of the single observation across pick-dates are only useful for examining the adequacy of the number of pick-dates. However, as long as the statistical interaction of Genotype x Location (GxL) is not a significant source of variation (which it isn't for at least some traits – see point 7 below), this sampling may be an efficient design for predicting average genetic potential across the growing region by using the mean across three locations. Significant differences were detected among selections and standard cultivars for most traits, suggesting that three locations is a sufficient number for selection trialing.

6. **Information from comparisons to standard cultivars**

Comparisons with particular standard cultivars are used in P2 to calibrate trial performance to known commercial performance. Probabilities can be assigned to whether each selection performs better (or worse) than each standard cultivar for each trait (Figure 2; Table 1). Confidence in the genetic potential of selections could be increased if there were more varieties (selections and/or standard cultivars) in common planted in each year to improve connectedness among trials and allow effects of age and season to be accurately identified and adjusted for. In 2009 and 2010, the same five standard cultivars were planted in each location in each year, which is expected to provide strong genetic potential discernment. However, two other ways to increase connectedness among plantings would be to (1) include elite selections from P3 or new cultivars from P4 rather than additional standard cultivars, or (2) planting P2 trials only every second year, thereby combining the selections that

would otherwise have been planted separately in two annual plantings, and halving the number of standard cultivar trees need. Given the long term activity that is apple breeding, it is unlikely the loss of a year of evaluation for those selections delayed a year would be a major detriment.

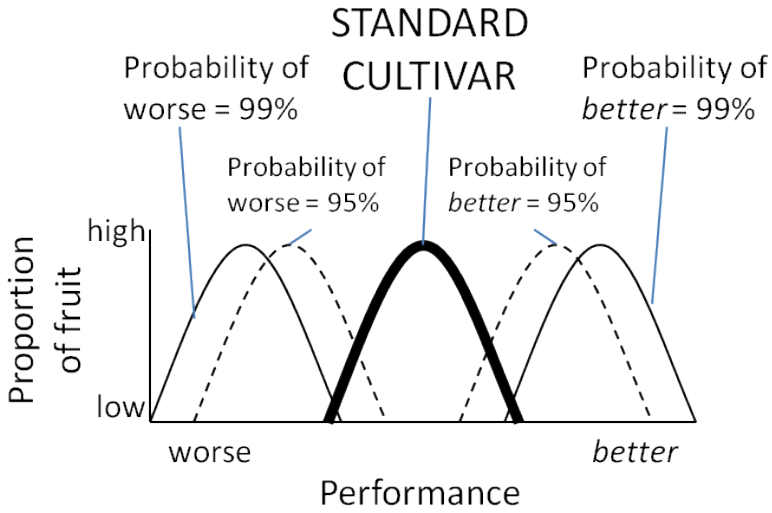

Figure 2: Visualization of what it means for a selection to outperform or underperform in comparison to a check cultivar. Probabilities can be quantified as to whether a selection's evaluated fruit (or some other important attribute) are statistically significantly better or worse than a standard cultivar. The standard cultivar is included in the same trials as selections.

No. of P2	Probability of performance relative to standard cultivar				Example cultivar
selections	Honeycrisp	Gala	Cripps Pink	Gala	also evaluated
1	equal	*99% better*	*99% better*	*99% better*	
2	equal	*95% better*	*99% better*	*99% better*	
12	equal	equal	*99% better*	*99% better*	Honeycrisp, Gala
3	equal	equal	*95% better*	*99% better*	Braeburn
2	equal	equal	*95% better*	*95% better*	
2	equal	equal	equal	*95% better*	
4	95% worse	equal	*99% better*	*99% better*	
1	95% worse	equal	equal	*95% better*	
2	95% worse	equal	equal	equal	
1	99% worse	equal	equal	*95% better*	
1	99% worse	equal	equal	equal	
2	99% worse	95% worse	equal	*95% better*	
1	99% worse	equal	equal	equal	
4	99% worse	99% worse	equal	equal	Golden Delicious, Cripps Pink, Fuji

Table 1: Adjusted mean performance (= genetic potential) for sensory crispness of WABP selections, 2004-2010 Phase 2 trials. Sensory crispness was measured on a 1-5 scale, where the adjusted (according to location, year, and storage) mean for standard cultivars were Honeycrisp = 3.8, Gala = 3.4, Cripps Pink = 2.3, and Fuji = 2.1.

7. **Information from multiple trial locations**

Performance at breeding trial locations appears predictive of commercial performance. Although P2 trial locations used are reasonably diverse, relative performance of varieties across those locations was stable for most fruit quality traits (and thus GxL [GxE] appears to be low). Because genetic correlations are therefore high among variety means across locations, and growers appear satisfied that one or more of these trial locations represent their own location, performance information from these multiple locations is likely to be predictive of performance in commercial orchards. This conclusion wouldn't have been valid if we'd found that one site was not well correlated with others – in that case the WABP couldn't tell what was "typical" and would need to replicate across more locations.

Although stability across locations indicates redundancy of locations, the current design has comfortable redundancy: evaluation over multiple locations provides insurance against unforeseen events leading to loss of information at any one location in a given year. Furthermore, relatively accurate predictions can be made for most fruit quality traits on less replication if resources in a given year are reduced.

8. **Information from particular trial locations**

A strong main effect of location was identified for all fruit quality traits examined. This finding underlies a strong recommendation for the WABP to determine and take into account the location mean effect when establishing genetic potential of selections. Not doing so would lead to incorrect estimates of genetic potential. An example of the implications: The current practice in P3 of comparing selections with standard cultivars growing in nearby orchards or at other locations rather than being established in the same planting may lead to under- or over-estimation of the true genetic potential of selections if there are environmental differences between the orchards (which according to our analyses is likely).

9. **Information from multiple years (season and tree age)**

As for locations, strong main effects of season and tree age were observed, and therefore these effects need to be calculated and adjusted for when establishing genetic potential of selections. Ranking of varieties appeared stable over seasons (and thus GxY [GxE] appears to be low). However, we were not able to separate the effect of tree age from the effect of season on the ranking of varieties.

Although an extensive examination was not undertaken due to time constraints, initial results suggest that fruit sampled from 2nd leaf trees are more variable than fruit sampled from older trees. It could not be determined if the apparent greater variation in fruit from the younger trees was due to ontological changes, improvement in the ability of field assessors to select mature fruit, or the greater number of mature fruit at later years enabling only mature fruit to be selected for assessment. However, the consistent ranking of varieties over assessment years suggests that the relative performance of varieties does not vary with age. Further investigations should be undertaken in this area.

One implication of the consistency of the ranking of varieties among seasons is that it may not be necessary to assess selections over many seasons. Another is that the current practice of eliminating some selections due to inferior performance in early years of P2 trialing appears suitable because it is unlikely that eliminated selections would start to show superiority in other seasons.

10. **Information on fruit maturity**

Fruit maturity should be either carefully standardized or else accurately measured for each sample and a mean-adjustment made when establishing genetic potential of selections for sensitive traits. A main effect of fruit maturity, as ascertained by starch levels, was generally more pronounced for

instrumental traits than sensory, perhaps because starch ratings are assessed on fruit that are also used for instrumental evaluation while sensory evaluations are done on separate sample of fruit from the same bulk. Certain traits were especially sensitive to fruit maturity (such as instrumental firmness and acidity and sensory sweetness). These relationships were generally consistent across varieties.

In the few location-year trials where sensory crispness had a significant relationship with fruit maturity, it was observed that crispness increased with fruit maturity – an interesting result indicating that WABP evaluations have successfully uncoupled the measurement of firmness and crispness at least for sensory measures.

It would be useful to examine how accurately the current sampling design estimates the starch rating of the bulk sample. Despite the intention to harvest fruit with the same maturity across pick dates, the starch rating of fruit from 2nd leaf trees increased with harvest date. However, this confounding relationship was generally avoided in older trees, probably because the greater number of fruit on older trees allows more careful choice of consistent fruit. Therefore, evaluations in 2nd leaf are not likely to be accurate for determining a selection's harvest window; efforts to establish the harvest window could be shifted to older trees only.

11. **Information on storage treatment**

Genetic potential of selections should not be calculated from an average of pre- and post-storage performance for traits that were sensitive to storage (such as firmness and acidity). In contrast, traits like shape, russet, lenticels, skin color, and even sensory tartness and crispness, were independent of storage condition. Only a few traits (especially instrumental firmness) showed evidence that storage affected the ranking of varieties, meaning that the relative genetic potential of varieties for a trait depends on if the apple is consumed fresh or after storage. Further investigations are required to identify if only particular varieties are susceptible to different storage conditions or it is a general re-ranking of all varieties.

For the majority of traits that didn't exhibit any interaction with storage, the ranking of varieties is essentially the same for fresh fruit as for fruit after storage. This lack of re-ranking under storage indicates that it may be inefficient to evaluate fruit quality both pre- and post-storage in P2 trials. As storage provides a useful indicator of susceptibility to storage disorders, fruit quality evaluation only after storage might be more efficient than the current practice. However, at-harvest fruit quality data collected in P2 provides an important baseline and depth of performance data in later years for decision confidence regarding handling and advancement of P3 elite selections.

12. **Information from multiple pick dates**

Multiple pick dates are used to help determine the length of the optimal harvest window – how long fruit can be harvested from a selection to provide superior or sufficient quality fruit to the market. P2 selections are picked at two to three pick dates that are a week apart. For those traits with high repeatability across pick dates, such as acidity and firmness (Figure 3), the P2 performance observed at any given harvest of ripe fruit (i.e., any pick date) is a good indicator of performance in general. Conversely, for those traits with low repeatability across pick dates, such as sensory sweetness, SSC, and sensory aroma (Figure 3), more replication is required. However, such replication should not be via additional pick dates as this is re-sampling of the same trees and thus is not independent replication. The next point describes improved replication strategies to gain a better measure of genetic potential for traits with low repeatability.

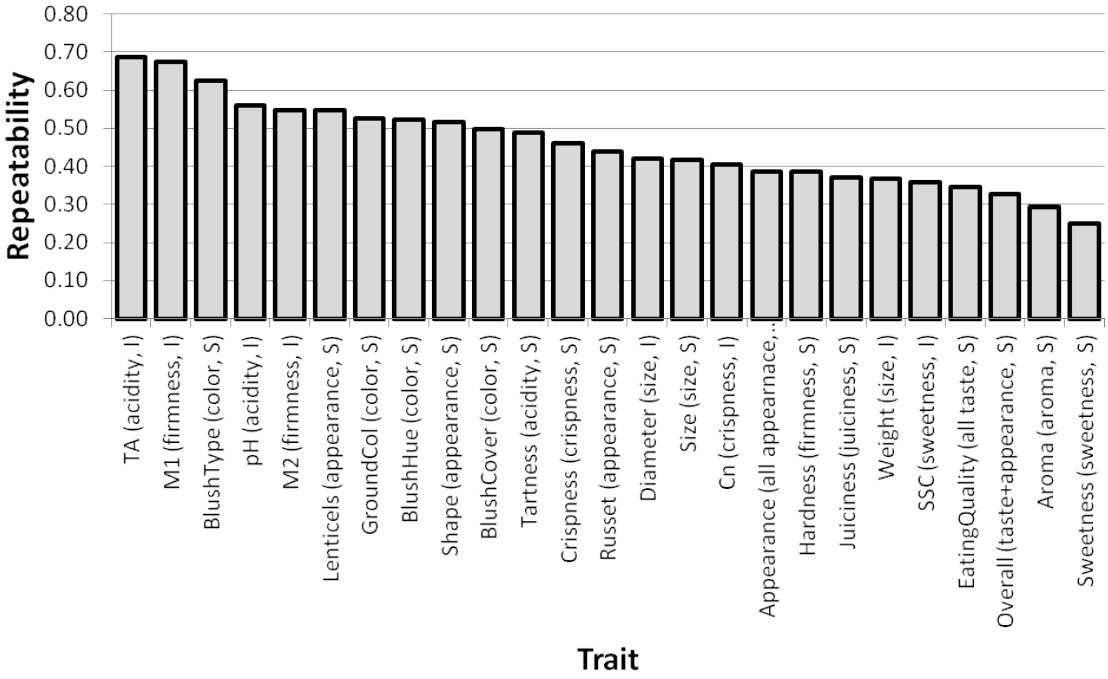

Figure 3: Repeatability of various fruit quality traits as determined from analysis of multiple pick dates in P2 trials from 2004-2010. "I" refers to traits determined from instrumental *measures, while "S" refers to* sensory *measures.*

13. **Information on trait heritability**

Heritability of a trait indicates the strength of genetic effects in determining the mean of a variety, once known environmental effects such as location, season, etc. have been adjusted for, compared to the strength of unpredictable environmental conditions. For highly heritable traits, the performance of a variety relative to other varieties observed at any given location, season, tree age, maturity, and storage condition is unlikely to change when the same varieties are observed under another situation, and so is a good indicator of performance in general (genetic potential). Conversely, for traits highly and unpredictably influenced by environmental conditions, the WABP needs greater replication than currently used to be confident that the observed adjusted mean is indicative of genetic potential.

Although the trial design did not allow assessment of heritability, multiple published studies have indicated that fruit firmness and acidity (and harvest date) have high heritability. Repeatability across pick dates, described in the previous point, was highest for instrumental measures of these same traits. Similar to published studies of heritability, we identified that sweetness (sensory and SSC) and aroma were more affected by undetermined environmental effects than all other examined fruit quality traits. Therefore, more replication is needed for gaining valid estimates of genetic potential for sweetness. Because of the bulked sampling method used in P2, the relative merits of increasing fruit number, tree number, or pooled sample number could not be determined, but one of these would be an efficient means of gaining a better estimate of genetic potential for this type of trait. Other replication-increasing approaches, such as more picks, locations, or years, would be less statistically robust or much more resource-consuming and thus less efficient.

14. **Information on each trait**

Some traits and performance levels for them are more important than others for commercial viability of a new cultivar. The WABP needs confidence that a selection performs above the minimum for all

traits (= achieves “essential”/baseline levels). The WABP also needs confidence that a selection performs exceptionally for one or more traits (= contains one or more “bells & whistles”). A trait target checklist has been developed (and is being refined) to allow systematic evaluation of such performance levels, especially for decisions regarding selection advancement out of P2 and P3.

15. **Information on genetically correlated traits**

Opportunities may exist for information on one trait to predict performance of another. Analyses of genetic correlations among traits to assess this have not yet been performed. For example, if TA and sensory acidity were calculated to be very highly genetically correlated, it would indicate that selection decisions need only take into account one of these measures of acidity. A high genetic correlation between a pair of traits would also indicate that measurements on both at the same time would be redundant and so dropping one would allow gain in efficiency (depending on the relative ease of such measurements and the opportunity/resource cost of missing other trait evaluations).

16. **Information from trained sensory and consumer panel evaluations**

Detailed evaluations of elite selections conducted by Carolyn Ross’s program focus on fine-scale determinations of differences among selections and check cultivars for fruit quality traits (trained sensory panels) and consumer preferences for fruit quality traits and trait combinations (consumer panels). Information from Dr. Ross’s studies has established a sensory profile baseline and identifies gaps in the current market portfolio of varieties. The information could be used in establishing objective trait priorities/weights at earlier stages of the WABP selection process. Measures obtained from trained sensory panels and preferences from consumer panels could be examined for correlation with P1 and P2 trait measures (sensory and instrumental) to determine how well the same traits are being evaluated. Such analyses have not yet been conducted.

17. **Information from the *Ma* locus**

Initial results indicate that the *Ma* locus is predictive for the traits of sensory crispness, sensory and instrumental firmness, and sensory juiciness at harvest. The test explained 28%-48% of the observed genetic variation for these traits and this was relatively stable across trial locations. Unexpectedly, the test provided no prediction of TA/tartness levels in selections, perhaps because of the truncated upper range in this elite germplasm. The test was also not predictive of instrumental crispness (Cn) or overall eating quality. The genetic test therefore appears to be a useful tool for guiding crossing and P1 seedling culling decisions for some fruit quality traits via marker-assisted selection, to help enrich progeny populations and their field-planted subsets with individuals that are expected to reach and in some cases exceed required baseline levels of texture genetic potential.

While there is evidence that some haplotypes were significantly better or worse than others for certain fruit quality traits at harvest, an alternative hypothesis is also plausible that the extreme haplotypes are due to alleles at other loci from specific sources. For example, the best two haplotypes for crispness are both from Honeycrisp, but superior Honeycrisp alleles elsewhere in the genome than the *Ma* locus may be providing superior crispness in its descendant selections. If the *Ma* locus does not contribute to the superiority calculated for Honeycrisp’s haplotypes, the worst the WABP can do by selecting for seedlings (grandchildren of Honeycrisp) carrying these haplotypes is lose seedlings with unknown genetic potential. However, the monetary cost of using the *Ma* locus test when the same seedlings have already been screened for other markers is marginal, and if the initial number of seedlings is greater than the WABP’s P1 capacity, there is little to be lost and much to be potentially gained by *Ma* locus screening. Furthermore, general use of the test to screen against poor haplotypes appears justified because the two poorest were single haplotypes from two different parental sources.

The *Ma* locus analysis provided the first quantitative genetics examination of predictive marker effects for WABP replicated trials, opening the door for further trait loci to be examined.

18. **Information from other trait loci**

We expect that other available genetic tests can be used to help predict genetic potential for fruit texture by explaining some of the genetic variation not covered by the *Ma* locus. However, the *ACS* and *ACO* gene tests were not very predictive of most traits in P2 trials. While the previously known alleles contributing low fruit ethylene levels tended to be associated with better firmness, especially at the Wenatchee trial location, their effects on texture were not anywhere as strong as those of the *Ma* locus. Combined analysis of the effects of the three trait loci on texture components may reveal that certain combinations of alleles are particularly predictive, but such analyses have yet to be performed. Several other potentially predictive genetic tests of fruit quality are available now but have yet to be tested for their predictability of P2 performance. From massive advances in 2011, the RosBREED project is poised in the first half of 2012 to discover and validate many new markers for apple fruit quality. Heavy involvement of the WABP in the RosBREED project will ensure these scientific breakthroughs are efficiently transferred to Washington apple industry benefit.

19. **DNA information from across the genome**

We expect to eventually build up comprehensive information from multiple trait loci to most accurately predict variety performance. Trait locus marker-based approaches as described above can be used to capture genetic variation explained by those markers. But in the meantime, while available trait locus markers explain only some of the observed genetic variation among varieties, a promising new approach called genomic selection (GS) may allow us to capture the majority of useful genetic variation for important traits to predict performance. The availability of many markers across the whole genome (such as the >5000 informative SNP markers from the RosBREED project's new genome scanning capability) makes GS possible. This promising approach should complement the WABP's existing MAB capability and to take advantage of advances in genomics technology. The accuracy of GS to predict new cultivar performance in commercial orchards depends on the size and comprehensiveness of the training dataset of performance information. Historical WABP performance data from P2 and P3 trials is a good start, and should be bolstered by existing cultivar trial data and RosBREED data. Performance-evaluated individuals in the latter have already undergone the necessary genome scanning (approx. $70 per individual if part of a larger coordinated effort). GS algorithms have yet to be developed for the WABP.

20. **DNA information on parentage and identity**

Performance is often viewed in light of the parentage of a selection/cultivar. DNA marker data (especially from genome scans) are useful to confirm or deduce parentage. In the absence of information on particular alleles at trait loci inherited from parents, parentage in general plays an as-yet unquantified role in new cultivar adoption decisions. Parentage is very important in breeding decisions – not in selection advancement decisions (unless a selection was intended to be descended from a unique trait source) but in crossing decisions. Even if the *Ma* locus is not predictive, our analyses in 2011 indicate that Honeycrisp is an excellent parent for crispness! Similarly, from other observations, Cripps Pink is an excellent parent for precocity, and many other traits have superior parental sources. The WABP would benefit from a systematic evaluation to determine the value of each parent and parental combination for providing particular levels (useful to approach this from the viewpoint of essential vs. bells & whistles) of each trait, which can be determined via calculations of general and specific combining abilities. Also, such information will be amplified in value for the WABP through use of RosBREED's Cross Assist tool (coming soon!) that directly uses such information to refine crossing possibilities.

Finally, simply confirming the identity of a selection or cultivar is a useful application of DNA testing and is a critical contributor to decision confidence for growers and the breeder when identity of a set of propagated trees is in any doubt.

EXECUTIVE SUMMARY

In 2011, a scoping project was undertaken to identify sources of information about genetic potential that can increase decision confidence in new cultivar development (breeding) and adoption (uptake by industry). Once identified and debated among project participants in various forums, we investigated methods to obtain within the breeding program and deliver to growers the most relevant decision-influencing information.

Some major findings in 2011 were that:

- The most important genetic-based drivers of grower adoption of new cultivars are consumer appeal, biotic stress resistance, yield, packout, and timing and length of the harvest and market windows.
- The WABP's current P2 trial design efficiently identifies genetic potential in selections for most fruit quality traits, but we have identified opportunities for improvement.
- Differences in performance among selections are relatively stable across P2 locations, and so the WABP can confidently predict orchard performance, but P3 data on postharvest performance is essential.
- Trial location, season, tree age, and storage each affect most fruit quality traits by increasing or decreasing the observed mean, but rarely change the relative ranking among varieties.
- Genetic potential for most fruit quality traits can readily be determined in few years, and selections that fail to perform well in early years of a trial can be safely discarded early.
- Effects of trial location, season, tree age, and storage must be calculated and adjusted for before genetic potential of a selection can be determined from a dataset that contains multiple levels of one or more of these factors.
- For certain traits that are highly unpredictably influenced by environmental conditions, such as sweetness, enhanced replication is required to obtain robust estimates of genetic potential.
- Available DNA tests currently used in the WABP can help predict performance for texture traits within and across WA growing regions.
- Pre-screening of parents and seedlings with predictive DNA tests for any traits currently evaluated in P1-P3 field trials is especially valuable for improving breeding efficiency.
- Opportunities exist to incorporate more DNA information for increasing decision confidence in new cultivar development.
- Multiple avenues of information delivery to growers and feedback to the WABP on new cultivar genetic potential for commercial success are important to maintain.

The 2011 project identified many opportunities for increasing decision confidence that warrant immediate attention in 2012 – some to refine and enact within the breeding program for impact in 2012, others to explore, test, and adapt to WSU's new cultivar development, release, and management program.

Engagement among all stakeholders in regional apple crop improvement is critical. Regular and systematic feedback between breeding and support program personnel and industry members, especially growers, is essential to the sustained success of the Washington apple breeding program and will amplify the return on investment and effort.

FINAL PROJECT REPORT

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
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City: Wenatchee
State/Zip: WA

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

Other funding sources:

Agency Name: Valent BioSciences, Agro-K
Amt. requested/awarded: (will partially defray harvest costs in 2010)

Total Project Funding: $9,320

Budget History

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

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; see Fig. 1).

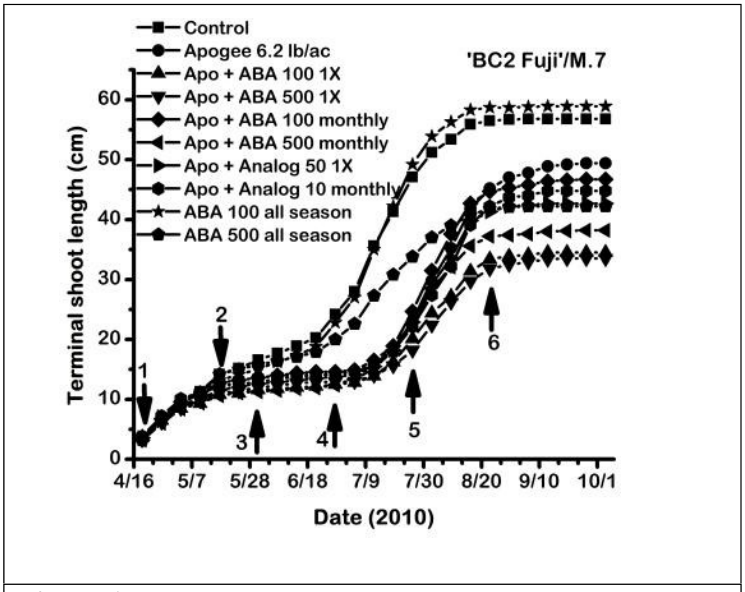

Figure 1

2. The full seasonal label rate of Apogee per acre was divided into two sprays applied 22 days apart (arrows 1 and 2 in Fig. 1). The first spray was made in late April and trees were retreated in mid-May (total of 6.2 lb. 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), despite application of the full seasonal labeled amount of Apogee.
3. Other Apogee-treated trees in the trial received one of several follow-up applications of either abscisic acid (ABA) or a chemically similar analog of ABA (arrows 3-6 indicate application dates) 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, applying the analog (50 ppm) once or 10 ppm monthly to previously Apogee-treated trees did not significantly reduce terminal shoot extension growth.
6. Treatments with Apogee, ABA and/or the analog showed no effects at all on flowering, fruit set, fruit growth, fruit size, fruit drop, fruit appearance or total yield.
7. ABA is reported to reduce stomatal aperture; this effect could contribute to reduced photosynthesis. However, even a season-long ABA program did not produce any signs of a significant reduction in photosynthetic production, which would most likely have manifested itself as a reduction in fruit size.
8. The latest application of ABA was made 24 Aug. 2010. Although other work of ours shows that ABA can stimulate defoliation, even a season-long ABA program that ended on 24 Aug. did not show any sign of producing early defoliation.

Significant findings 2011:

1. In 2011, Apogee was applied at the equivalent of 3.1 lbs. per acre over the season (half the full labeled rate). We chose this half rate because the results of 2010 studies clearly showed no advantage in terms of shoot growth control to applying twice as much of this product. The seasonal per-acre amount of Apogee was either divided into 4 equal spray amounts (arrows 1,2,4,6 indicate Apogee or Apogee/ABA tank-mix spray dates) or applied in 3 sprays as ¼, ½ and ¼ of the seasonal labeled amount (arrows 1,2,4 indicate Apogee or Apogee/ABA tank-mix spray dates). Applying a greater concentration of Apogee as the treated shoots were approaching their first period of controlled elongation was carried out to determine if the mid-summer period of non-elongation could be extended with this larger Apogee dose.

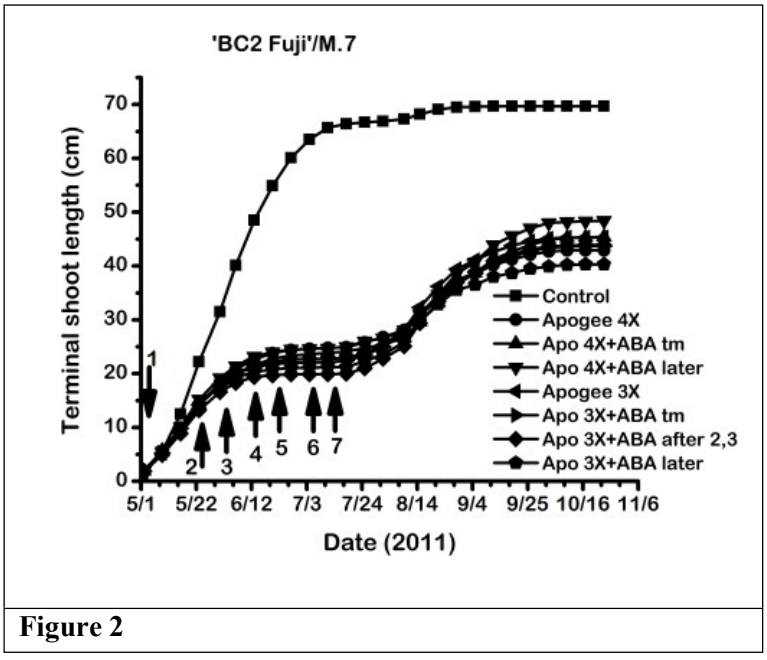

Figure 2

2. ABA was applied either as a tank-mix with Apogee (Apo4X+ABA tm or Apo3X+ABA tm, see Fig. 2) or as a follow-up application after the last 2 Apogee sprays. The amount of ABA applied in any spray was adjusted so that the equivalent of a total of 500 mg/liter was applied in each treatment.
3. The growth graph (Fig. 2) clearly shows that shoot growth behavior in 2011 was controlled by Apogee with little impact from any of the ABA application strategies. Shoot growth control in 2011 was better with the half-rate program than it was in 2010 with twice the amount of Apogee. The reasons for these observations are unknown.
4. For the second year in a row, treatments with Apogee and/or ABA produced no effects on flowering, return bloom, fruit set, fruit size or total per tree yield (measured as total number of fruit per tree). The likelihood of significant suppression of photosynthesis due to effects of ABA on stomatal aperture appears to be non-existent.

Methods:

Two trials were initiated in 2010 and one in 2011 to carry out follow-up trials to test various ways of combining Apogee and ABA applications that might produce some form of synergistic or interactive effects on apple terminal shoot growth extension or terminal-bud formation.

Results and discussion:

One of the key questions tested in 2010 was to what degree might ABA alone beneficially affect apple shoot extension growth. Applying 500 mg a.i./liter ABA alone 6 times to 'Fuji' apple trees did not produce satisfactory reduction in vegetative shoot extension. Applying the full labeled amount of Apogee in 2010 also did not produce acceptable control of growth. Hence the notion of examining Apogee/ABA tank-mixes and follow-up ABA treatments became the focus of the 2011 growth-control program. It is clear from Fig. 2 that no tested strategy of combining ABA and Apogee applications was effective for producing acceptable shoot extension control in 'Fuji' apple in 2011. It appears safe to conclude that ABA is unlikely to have a useful role to play in management of vegetative growth in apple.

Acknowledgements:

The assistance and support of the following people and organizations is gratefully acknowledged: Dr. Greg Clarke, Ken Dart, Tom Gausman, Jeff Henry, Dr. Peter Petracek, Richard Scranton, AgriMACS, Agro-K, Inc., BASF Inc., Valent BioSciences, Washington Tree Fruit Research Commission, Whiskey Ranch Orchards, and the WSU Agricultural Research Center.

Reports Published:

Elfving, D.C., D.B. Visser and J.L. Henry. 2011. Gibberellins stimulate lateral branch development in young sweet cherry trees in the orchard. **International Jour. of Fruit Sci. 11:41-54.**

Elfving, D.C. 2010. Plant bioregulators in the deciduous fruit tree nursery. **Acta Horticulturae 884:159-166.**

Elfving, D.C. and T.R. Schmidt. 2010. Bioregulator sprays. p. 133-146. In: M. Bush (coord.), **2010 Crop Protection Guide for Tree Fruits in Washington. EB 0419.**

Executive Summary

1. The amount of Apogee applied to an apple orchard is not directly related to the degree of shoot-growth control observed. It is likely that seasonal weather patterns may play an important role.
2. At cost-effective concentrations, abscisic acid (ABA, ProTone, Valent BioSciences) does not appear to produce effective control of vegetative growth in apple, either alone or in combination with Apogee.
3. ABA does not demonstrate the ability to encourage terminal-bud formation in actively-growing apple shoots.

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

Project Title: Programs to increase packouts of apples

PI: Ines Hanrahan
Organization: Washington Tree Fruit Research Commission
Telephone: 509 669 0267
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City/State/Zip: Yakima, WA, 98901

Cooperators: Tory Schmidt, Felipe Castillo, Tom Auvil, Jim McFerson, WTFRC, Wenatchee, WA, James Mattheis and Janie Countryman, USDA-ARS, Wenatchee, WA

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

Item	2010	2011	2012
Salaries	16,000	7,867	7,867
Benefits	5,120	2,439	2,439
Wages	7,000	7,585	7,585
Benefits	1,200	3,778	3,778
Equipment + supplies	2,200	100	100
RCA rental	12,600	12,600	6,300
USDA rental	750	0	0
Travel	4,000	2,013	2,013
Total gross costs	48,870	36,382	36,382
Reimbursements	(14,000)	(7,000)	NA
Total net costs	34,870	29,382	**30,082**

Footnotes:
Salaries: include proportional time spent on outlined projects for Hanrahan, Castillo, Schmidt
Wages: covers time slip expenses, sunburn: July-Oct.; physiological disorders and Honeycrisp storage all year; benefit rate at 42%
Supplies: fruit donated, storage boxes donated, proportional cost for quality lab incidentals
RCA rental: numbers based on fiscal year (@ approx. $6,300/room/year)
USDA rental: access to packing line and storage space for equipment
Travel: fuel costs to travel to and from trial sites (total of 2,750 miles @55cents/mile) and vehicle maintenance ($500)
Reimbursements: monetary contributions by chemical suppliers

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

OBJECTIVES

1. Compare sunburn protectant efficacy in apple.
2. Evaluate effectiveness of sunburn protectants to inhibit blush development.
3. Determine the effect of Raynox on development of delayed sunburn in storage.
4. Determine factors influencing the storage potential of Honeycrisp apples.
5. Devise methods to minimize disorder development when storing Honeycrisp apples.
6. Expand collaborative efforts with other research programs working on fruit quality management.

SIGNIFICANT FINDINGS

Sunburn protectants available to the industry work well in reducing the incidence of sun damaged fruit, but adequate coverage remains a problem, especially in v-trellis orchards (Table 1, 2).

Early season blush was not related to incidence of blushed fruit at harvest. The application of Raynox did not influence blush development (Table 2).

Delayed sunburn appeared between 2-4 months of storage, even on clean fruit (Table 3). In-season sunburn protectant application sometimes reduced the amount and severity of delayed sunburn.

The fruit maturity parameter most affected by crop load level and location was titratable acidity (Tables 4, 5).

Soft scald in storage developed in one site (Gleed), where it was highly correlated to maturity at harvest (Tables 6, 7).

No clear correlation could be found between crop load, harvest timing, storage treatment and taste of fruit after 4 months of storage.

We did not observe major differences in fruit exposed to varying amounts of time at 50F in this experiment, especially in CA (Table 8).

Ongoing collaborative efforts across disciplines, institutions, and regions leverage funding and increase relevance and impact of research (Table 9).

METHODS

Sunburn suppression: Two contract sunburn trials subsidized by a private chemical company were established in 2011 (Granny Smith in Sawyer and Wapato) testing 2 sunburn protectants (Raynox, organic Raynox). All materials were applied four times starting on July 6, according to each product's respective labeled rate. A third treatment of five consecutive Raynox applications was added in the Wapato site. Around each product application time, on-tree sunburn readings were performed on 200 fruit/plot in the most exposed zones of the trees.
Additionally, blush readings on the tree were performed on tagged fruit. At harvest, individual fruit were graded for sunburn according to the Schrader/McFerson system (0 = clean, 6 = necrosis). Postharvest evaluations included common fruit maturity parameters, background color grade, sunburn, and russet incidence.

Delayed sunburn: Trials were set up to test the onset of delayed sunburn in storage as influenced by Raynox in 2010 (Granny Smith, Wapato) and 2011 (both sites). Clean fruit was placed in cold rooms with RA and CA conditions and will be evaluated after different periods of time.
Honeycrisp storage/physiological disorders: In 2010, we sampled fruit from 3 mature, annually bearing Honeycrisp orchards in Washington that were under similar horticultural management, but in different growing regions (early/hot, medium, late/cool). In each location, we marked trees based on crop load: low = 2-3frt./cm^2 TCSA, medium = 5-6 frt./ cm^2 TCSA, high = 8-9 frt./ cm^2 TCSA and sequentially harvested the trees (up to 3 picks). Fruit was held for 1 week at 50F before being stored at 36F in regular atmosphere (RA), controlled atmosphere (CA: 0.5% CO_2, 2% O_2) for up to 10 months. Subsamples of fruit were pulled to evaluate storage performance frequently. In addition we performed one experiment delaying the time at 50F for up to 3 weeks in 2010. From 2009-11, we observed several lots in accelerated cold storage (storing fruit at 33F immediately after harvest for the development of soft scald. In addition, we are developing a starch chart and have monitored the occurrence of uneven red color development (blotchiness) on the tree.

RESULTS & DISCUSSION
Sunburn suppression: Sunburn is the primary physiological cause of cullage, sometimes damaging up to 50% of the fruit in a given orchard. The WTFRC revisited the question of sunburn protection product efficacy from 2008–2011, as several new products were introduced to the market (Table 1). Since 2008, eleven trials with 3 different varieties (Granny Smith, Golden Delicious, Braeburn) tested a variety of sunburn protectants (Raynox, organic Raynox, Cocoon, Eclipse, FruitShield, Invelop, Raynox Plus, SunGuard, Surround WP, SunShade and several proprietary formulations). Sunburn protectants tested over the years increased the percentage of sunburn-free fruit (data not shown). WTFRC trials have shown calcium-based products (Eclipse, FruitShield) to perform as well as industry standards (Raynox, Surround WP) (data not shown).

Table 1. Examples of commercial sunburn protectants currently available.

Active Ingredient	Commercial products available
Plant wax	Raynox Plus
Kaolin	Surround WP, Cocoon
Calcium carboxylic acids	Fruit Shield
N-pinolene	VaporGard
Calcium carbonate	Diffusion, Mask, Microcal, PurShade, Invelop, SunGuard, Eclipse, SunShade

In 2011, we scaled back our efforts and did product evaluation for one company on a contract basis. The field trials had varying degrees of sunburn pressure (54% in top portion of trees vs. 9-24% in middle portion of untreated controls) (Table 2). Based on harvest on-tree readings in the upper portion of the trellis, no significant treatment effects were observed. Fruit readings in the middle portion of the trellis showed a reduction of fruit affected by sun damage in the Sawyer site by both Raynox and the organic Raynox (Table 2). These differences were due to reductions in the number of fruit with slight sun damage (Y1).
Both trials in 2011 were conducted in v-trellis orchards. We continue to observe problems with adequate coverage in the top portion of the inside of the trellis, even with increasing rates of water/acre (130 gal/acre in 2011). We are planning to investigate the use of angled nozzles in 2012. Especially with high density systems, it is important to fill in any missing trees to avoid damaging fruit on opposite wires.

Table 2: Sunburn suppressant effects on harvest sunburn incidence, background color grade, and blush. Granny Smith/M9 and M26. WTFRC 2011.

Location	Treatment	Color grade	Blush	Sunburn incidence[b]		
		Green[a]		Middle		Top
				Sunburn	Y1	Sunburn
		(%)	(%)	(%)	(%)	(%)
Wapato	Organic Raynox	83 ab	52 ns	6 ns	4 ns	57 ns
	Raynox	88 ab	45	10	8	53
	Raynox 5 times[c]	89 a	45	11	6	50
	Control	75 b	47	9	5	53
Sawyer	Organic Raynox	91 ns	3 ns	11 ab	8 b	53 ns
	Raynox	89	2	6 b	5 b	54
	Control	83	2	24 a	18 a	54

[a] Experico scale (0.5-5, only 0.5 shown); www.experico.com.za
[b] at harvest readings of 30 fruit/ rep. in the middle section (4-5 ft.) and 200 frt. in the top section (6-9ft.) of the trellis
[c] fifth application 2 weeks prior to harvest

Delayed sunburn: In 2010, 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 four months of storage, 17-67% of fruit in each sunburn class showed delayed sunburn (Table 3), even if fruit was clean at harvest. Little change occurred in continued storage. Unlike 2009, when Raynox treated fruit showed up to 30% less delayed sunburn, no treatment effects were observed in the 2010 season.
Delayed sunburn is a major cause for cullage, and a deeper understanding of the lot-to-lot variability at harvest could increase packouts and drive storage decisions.

Table 3: Sunburn suppressant effects on sunburn classes after 4 and 6 month RA storage. Granny Smith/M26. WTFRC 2010.

Storage time	Treatment	Sunburn class*				
		0	1	2	3	4
4 months	Raynox	19	72	61	88	0
	Control	23	58	67	17	na
6 months	Raynox	21	80	80	100	44
	Control	26	72	73	50	na

*classes according to Schrader/McFerson scale

Honeycrisp storage: High demand and premium pricing has led to rapid increases in Honeycrisp acreage in Washington. 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 2010 we focused on harvest timing and started to investigate the influence of crop load and delayed cooling to the final storage temperature.

Crop load and orchard location. Ranges of several fruit quality parameters for fruit from one orchard location (Gleed) at harvest are shown in Table 4. The fruit maturity parameter most affected by crop load level and location was titratable acidity (TA) (Tables 4, 5). Low TA at harvest and subsequently through storage was noted for Frenchman Hills compared to other sites (Table 5). Very little soft

scald developed over time for the Frenchman Hills and Naches Heights locations (example in Table 6). Harvest time and crop load did not affect soft scald appearance in these two sites. The most consistent treatment showing low soft scald levels, besides the initial one week at 50F, has been CA.

Table 4: Selected fruit quality parameters at harvest. Honeycrisp/M9. Gleed, WA. WTFRC 2010.

		WEIGHT	SUGARS	ACIDS	FIRMNESS	COLOR	STARCH
Pick	Crop load	(g)	(% Brix)	(% malic acid)	(lbs)	(1-5)	(1-6)
1st	low	321	12.6	0.550	14.1	3.0	2.7
	medium	252	12.4	0.521	13.3	3.4	2.6
	high	244	12.5	0.496	13.6	3.8	3.5
2nd	low	315	12.8	0.559	13.5	1.5	3.1
	medium	212	13.2	0.463	13.4	1.6	3.4
	high	208	13.0	0.444	13.4	1.2	4.8
3rd	low	354	13.4	0.570	13.1	2.6	4.7
	medium	275	12.8	0.479	12.2	2.3	4.9
	high	232	12.9	0.445	12.9	2.2	5.3

Table 5: Honeycrisp titratable acidity (% malic acid) at harvest. WTFRC 2010.

Pick	Crop load	Gleed	Frenchman Hills	Naches Heights
1st	low	0.550	0.441	0.576
	medium	0.521	0.381	0.476
	high	0.496	0.438	0.460
2nd	low	0.559	0.360	0.478
	medium	0.463	0.380	0.461
	high	0.444	0.348	0.382
3rd	low	0.570	0.303	na
	medium	0.479	0.300	na
	high	0.445	0.243	na

The Gleed orchard behaved differently where soft scald development was strongly related to maturity at harvest (Table 7). Fruit from the last pick developed high amounts of soft scald in storage, regardless of storage treatment and/or time in storage. A set of fruit including all crop loads and storage treatments from the second pick at Frenchman Hills was sent to Pullman to be tested by a trained panel after four months of storage (data not shown). Results corroborated what had been observed in frequent fruit tastings during quality evaluations in the Mattheis lab: no major differences between treatments were perceptible. In general, panelists rated fruit as sweet, crisp, firm and juicy. Panelists also found fruit to be faintly mealy or astringent with intermediate apple flavor and sourness.

Table 6: Soft scald development in storage for second pick of medium crop load. Frenchman Hills, WA. WTFRC 2010.

Cropload	Storage	Storage time in months				
		2	**4**	**6**	**8**	**10**
Medium	RA	0	0	6	0	22
	CA	0	0	0	6	6
	MCP/CA	0	11	11	27	11
	MCP/RA	0	6	0	7	0

Table 7. Soft scald development after 4 and 8 months of storage. Gleed, WA. WTFRC 2010.

		Harvest 1		Harvest 2		Harvest 3	
Cropload	**Storage**	**4**	**8**	**4**	**8**	4	8
Low	RA	6	0	6	11	6	50
	CA	6	0	0	0	0	43
	MCP/CA	0	0	6	0	31	31
	MCP/RA	0	0	11	0	19	na
Medium	RA	0	0	0	6	56	56
	CA	0	6	6	6	29	53
	MCP/CA	0	0	0	20	31	47
	MCP/RA	0	0	6	0	56	67
High	RA	0	na	0	0	50	44
	CA	0	0	0	0	72	61
	MCP/CA	na	na	0	na	72	50
	MCP/RA	na	na	0	0	76	50

Delayed cooling. Keeping Honeycrisp apples at 50F for one week has been an effective method to delay and/or reduce the onset of soft scald. However, longer periods in less than minimum acceptable temperatures can lead to advanced maturity and the onset of bitter pit. In reality, storage rooms are not always filled at the same predetermined time interval. Table 8 shows results of a lone experiment performed in the 2010 storage season. Fruit at maturity was considered optimum for longer storage (TA>0.5; some remaining starch, good firmness >14 lbs. etc.). After 4 months in storage, all fruit exhibited acceptable firmness, SSC, and TA. No discernible differences were observed between storage treatments for disorder development. If storing fruit in RA, the time in delayed cooling did not influence greasiness after 4 months in storage, as all batches were greasy. Fruit stored in CA (with or without 1-MCP) was not greasy, when stored for 4 months after one week at 50F. Longer periods at 50F led to greasiness levels (22-39%) which were lower than comparable levels from RA. In conclusion, we did not observe major differences in fruit exposed to varying amounts of time at 50F, especially when storing batches in CA.

*Physiological disorders***.** We have observed the on-set of softscald for several orchards over a 3 year period to understand year-to-year variability. Fruit in this experiment was placed at 33F immediately after harvest and observed in weekly intervals for up to 3 months (data not shown).
Based on field observations from 2010, we started to observe several orchards during the last weeks before harvest to determine the extent and the development of uneven red coloring of fruit (data not shown).

After receiving multiple requests from industry, we started working on a Honeycrisp specific starch scale in 2010 and continued this past harvest season. We are currently finalizing a starch scale for Honeycrisp and will have it available in the 2012 harvest season.

Table 8. Storage performance of Honeycrisp apples held 1-3 weeks at 50F and 4 month storage. WTFRC 2010.

	Firmness	SSC	TA	Starch	BP	Int. brown.	Softscald	Grease
	Lbs.	% Brix	% malic acid	(0-8)	(%)	(%)	(%)	(% severe)
At harvest	16.6	13.2	0.501	6.7	0	0	na	na
1 week 50F+RA	15.5	12.8	0.325	-	6	0	0	44
1 week 50F+CA	15.7	12.6	0.371	-	0	0	11	0
1 week 50F CA+MCP	15.4	12.9	0.331	-	10	0	0	0
2 weeks 50F+RA	15.5	12.7	0.352	-	0	0	0	44
2 weeks 50F+CA	15.4	13.2	0.361	-	6	0	0	39
3 weeks 50F+RA	15.6	13.0	0.352	-	0	6	6	61
3 weeks 50F+CA	15.7	12.5	0.369	-	6	0	0	22

Collaborative research

We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to pre-and postharvest fruit quality management (Table 9).

Table 9. 2011 WTFRC collaborations on external pre- and post- harvest fruit quality management projects.

COLLABORATOR(S)	PROJECT	COMMENTS
Rudell	Disorder toolbox	Cooperator on SCRI project
NSure/Sensitech*	Maturity and disorder ID	WA field testing
PickerTech/Oxbo*	Picking machine	WA field testing
Killinger	Packingline microb. safety	Field support (see AP-09-906)
Wetherington	Microbial baseline levels	Cooperator on CPS project
Brunner, MSU	Solid set spray system	Cooperator on newly approved SCRI project

*project costs covered by companies

Outreach (2010-11)

Talks: Update on Honeycrisp storage (Yakima Pom Club, January 2010)
WTFRC Internal Program (Horticulture seminar, Pullman, April 2010)
Storing Honeycrisp (CA clinic, Michigan, August 2010)
Honeycrisp storage (Yakima Pom Club, September 2010)
Honeycrisp storage update (Yakima Pom Club, October 2011)
Crop physiology lecture series at Heritage University (November 2011)

Papers/abstracts:
Extending the Honeycrisp marketing season in Washington State (ASHS, July 2010)
A Diagnostic Toolbox for Integrated Management of Apple Postharvest Necrotic Disorders (Rudell et al.; ASHS, 2011)

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

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

PI:	Jim Mattheis	**Co-PI**:	Dave Rudell
Organization:	USDA, ARS	**Organization**:	USDA, ARS
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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 awarded (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. Ethanol and ethyl ester accumulation increased with decreased O_2 content ('Delicious') or increased CO_2 content ('Fuji'). An increase in ethyl esters was detected prior to increased ethanol.

3. Production of a number of other 'Delicious' volatiles remained low or decreased in chambers held at 0.2% compared to 0.7 or 1.5% O_2.

4. Relationships between CA chamber ethylene, nitric oxide and O_2 or CO_2 concentration were apparent.

5. Volatile compounds in commercial CA rooms containing 'Delicious', 'Fuji', and 'Granny Smith' apples were similar in identity to those detected in research CA chambers, and compounds previously identified as possible predictors of superficial scald were detected in both research chambers and commercial CA rooms.

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. All fruit were 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 ºF 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.

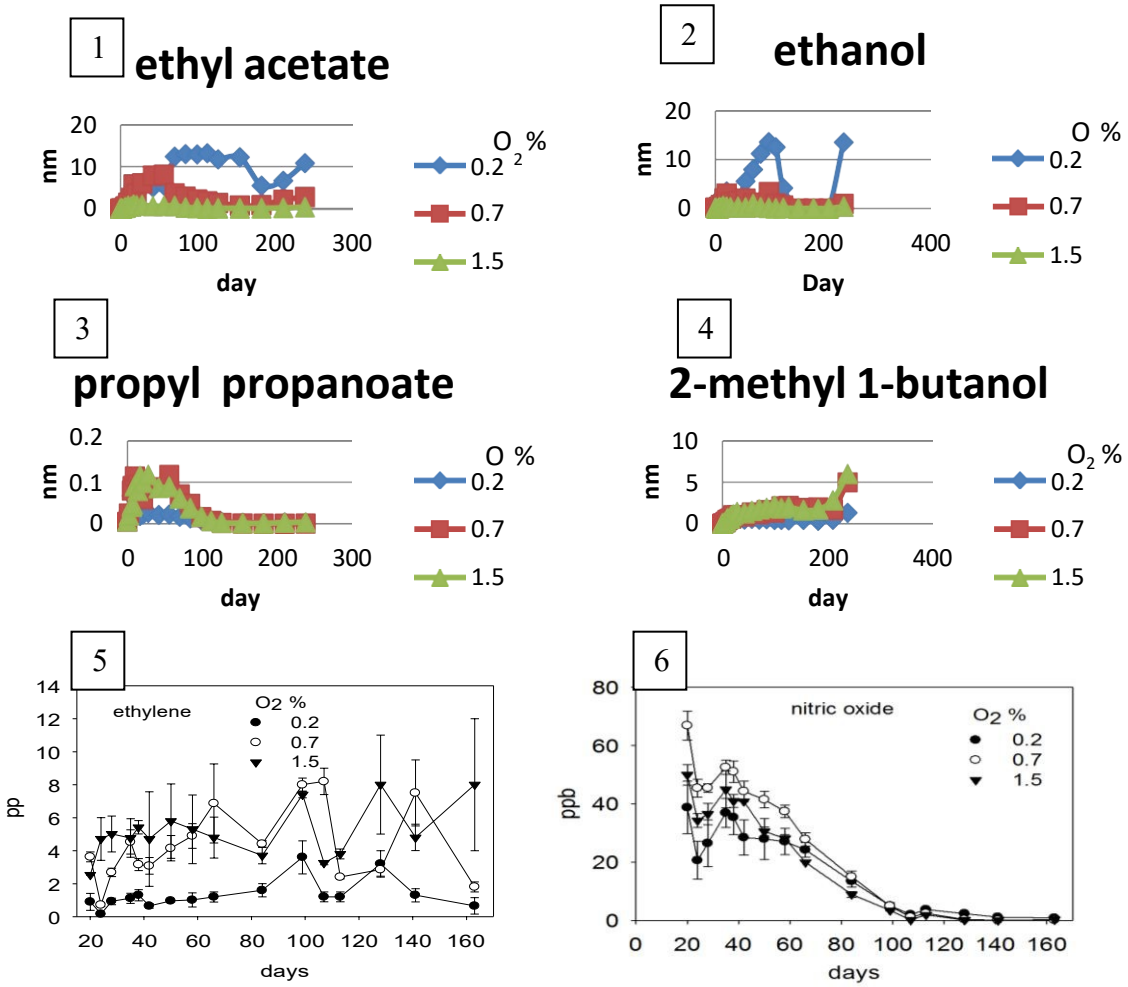
Year 3: *Objective 3:* Validate results from years 1 and 2 using multiple lots for a subset of cultivars based on strength of the first two year's results. Initiate studies to evaluate system capacity to mitigate injury development by relieving CA low O_2 or high CO_2 stress after stress- related volatile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

'Delicious' apple volatile accumulation during low O_2 storage. Ethyl esters, particularly ethyl acetate, accumulated after the first week in chambers held at 0.2 or 0.7% O_2 (Fig. 1). Ethanol also accumulated but peak concentration at 0.2% O_2 did not occur until 100 days after harvest (Fig. 2). Accumulation of several esters decreased with decreased O_2 concentration including propyl propanoate (Fig. 3). At 0.2% O_2, 2-methyl-1-butanol did not accumulate relative to the 0.7% and 1.5% O_2 chambers (Fig. 4). Ethylene and nitric oxide (NO) content were also related to chamber O_2 concentration (Figs. 5, 6). Ethylene and NO were both lowest for most of the first 60 days in chambers held at 0.2% O_2. Fruit stored at 0.2% O_2 had a pineapple-off flavor at removal from CA.

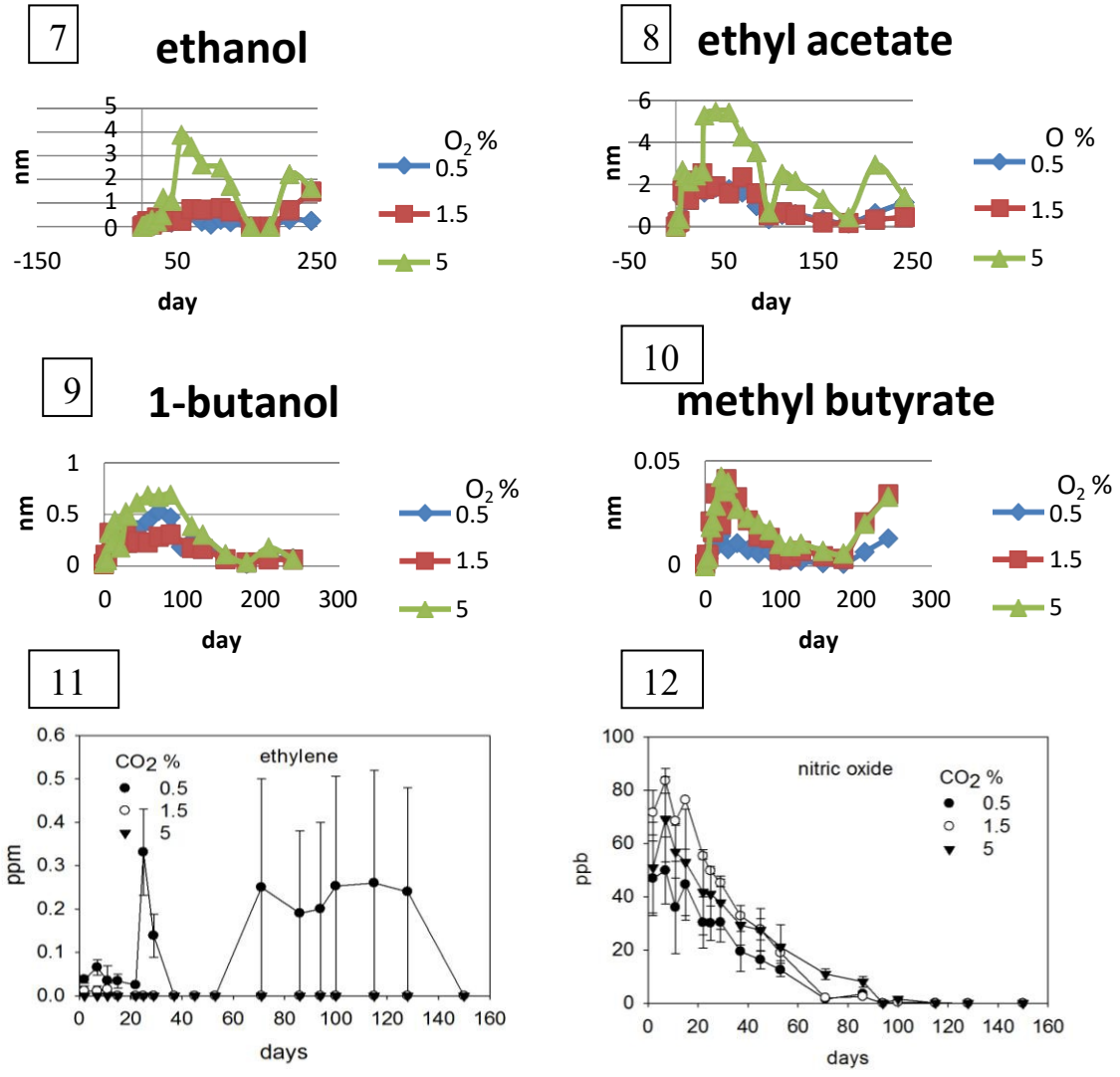
This off-flavor was not present after fruit were held 7 days at 70 ºF. Low oxygen injury (coretex-, core-browning) was present after 4 and 8 months in fruit stored at 0.2% O_2.
The results indicate fruit volatile production changes in response to chamber O_2 concentration with production of ethyl volatiles increasing as ethanol becomes abundant. As ethanol accumulates, production of other volatiles decreases including various non-ethyl esters and alcohols other than ethanol. This pattern reflects a metabolic response to the alcohols present in fruit as esters are produced in part based on the alcohols available at any point in time. The accumulation of ethanol in the 0.2% O_2 chambers may indicate oxygen is too low to allow metabolism of ethanol to other volatiles. A pattern of increased ethyl volatiles with decreased O_2 content indicates that even at moderately low (0.7% O_2) sufficient ethanol is formed to alter the pattern of volatile production relative to higher O_2 concentrations.



Figures 1-6. 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™ bags.

'Fuji' apple volatile accumulation during high CO_2 storage. Ethanol and related ethyl ester accumulation occurred in chambers as CO_2 increased from 0.5 to 5% (Figs. 7,8). The accumulation of ethanol was slower when compared with ethyl acetate and other ethyl-esters. A decrease in other alcohols and esters with increased CO_2 content was not as pronounced as for 'Delicious' apples stored in ultralow O_2 (Figs. 9,10). Cahmber volatile accumulation increased with increased CO_2 content. The volatile accumulation patterns for chambers at 0.5 and 5% were very different with the pattern for the 1.5% CO_2 chambers intermediate, having some but not all features of chambers held at 0.5% or 5% CO_2.

Small amounts of ethylene were initially detected in chambers held at 0.5% and 1.5% CO_2, respectively, however, no ethylene was detected in chambers held at 5% CO_2 (Fig. 11). Nitric oxide was detected regardless of CO_2 concentration with the amount decreasing to a non-detectable amount near 100 days in storage (Fig. 12). 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 through 9 months storage. The odor is distinct from that detected in 'Delicious' chambers held at 0.2% O_2.



Figures 8-12. 'Fuji' volatile accumulation during CA storage. Fruit were held in 1% O_2 with 0.5, 1.5, or 5% CO_2.

'Granny Smith' apple volatile accumulation during air or CA storage. Fruit were stored in air or CA (1% O_2, 1% CO_2) with or without pre-storage DPA (2000 ppm) treatment. Through 8 months storage, volatile compounds typical of 'Granny Smith' were detected with amounts lower in CA chambers. Several volatiles previously related to scald development were 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' and 'Fuji', ester accumulation increased throughout the first 8 weeks CA storage of 'Granny Smith'. Superficial scald symptoms developed on fruit stored in air or CA and volatile accumulation indicative of scald preceded symptom development.

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

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

All volatiles detected (46) in commercial rooms have been previously identified to be present in research CA chambers containing the same cultivars. Amounts of volatiles detected in all commercial room samples were low compared to samples collected from CA chambers. Of the 3 cultivars, volatile content in the 'Fuji' room was highest followed by 'Delicious' and then 'Granny Smith'. 2-methyl-1-butanol was the most abundant volatile collected in all three rooms, and ester production was low in all rooms. Several volatile compounds that have been associated with future development of superficial scald were detected in the commercial 'Granny Smith' room. 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 **YEAR**: 1 of 3

Project Title: Testing biomarker-based tools for scald risk assessment during storage

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City/State/Zip: Wenatchee, WA 98801

Cooperators: Dr. Bruce Whitaker

Total Project Request: **Year 1:** $24,750 **Year 2: $24,750** **Year 3:** $24,350

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 proposed project extends and compliments the activities of this SCRI project.

Agency Name: AgroFresh, Inc.
Amt. awarded: $232,253 (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).

Budget

Organization Name:USDA-ARS **Contract Administrator:** Chuck Myers

Telephone: (510)559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2011	2012	2013
Salaries			
Benefits			
Wages	$15,038	$15,038	$5,263
Benefits	$4,962	$4,962	$1,737
Equipment			
Supplies[1]	$1,000	$1,000	$1,000
Travel			
Miscellaneous[2]	$3,750	$3,750	$3,750
Miscellaneous[3]			$12,600
Total	$24,750	**$24,750**	$24,350

Footnotes:

[1]Liquid nitrogen for sample processing and instruments; sample vials.

[2]25% service and maintenance for laboratory instruments used in project.

[3]Storage rental fee (2 rooms at $6,300 each per season)

Objectives:

1. Determine if scald risk assessment tools indicate when delayed CA imposition leads to high scald risk and high scald incidence.
2. Indicate if and when scald risk is high during CA storage based on risk assessment tools and determine if storage conditions can be changed to alter biomarker levels and scald incidence.
3. Assess effectiveness of scald-risk assessment tools in pilot scale and commercial CA storages.

Goals and activities for the next year:

Real-time scald risk monitoring of CA stored fruit to indicate when scald risk due to adverse storage conditions (too high oxygen) and if altering conditions (reducing oxygen levels) will alter the storage outcome by reducing scald or extending the scald-free storage period.

SIGNIFICANT FINDINGS:

1. Test validated 21 out of 25 scald risk assessment biomarkers (SRABs) continued to be useful to assess scald risk.
2. Levels of 13 SRABs most effectively reflected scald risk altered by delaying CA.
3. Candidate biomarker levels correlated with scald risk as early as 3 months after CA imposition where scald was first detected starting at 9 months.

METHODS:

Equipment and Cooperative Summary: Tissue sampling, and analysis of biomarkers using analytical instrumentation (gas and liquid chromatography-mass spectrometry) will be performed at ARS-TFRL, Wenatchee. Storage experiments will be performed both at ARS-Wenatchee and in commercial storages. Additional chemical identification and molecular characterization will be performed in cooperation with Dr. Bruce Whitaker (BARC, USDA-ARS, Beltsville, MD). New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

Experimental Plan
Procedures:
Year 1
Relationships between delayed CA and levels of candidate scald biomarkers
'Granny Smith' apples will be harvested 1 month prior to commercial harvest to improve the likelihood of maximum scald susceptibility. Firmness, internal ethylene concentration, soluble solids, and titratable acidity will be evaluated at harvest. Apples will be stored at 33 ^{o}F in air for 0-1 month upon which subsets will be transferred to CA (1% O_2: 1% CO_2). Scald risk assessment biomarker (SRAB) levels were evaluated monthly from 0-6 months. Scald incidence and severity will be evaluated immediately after removal from storage (from 2 to 10 months) and following 7 days at 68 ^{o}F.

Year 2
Test whether biomarker levels and scald incidence can be altered by altering CA conditions mid-storage.
'Granny Smith' apples will be harvested 1 month prior to commercial harvest. Fruit maturity and quality will be evaluated at harvest. Apples will be stored at 33 ^{o}F in CA at 1% or 5% O_2 (all 1% CO_2). Scald risk assessment biomarker levels will be monitored monthly. When biomarker levels indicate scald risk is increasing, a portion of the fruit stored at 5% O_2 will be pulled down to 1% O_2.

Biomarker evaluation will continue to indicate if levels return to "normal" levels. Scald will be evaluated in all treatments at 0 and 7 days (at 68 °F) after 3, 6, and 9 months storage.

Year 3

Biomarker-based scald risk-assessment under scaled-up test conditions

'Granny Smith' apples will be taken from 5 commercial lots with varying scald-susceptibility histories. Multiple bins of fruit from each lot will be stored in commercial pilot scale rooms (40 bin) or larger rooms at 33 °F in CA at 1% or 5% O_2 or 1% O_2 after a delay of 1 month in air. Scald risk assessment biomarkers will be measured monthly from 1-9 months. Scald will be evaluated in all treatments at 0 and 7 days (at 68 °F) after 3, 6, and 9 months storage.

Biomarker-based scald risk-assessment under commercial conditions

'Granny Smith' apples will be sampled from 5 lots from each of 3 organic commercial long-term CA rooms. Scald risk assessment biomarkers will be measured monthly from 1-9 months. Scald will be evaluated in all treatments at 0 and 7 days (at 68 °F) after 3, 6, and 9 months storage.

RESULTS & DISCUSSION

Scald risk assessment following delayed CA storage and DPA treatment

Scald was reduced or eliminated by DPA drenches (Figure 1). Scald symptoms developed on air-stored fruit beginning at 6 months and on CA stored fruit beginning at 9 months. Delayed (up to 1 month) CA had little relationship with final scald incidence and severity after 10 months storage in 1% O_2 plus 7 days at 68 °F. Levels of 21 out of 25 SRABs increased at least 3 months prior to scald symptom development at 9 months in CA storage (not shown). Levels of 13 of these SRABs were highly reflective of final incidence and severity of scald symptom development while SRABs did not increase in fruit that didn't develop scald (Table 1). For example, elevated scald risk was actually detectable by measuring SRAB N24 at 2 months in air, air+DPA, and CA fruit not treated with DPA, all treatments which developed scald by 10 months storage (Figure 2).

Rather than relying on changing levels of just 1 or 2 SRABs, we envision monitoring multiple SRABs to provide a more complete risk assessment. Using multiple SRABs may be a useful way to monitor many different indicators of the causes and effects of scald, painting a more complete picture of the condition of the peel during storage. For instance, many of the SRABs that closely reflect final scald levels may reflect levels of the oxidative stress that ostensibly leads to scald development. By monitoring these SRABs, we are actually providing a direct assessment of damage caused by adverse storage conditions that lead to scald.

It remains to be seen whether differences are reflected among susceptible and non-susceptible lots or under commercial CA conditions. We expect considerably more candidate biomarkers will be discovered as a result of our gene expression analysis efforts underway as part of the companion SCRI project, Postharvest Toolbox. We also plan to begin to employ easy platforms or protocols for monitoring some of these risk assessment protocols in the final year of this project.

Real-time scald assessment (progress)

Tests designed to indicate whether detection of high scald risk can be used to indicate when storage environment should be changed and whether these changes actually extend the symptom free life of the product. Already, 3 months of evaluation will have been completed. In the second month, increased scald risk was indicated by 3 SRABs in fruit held at 5% O_2.

Other progress

In collaboration with Dr. Bruce Whitaker, a group of unknown candidate biomarkers and other apple peel chemicals in our data base have been identified as p-coumaryl acyl esters. These apple peel chemicals are part of the waxy surface layer and are likely part of the wax structure. A few of these compounds have potential as biomarkers.

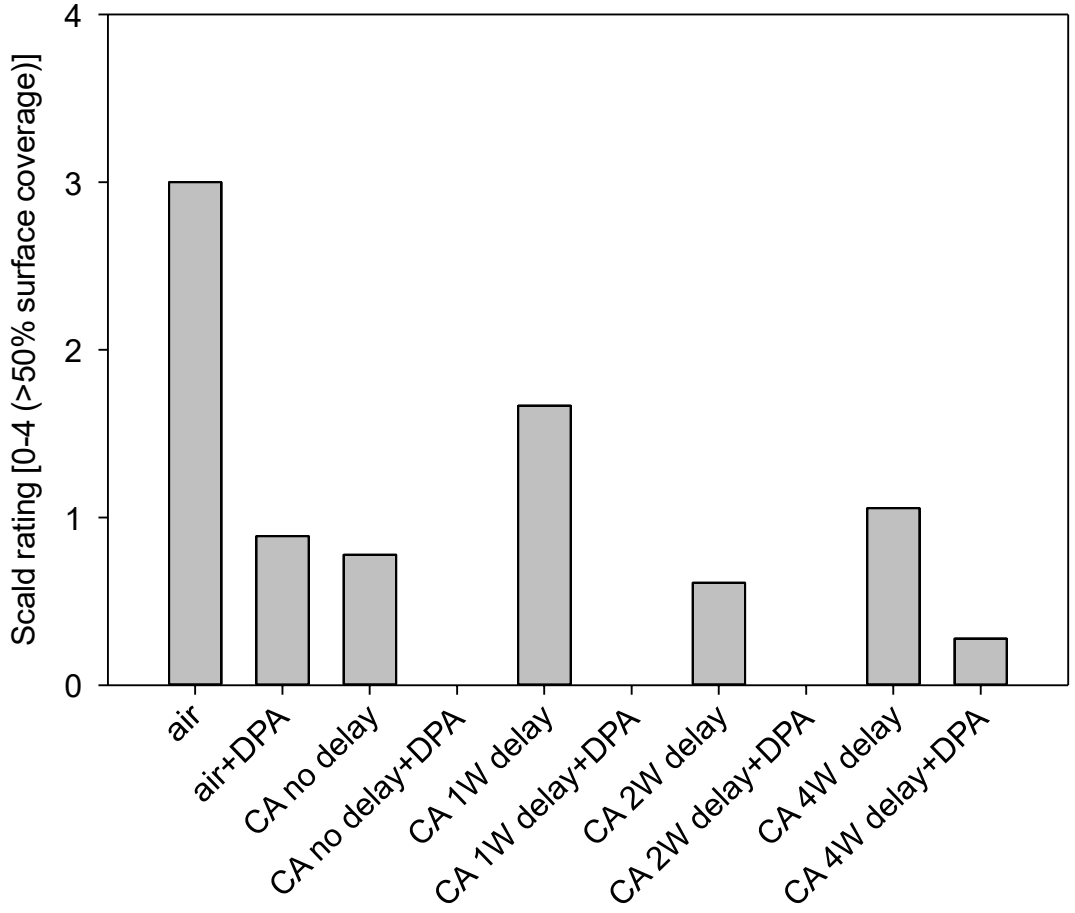

Figure 1. Superficial scald severity following 10 months air or 1% O_2, 1% CO_2 CA storage at 33 °F. Delaying CA imposition by 4 W reduced the effectiveness of at harvest DPA drench although had no relationship with the final scald severity of non-DPA treated fruit. Scald symptoms first showed in air stored fruit at 6 months and 9 months in some CA treatments which was relatively late in comparison with other years.

Table 1. A list of CA scald risk assessment biomarkers (SRABs) that best reflected scald levels following 10 months air or 1% O_2, 1% CO_2 CA storage at 33 °F. The "x" beside the each SRAB designates when and which biomarker levels best correlate (R > 0.70) with final storage outcome. Different SRABs likely reflect scald risk for different reasons and it is expected that evaluating multiple SRABs will provide the most accurate assessment. Scald symptoms began developing on CA stored fruit at 9M.

SRAB	3M	6M
N24	x	x
N30		
N34	x	x
N110		
N122		
N124		
N157		x
N172		
N210		
N211		
N220		
N222		
NC49		x*
MHOH		x
MHO	x	x
N187		
NHB31		
N248		
NHA34		x*
Sitogl182		x
Camgl182		x
Sitogl183		x
Sitogl180		x
N192		x
Sitogl160		x

*SRAB levels reduced with increased scald risk.

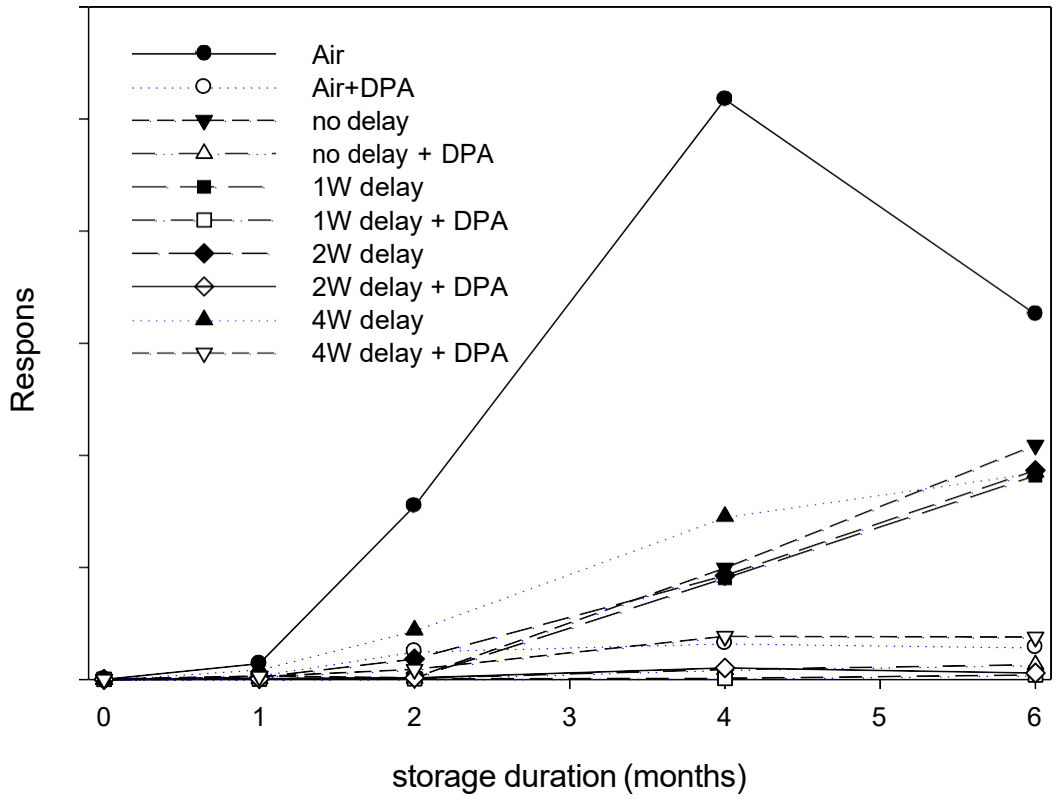

Figure 2. Changes in peel levels of scald risk assessment biomarker (SRAB) N24 during 6 months of storage. Levels increase starting as early as 2 months representing final scald symptom development at 10 months + 7 days. This and similar SRABs are directly linked to the damage provoked by storage conditions that lead to scald. These SRABs can also be monitored using less expensive, easily adapted techniques.

CONTINUING PROJECT REPORT **YEAR**: Year 3 of 3(Extension)
WTFRC Project Number: AP-09-906

Project Title: Validation of fresh apple packing food safety interventions

PI:	Karen Killinger, Ph.D.	**Co-PI (2):**	Richard Dougherty, Ph.D.
Organization:	WSU/ School of Food Science	**Organization**:	WSU/ School of Food Science
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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 arrived in 2011 for chlorine and chlorine dioxide experiments.

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

Item	2009 (funded)	2010 (funded)	2011 (funded)
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
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	50,990	53,030	55,152

Footnotes:
[1] Additional funds are not requested

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) and chlorine on whole, fresh apples using industry-relevant concentrations and exposure durations as indicated by currently available scientific literature.

- In Year 3, laboratory experiments focused on chlorine, using oxidation-reduction potential (ORP) in millivolts (mV) for measurement of chlorine activity. Treatment combinations included a water treatment, three chlorine concentrations (665, 750 and 850 mV ORP), and three application times (2, 3.5 and 5 minutes) to represent time of exposure in dump tanks.
    - Based on industry input of common practices, an additional treatment of phosphoric acid buffer (approximate pH 3.5) was included to represent treatment of apple varieties when chlorine is not used in the dump tank.
    - Three to five replications for generic *E. coli* and pathogenic *E. coli* O157:H7, respectively, have been completed. Data indicates that additional replications are needed to ensure statistical validity, so reported findings should be considered preliminary. Chlorine (665, 750 and 850mV ORP) and phosphoric acid treatments produced less than a 90% reduction of pathogenic *E. coli* O157:H7.

- Differences between the responses of pathogenic *E. coli* O157:H7 and generic *E. coli* may exist. In Year 3 laboratory experiments, pathogenic *E. coli* appeared to adhere to apples to a greater extent than nonpathogenic *E. coli* which may have affected the ability of chlorine to reduce pathogenic *E. coli* levels. Seasonal variation may also contribute to bacterial adherence to apples.
    - It is important to understand differences between pathogenic and non-pathogenic *E. coli* so that accurate estimation of pathogen reduction with commercial scale interventions (analyzed with non-pathogenic *E. coli*) can be performed.
    - An increased understanding of the response of pathogenic *E. coli* on apples and the potential influence of apple physiology on bacterial interactions provides the industry with advanced knowledge to enhance food safety efforts.

- In Year 3, commercial scale studies for PAA were initiated. Preliminary results indicated that 80ppm PAA produced a 0.6 $\log_{10}$ reduction and a double spray bar 80ppm PAA treatment produced a 0.8 $\log_{10}$ reduction in generic *E. coli*. Reductions were less than 0.5 $\log_{10}$ for 40 and 60ppm PAA.

- Laboratory PAA results produced greater microbial reduction; concentrations and exposure durations based on industry practices resulted in 0.5 – 1.3 $\log_{10}$ reduction of *E. coli* O157:H7.

- 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:

Chlorine Laboratory Validation Studies. In Year 3, laboratory validation work was designed to examine chlorine antimicrobial treatments similar to dump tank applications. Additionally, industry input indicated that a treatment of phosphoric acid alone should be included to mimic dump tank treatment of apple varieties that do not involve chlorine. Therefore, the compounds and concentrations tested included: water, phosphoric acid (approximate pH 3.5) and 3 concentrations of chlorine as measured by ORP (665, 750 and 850 mV). Three application times were examined (2, 3.5 and 5 minutes) and both pathogenic *E. coli* O157:H7 and non-pathogenic (generic) *E. coli* were examined. In each experiment, 16 treatment combinations were examined with 5 apples in each of the 16 treatments. Appropriate dilutions were 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 were incubated at 95˚F and enumerated manually.

Peroxyacetic Acid (PAA) Commercial Scale Validation Studies. In Year 3, four experiments at commercial packing facilities were conducted to assess PAA at the spray bar. Discussions involving industry representatives, chemical suppliers and WSU personnel identified several parameters of interest for examination in addition to PAA concentration, including: low and high pressure nozzles, use of a water rinse, use of soap, and use of a double spray bar. It was determined that all of these factors could not be addressed adequately given time and resources. Experiments focused on examining 40, 60 and 80ppm PAA alone in all four replications. Use of high and low pressure nozzles was examined in two replications and a double spray bar application was examined in two replications.

Apples were inoculated at WSU and transported in coolers containing ice to the packing facilities. Inoculated controls were measured immediately after inoculation at WSU, after arrival at the packing facility and apples were collected off the packing line prior to the spray bar treatment. This examined the potential for microbial growth during transport and throughout the experiment at the packing facilities. Inoculated apples were placed on the packing line and twenty apples were collected prior to and after the spray bar for each treatment. Apples that were collected after the spray bar were allowed to travel down the packing line to the fans to account for potential residual antimicrobial activity from PAA treatment. Apples were sampled immediately after collection and the diluent was chilled for transport to WSU. Samples were diluted appropriately and plated on VRBA for enumeration of generic *E. coli*.

PAA Laboratory Validation Studies. In Year 2 laboratory studies, apples were placed directly in PAA (40, 60 or 80ppm) for 5 seconds followed by removal and 4 exposure times (10, 25, 40 or 60 seconds) to mimic time on a conveyance system, based on industry input and plant visits. 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. In Year 1, apples were placed directly in PAA for 30, 60 and 120 seconds.

Food Safety Outreach and Education. In Year 3, the workshop, "Farm Production Practices for Food Safety" featured 4 national speakers, 3 regional speakers and 4 WSU faculty. The workshop was attended by several representatives of the tree fruit industry. Impacts of the workshop are in preparation, but initial comments indicate that the workshop was highly informative for participants. In Year 1 the workshop "Safety of Northwest Produce" was offered and the impacts of this workshop are discussed in a new proposal submission. Presentations were also delivered at the Washington State Horticultural Association meetings in 2010 and 2011.

Chlorine Dioxide Laboratory Validation Studies. A project continuation was requested to complete laboratory validation of chlorine dioxide. A series of experiments focusing on oxidation reduction potential (ORP) of chlorine dioxide will be performed. ORP levels of the aqueous solution will be adjusted to 665, 750 and 850mV. To mimic dump tank application times, apples will be directly exposed for 2, 3.5 and 5 minutes. To mimic spray bar application times, a design similar to the PAA Year 2 study will be utilized unless industry guidance warrants alterations to the study design. Studies will utilize pathogenic and generic *E. coli* using a similar experimental design as described above. 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 VRBA for enumeration of generic *E. coli*. Plates will be incubated at 95˚F and enumerated manually.

Results and Discussion:

Chlorine Validation: Laboratory Study. Three and five replications for generic *E. coli* and pathogenic *E. coli* O157:H7 have been completed, respectively. Data indicates that additional replications are needed to ensure statistical validity, so reported findings should be considered preliminary. Clear trends for application times (2, 3.5 and 5 minutes) are not yet apparent. Statistical analysis with increased replications will clarify the effects of application time.

Differences between the responses of pathogenic *E. coli* O157:H7 and generic *E. coli* may exist. Pathogenic *E. coli* appeared to adhere to apples to a greater extent than nonpathogenic *E. coli*. All treatments (water, phosphoric acid, 665, 750 and 850 chlorine) had lower reductions of pathogenic *E. coli* (0.2 – 0.9 $\log_{10}$ reduction, less than a 90% reduction) compared to generic *E. coli* (0.6 – 1 $\log_{10}$ reduction).

All chlorine treatments (665, 750 and 850mV ORP) for generic *E. coli* produced reductions that were in the same range as reductions achieved with water. For pathogenic *E. coli* O157:H7, the water treatment produced a greater reduction than treatments with 665, 750 and 850mV ORP chlorine. Similarly, phosphoric acid produced results that were equivalent to water for generic *E. coli* while water produced greater reductions (average 0.85 $\log_{10}$ reduction) than phosphoric acid (average 0.5 $\log_{10}$ reduction) for *E. coli* O157:H7. Current data indicates that chlorine and phosphoric acid treatments produced less than a 90% reduction of pathogenic *E. coli* O157:H7.

Laboratory data will continue to be collected to build a data set for statistical analysis. Coupling laboratory data with inoculation studies with nonpathogenic (generic) *E. coli* under commercial packing conditions would strengthen understanding of the ability of chlorine to reduce bacterial levels on whole, fresh apples.

Peroxyacetic Acid (PAA) Validation: Commercial Scale Study. Four experiments at commercial packing facilities to assess PAA at the spray bar were conducted. Data from the first two replications indicated that bacterial adherence was reduced in 2010 compared to 2008 and 2009 using the same methods; this observation coincided with an observation from an industry partner that fungicide residue adherence on apples was also reduced in the same year. Therefore, for replications 3 and 4, the concentration of the inoculum was doubled. Statistical analysis of the data is needed to determine whether the first two replications include outliers that would prevent their inclusion in the dataset; it is likely that further replications would be needed to draw statistical conclusions.

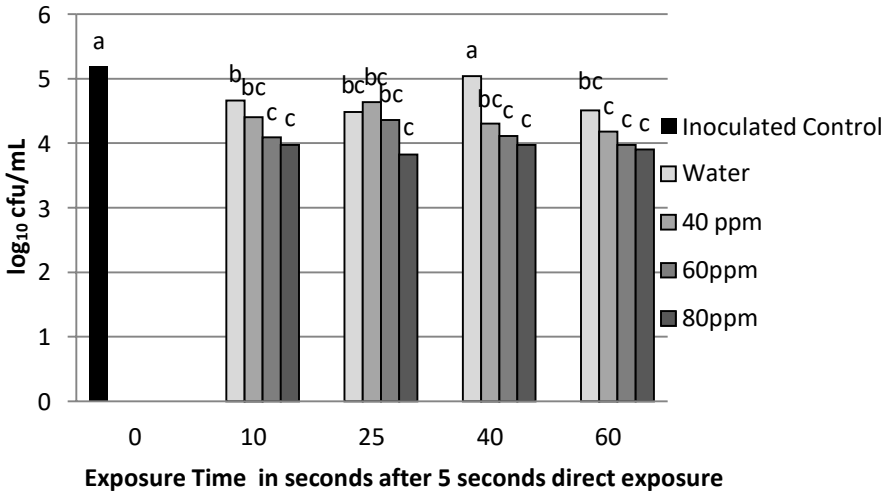
Experiments focused on examining 40, 60 and 80ppm PAA using high pressure nozzles (the more common industry practice) in all four replications. Data from the first two replications suggested that at 40ppm PAA, high and low pressure nozzles did not affect reduction of generic *E. coli* levels, so the last two replications examined use of a double spray bar at 80ppm PAA application.

Data examining inoculated apples prior to and after the spray bar will be discussed from the last two replications. The water treatment produced a 0.1 log reduction and treatment with 40ppm PAA and 60 ppm PAA produced a 0.3 and 0.2 log reductions, respectively. However, 80ppm PAA

achieved a 0.6 log reduction and a double spray bar treatment with 80ppm PAA achieved a 0.8 log reduction. It is interesting to note that all treatments achieved greater and in some cases statistically significant reductions in the laboratory studies (results described below). Therefore, greater reductions using PAA in commercial settings are possible.

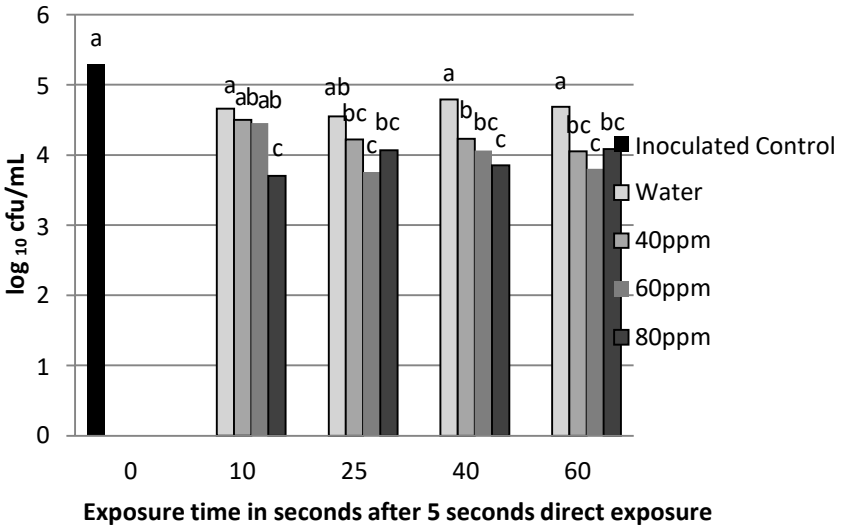
Peroxyacetic Acid (PAA) Validation: Laboratory Study. The laboratory results of Year 2 indicated at concentrations and exposure durations currently used in the industry, PAA treatment can result in at least a 0.5 – 1.3 $\log_{10}$ reduction of *E. coli* O157:H7 (Figure 1). At two levels of exposure time (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).



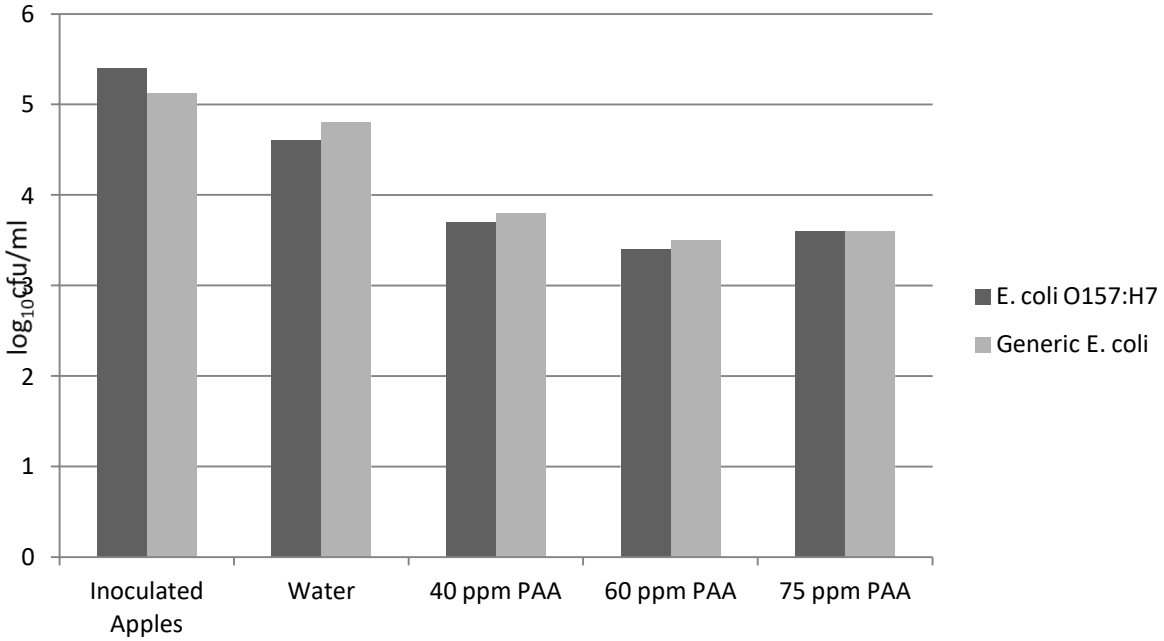
[a-c] Values that do not share a common superscript differ significantly ($p<0.05$)

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 ($p<0.05$)

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=1000fu/ml).



Summary.

These results provide valuable data to packers to assess the effectiveness of food safety interventions. Regulatory changes associated with the Food Safety Modernization Act will require examination of microbial preventive controls and the ability to provide supporting documentation to regulators and third-party auditors. The ability to document the effectiveness of current antimicrobial treatments as food safety interventions 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 or has been performed on other produce items.

It is important to understand the effectiveness of current interventions alone and then examine the combined effect of food safety interventions along with other packing steps. Economic benefits include the ability to demonstrate to customers that food safety interventions address potential food safety risks. 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.

CONTINUING PROJECT REPORT **YEAR**: 2012

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**: 87,101 **Year 2**: 100,724 **Year 3**: 129,855

WTFRC Collaborative expenses:

Item	(2010)	(2011)	(2012)
Salaries[2,3]	29,500	30,500	30500
Benefits[2,3]	9,440	9,760	10360
Crew Wages[3]	25,880	26,655	59750
Crew Benefits[3]	8281	8529	9970
Stemilt RCA room rental	8400	8400	8400
			0
Shipping			0
Supplies[4]		10880	3000
Travel[1]	5600	6000	7875
Miscellaneous			0
Total	87,101	100,724	129,855

Footnotes:
[1]Fuel and maintenance
[2]Salaries and benefits for Auvil, Hanrahan, Schmidt, and Castillo apportioned to this project.
[3]Harvest, storage and fruit quality lab labor for Phase 3 increased significantly in 2010-11(10 genotypes off 3 sites) crop and induced a reduction in scope and scale of P3 trials for 2011-12 crop (6 genotypes 4 sites) 2013-14 season may only have 1 or 2 P3 genotypes.

[4]Phase 3 trees for WABP

OBJECTIVES:

1. Evaluate apple rootstocks, particularly disease resistant rootstocks, in commercial settings in Washington State with replant conditions.
2. Integrate the processes of evaluation and industry adaptation.
3. Refine protocol for P3 scion evaluation program.

Scion evaluation accomplishments

- 2011 season provided fruit storage and handling trials. Plus fruit for waxing and market samples for WA 2.
- Conduct field day at Quincy P3 site.
- Discontinued P3 trials with WSU 7, 48 and 49. WSU 30 withdrawn due to virus infection.
- IAC gave WSU 17,19 and 36 at least one more evaluation season.
- IAC advised WTFRC to disengage from further commercialization of WA 5.
- WSU 38 has P4 trees budded in the nursery. Half are on G.41 rootstock
- Harvested and stored fruit for the Sensory panels at Pullman. Commercial varieties include Jazz and Ambrosia. Gala is the standard variety. WSU 38, WA 2 and WSU 19 are the breeding program genotypes this year. Will deliver half in January and the other half in April.

Significant Rootstock Findings

- Availability of Geneva rootstocks is finally approaching 1 million. Demand is significantly greater than supply.
- G.214 will grow larger canopy than M.9 337 and was released in 2010. G.214 received a set back when it became apparent that the tissue culture material shipped to Oregon was not true to type.
- 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. It appears to calm with crop and may be easier to manage than M.9 Nic 29.
- G.41, G.890, G.11, G.214, G.935 are all fireblight resistant.
- In tough replant sites, G.41, G.214, G.890, G.30 and G.935 are improving consistency of performance.
- G.11 and G.935 are not woolly aphid resistant.
- G.11 is not replant tolerant. It is reasonably fire blight resistant and out performs 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.
- G.202 and G.222 have some availability. Neither demonstrate enough benefit or advantage to promote over the elites listed above.
- G.30 and G.890 should be tested with Red Delicious to see if fruit shape is adversely affected as with M.9 or M.26. Both of these rootstocks may dramatically improve Red Delicious performance on replant sites.

Figure 1: 2006 Wapato Gala accumulated yield in bins per acre 2007-2011.

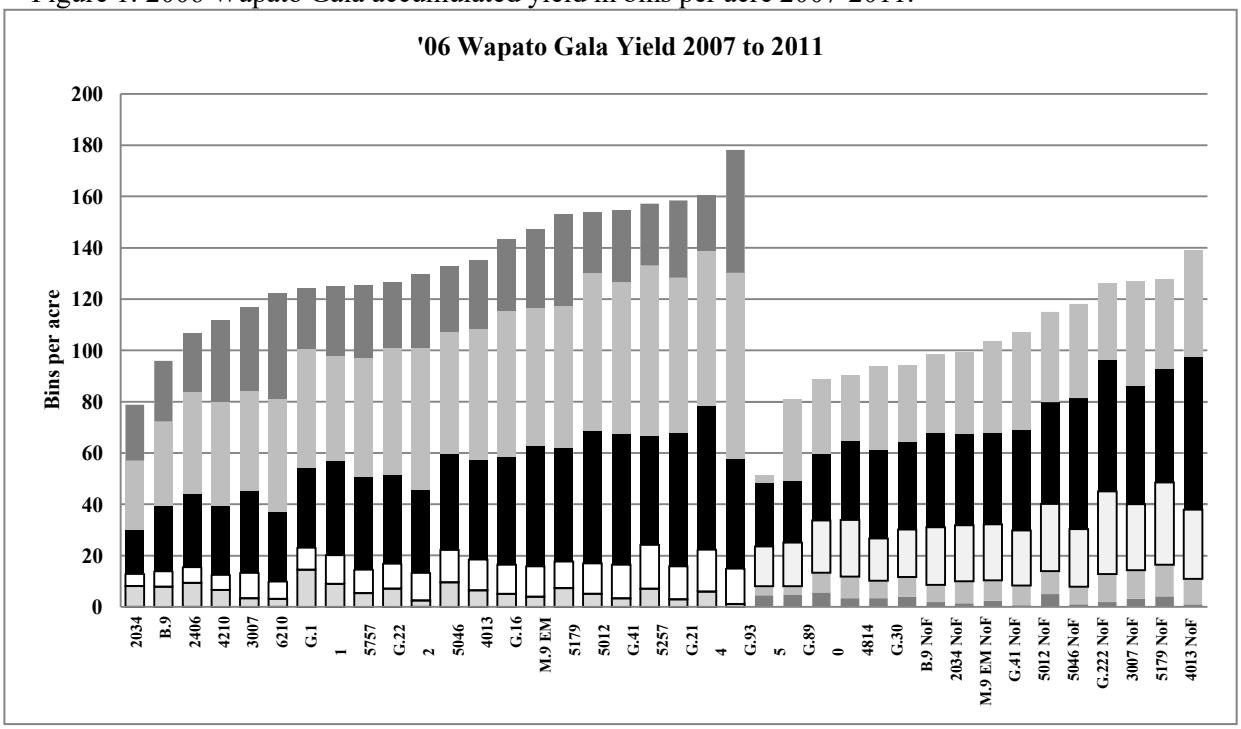


Figure one's data shows that if the new production canopy is short of growth for any reason, it may never catch up. In this case, in addition to no fumigation the trial had irrigation issues. The nonfumigated plot's poor performance in the first two seasons was multiplied by lack of water. Note that 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

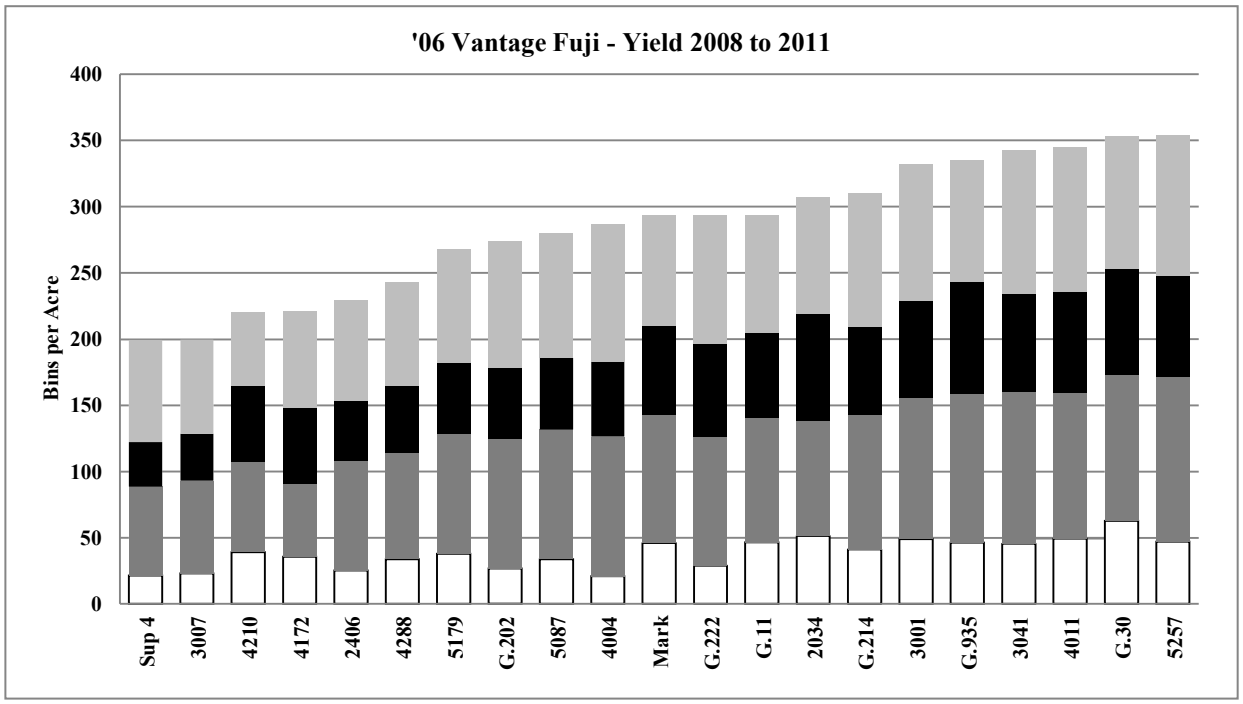
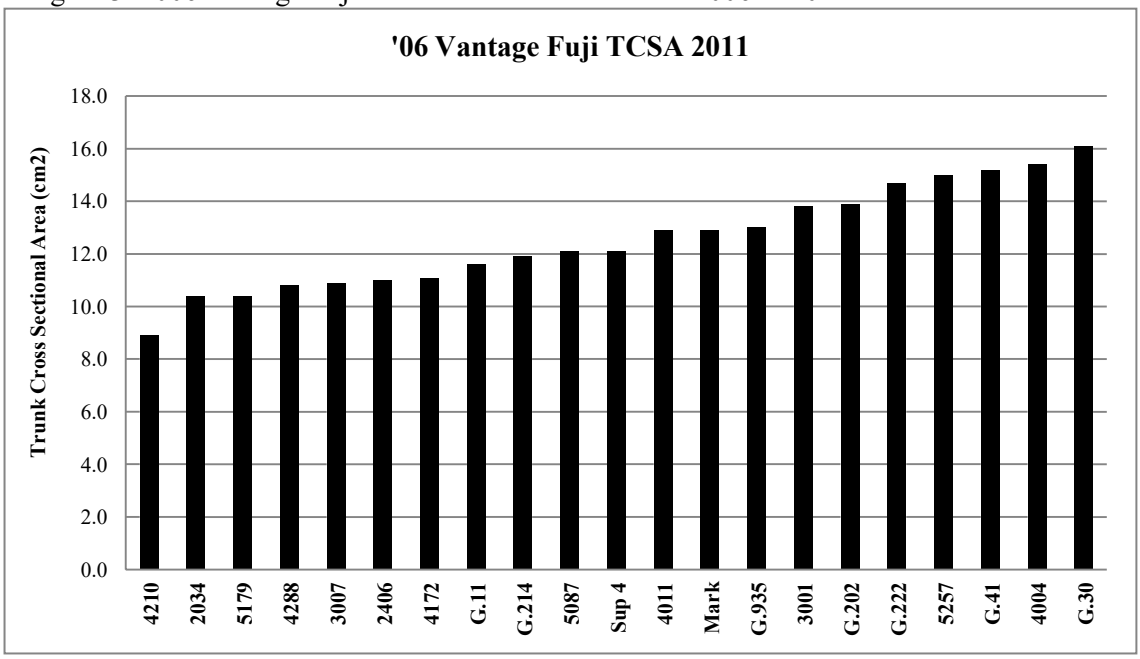


Figure 3: 2006 Vantage Fuji Trunk circumferences from 2006 to 2011



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.

G.30's performance and management difficulties in the nursery may be improved by bench grafting the liner prior to placing in the finshed tree nursery row.

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

Project Title: 'WA 2' plant variety rights applications

PI:	Kate Evans	**Co-PI:**	Tom Kelly
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City:	Wenatchee	**City:**	Pullman
State/Zip:	WA 98801	**State/Zip**:	WA 99163

Cooperators: Clean Plant Network, Prosser; Tom Auvil, WTFRC

Total Project Request: **Year 1:** 9,150 **Year 2: 10,650** **Year 3:** 3,440

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU; WSURF **Contract Administrator:** Kevin Larson; Tom Kelly
Telephone: 509 663 8181; 509 335 1210 **Email address:** kevin_larson@wsu.edu; kellytj@wsu.edu

Item	2011-2	2012-3	2013-4
Supplies	250	250	250
Travel	0	0	0
Plot Fees	0	0	0
Miscellaneous	0	0	0
- **Quarantine costs**	8,000	0	0
- **PVR application fees**	900	10,400	3,190
Total	9,150	**10,650**	3,440

Footnotes:
Supplies will cover collection and postal charges for distribution of propagating wood plus the issue of phytosanitary certificates.

OBJECTIVES

1. To establish CVT material of 'WA 2' in the PVR process in selected territories (see "Methods" section below) as a prelude to applying for PVR in those territories, which is the only way to protect 'WA 2' outside the USA and control its possible release.
2. To apply for PVR for 'WA 2' in the EU and in the countries mentioned below, as is feasible, by project's end.

SIGNIFICANT FINDINGS

- Propagating wood was sent to the EU from the University's Sunrise orchard; unfortunately propagation failed so this will be repeated in 2012.
- CVT propagating wood is being sent to Chile, South Africa, Australia and New Zealand in winter 2011 from the Fruit Tree Clean Plant Network in Prosser for establishing trees in quarantine.

METHODS

CVT propagating wood of 'WA 2' was sent from the Fruit Tree Clean Plant Network in Prosser to selected quarantine establishments in the EU, Chile, New Zealand, Australia, and South Africa. Quarantine is expected to take up to 2 years depending on the territory. Trees will be propagated for PVR testing and the initial application submitted in a timely manner as appropriate.

Agreements are in place and discussions have been initiated with in-country agents for each of these territories (a requirement for PVR applications). A strategy for testing 'WA 2'and controlling possible release will also be discussed with a view to having some of the PVR costs covered in this proposal reimbursed or at least some of the future annual PVR payments taken over by licensees. Beyond that, any unreimbursed funds spent under this proposal for obtaining and maintaining PVR protection would be recoverable from future royalties of that cultivar coming in to WSURF.

RESULTS & DISCUSSION

Under the agreements, agents acting on behalf of WSURF within each of the territories noted above will oversee the quarantine process and PVR application for 'WA 2'.
Propagating wood was sent to the EU quarantine establishment in the Netherlands from Sunrise orchard in April 2011; the EU, unlike other areas, will accept wood from the University orchard rather than having to go through the Clean Plant Network. Unfortunately these grafts failed, possibly as the grafting was late in the season, so wood will be sent early in 2012.
Propagating wood from the Clean Plant Network in Prosser was required for distribution to the other territories; this is being sent in winter 2011 as requested.
It is expected that even with the failure of propagation in the EU, the application for PVR in each of the territories will still be submitted in a timely manner.

CONTINUING PROJECT REPORT **YEAR**: 2 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: $30,439**

Other funding sources None

WTFRC Collaborative expenses: None

Budget 1
Organization Name: WSU **Contract Administrator: Carrie Johnston**
Telephone: 509-335-4564 **Email address: carriej@wsu.edu**

Item	2010	2011	2012
Salaries[1]	22,014	22,895	24,542
Benefits[2]	1,881	1,956	1,897
Wages			
Benefits			
Supplies[3]	4,000	4,000	4,000
Travel			
Total	$27,895	$28,851	**$30,439**

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 stored commercial apple varieties, specifically by performing trained sensory panel analysis.

SIGNIFICANT FINDINGS:
The trained panel was able to determined differences in the sensory properties of the apples. A summary of the five apples' attributes:

- Fuji – high perceived sweetness, apple flavor, crispness, firmness, juiciness
- Gala – moderately perceived sweetness, moderately high apple flavor, crispness and juiciness, high firmness
- Honeycrisp – moderately perceived sweetness, low perceived sourness, moderately high apple flavor, high crispness and juiciness, moderate firmness
- Pink Lady – moderately perceived sweetness, sourness and juiciness, moderately high apple flavor and crispness, high firmness
- Granny Smith – low perceived sweetness, high perceived sourness and astringency, moderately perceived crispness and firmness

METHODS:
Apples: In 2011, the varieties to include in this study were chosen in collaboration with the WTFRC: Honeycrisp, Auvil Stripe Gala, Fuji, Granny Smith, Cripps Pink/Pink Lady. In order to minimize the effects of environment and management, all the fruit was sourced from the Auvil Vantage orchards by the WTFRC staff. The samples were delivered to Pullman on 01/14/11 and then stored in a cold room at 37F until the panel assessment on 01/19/11. A chain of custody document was completed for each sample. Fruit from each sample were also tested by the WABP lab in Wenatchee on 01/13/11. New apple selections were not evaluated in 2011, but fruit from the 2011 season will be evaluated in early2012.
In 2012, selections from phase 2 and 3 of the apple breeding program will be chosen for evaluation by the trained panel. Fruit will be sorted and delivered to Pullman 3 days before the scheduled sensory testing. In Pullman, apples will be 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 will be performed.

Trained Panel Evaluation: In 2011, the trained panel was composed of 10 individuals. The panelists were trained over 11-15 hours using techniques described by Meilgaard et al. (1999). In addition, a refresher course was conducted over three extra hours, using the same techniques. 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 on 01/19/11. 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. In 2012, these same training protocols and evaluation conditions will be employed.

2012 Consumer Evaluation Panels: The first consumer sensory evaluation panel will be conducted in the sensory evaluation facility at WSU using 100 consumers on each of 2 days. Evaluations will take place in individual sensory booths equipped with laptop computers. Consumers will be presented with 1/8 apple of the apple selections. Two standard cultivars (probably Honeycrisp and Gala) will be presented as controls. Consumers will indicate 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 will be evaluated by the panel using a 7-point scale (1 = dislike very much, 7 = like very much). Results will be collected and analyzed as described above. We also plan on conducting a 2-3 off-site consumer evaluation panels in the Wenatchee area in Spring 2012 to gather additional consumer acceptance data for 6 of the selections. These evaluations will be conducted using standard central location testing methodology.

RESULTS and DISCUSSION:
Table 1 details the harvest dates and storage conditions of the fruit samples.

Specific differences in sensory attributes were found between apples. Specific differences between specific attributes and cultivars will be discussed in more details below.

Based on sweetness, Fuji and to a lesser extent, Gala, were found to be significantly sweeter than the rest of the apple varieties (**Figure 1**). Granny Smith was significantly less sweet than the rest, with Gala, Honeycrisp, and Pink Lady all being found to be generally similar in their sweetness. The analytical data, shown at the right of the figure, reflect similar trends as the trained panel.

Based on sourness, Fuji, Gala, and Honeycrisp were all statistically similar and formed the least intense group (**Figure 2**). Granny Smith was rated the most intense, with Pink Lady falling in the middle of the two groups.

Fuji's rating for apple flavor was found to be the highest, with Pink Lady being slightly lower (**Figure 3**). Granny Smith was the lowest. Gala, Honeycrisp and Pink Lady were similar in their intensity of apple flavor intensity, falling in the moderate group.

In astringency, all of the samples were similar, except for Granny Smith, which was significantly more astringent than the rest of the samples. This difference is clearly visible in **Figure 4**.

Crispness was split into three groups (**Figure 5**). Fuji and Honeycrisp were the most crisp. Gala and Pink Lady were slightly less crisp (but not significantly different). Finally, Granny Smith was the lowest in perceived crispness.

For firmness, Honeycrisp was the least firm apple sample, and Pink Lady was the most firm (**Figure 6**). Fuji and Gala were slightly less firm than Pink Lady, and Granny Smith was similar to Honeycrisp. The analytical data support these findings.

Pink Lady and Granny Smith were found to be the least juicy apple samples (**Figure 7**). Fuji and Honeycrisp were the highest in juiciness, closely followed by Gala.

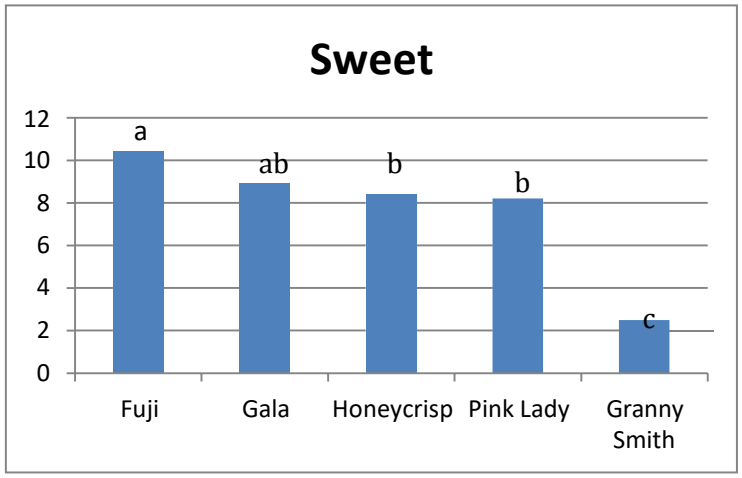
Finally, mealiness was found to be very similar among all of the samples (**Figure 8**). There was very little variation in mealiness among all five samples.

Overall, these results show that apple selections do differ in their sensory properties, with the greatest differences being observed for sweetness and sourness of the apples. These data provide a useful baseline for future comparison to new selections.

Tables and Figures

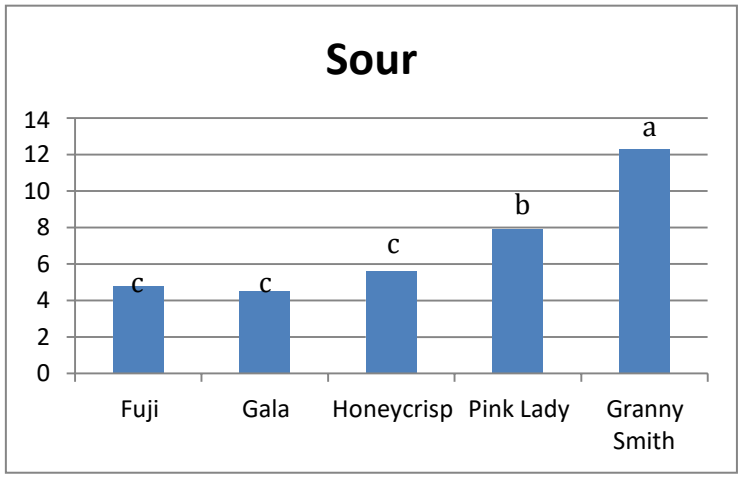
Table 1. Harvest dates and storage regimes of the fruit samples. All samples were removed from CA on 01/04/11 and stored in RA until delivered to Pullman on 01/14/11.

	Harvest date	Storage regime	Comments
Fuji	10/06/10	MCP / CA	
Gala	08/26/10	MCP / CA	Penbotec drench
Honeycrisp	09/01/10	MCP / CA	Scholar drench; 11 days at 50F pre-cold storage
Pink Lady	10/26/10	MCP / CA	
Granny Smith	10/06/10	MCP / CA	



	% Soluble solids
Fuji	14.75
Gala	13.93
Honeycrisp	13.74
Pink Lady	14.51
Granny Smith	11.56

Figure 1. Separation of the five apple varieties based on sweetness as evaluated by the trained panelists (n=10). Different letters indicate significant differences between varieties ($p<0.05$).



	Titratable acidity
Fuji	**0.46**
Gala	**0.39**
Honeycrisp	**0.48**
Pink Lady	**0.64**
Granny Smith	**0.79**

Figure 2. Separation of the five apple varieties based on sourness as evaluated by the trained panelists (n=10). Titratable acidity was measured as mg/l malic acid. Different letters indicate significant differences between varieties ($p<0.05$).

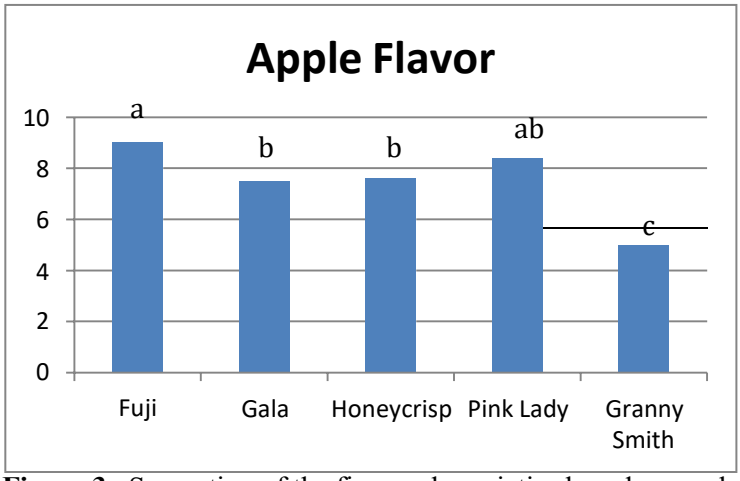


Figure 3. Separation of the five apple varieties based on apple flavor as evaluated by the trained panelists (n=10). Different letters indicate significant differences between varieties ($p<0.05$).

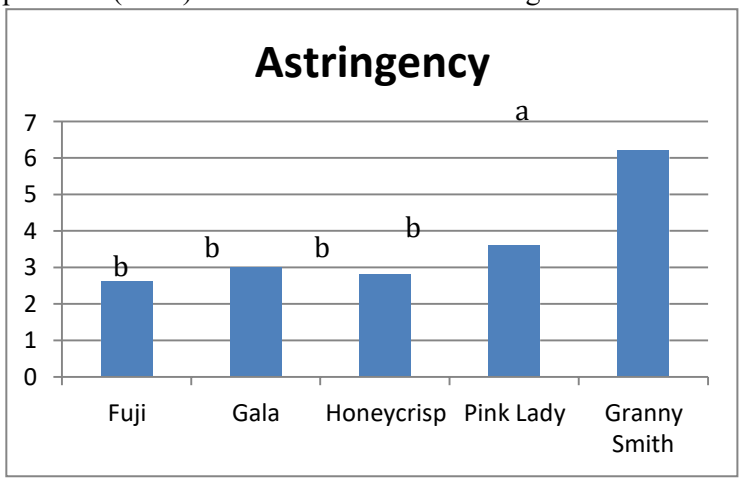
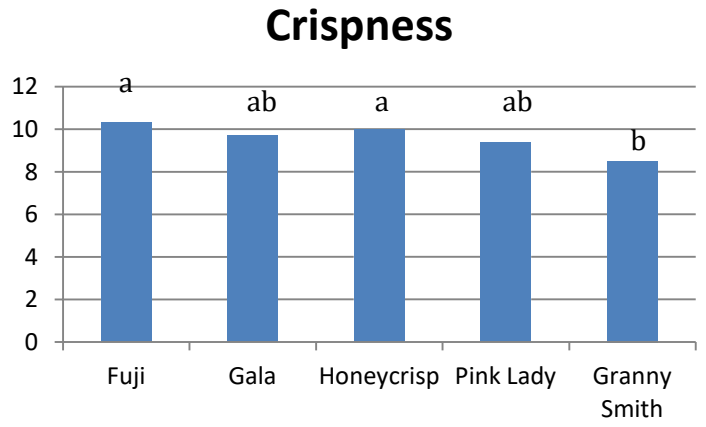
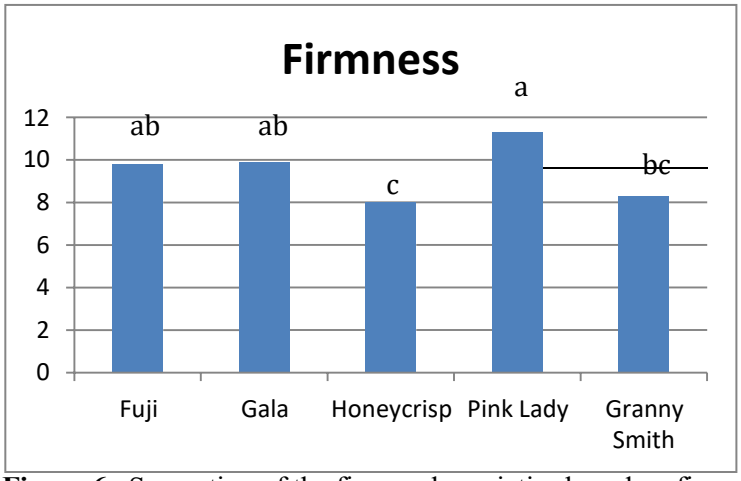


Figure 5. Separation of the five apple varieties based on astringency as evaluated by the trained panelists (n=10). Different letters indicate significant differences between varieties ($p<0.05$).



	DigiTest Cn
Fuji	378
Gala	398
Honeycrisp	386
Pink Lady	221
Granny Smith	279

Figure 5. Separation of the five apple varieties based on crispness as evaluated by the trained panelists (n=10). The DigiTest data refers to the Cn (crispness) value which is representative of the tearing of the fruit as the probe goes through it and is therefore theoretically similar to the energy released during a bite. Different letters indicate significant differences between varieties ($p<0.05$).



	DigiTest Firmness (M2 lb)
Fuji	25.57
Gala	24.33
Honeycrisp	26.07
Pink Lady	27.19
Granny Smith	23.29

Figure 6. Separation of the five apple varieties based on firmness as evaluated by the trained panelists (n=10). The DigiTest data relates to the pressure of the bulk of the edible flesh (from 0.32 inches below the skin to just outside the core). Different letters indicate significant differences ($p<0.05$).

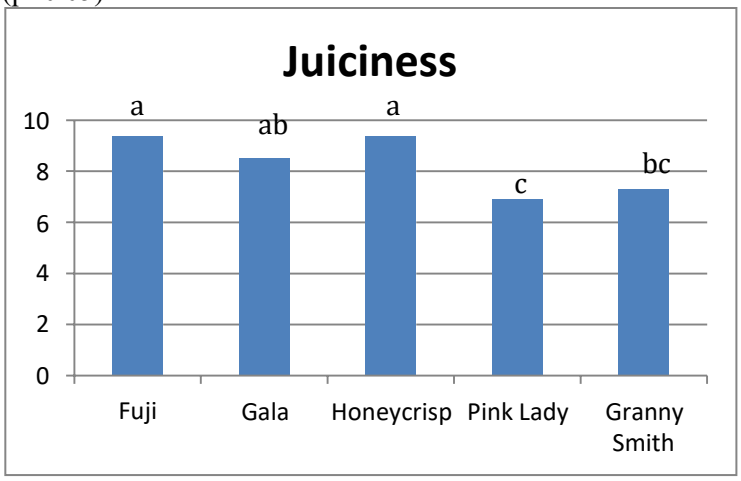
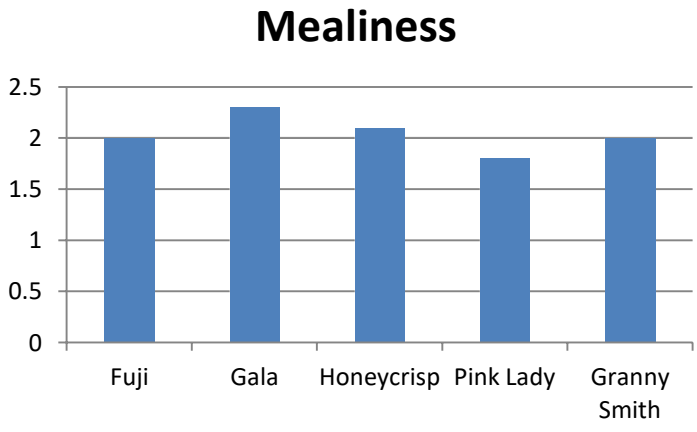


Figure 7. Separation of the five apple varieties based on juiciness as evaluated by the trained panelists (n=10). Different letters indicate significant differences between varieties ($p<0.05$).



	DigiTest Co
Fuji	0.004
Gala	0.001
Honeycrisp	0.023
Pink Lady	0.002
Granny Smith	0.007

Figure 8. Separation of the five apple varieties based on mealiness as evaluated by the trained panelists. The DigiTest data refers to the Co value which indicates the relaxation rate or creep of the apple flesh.

CONTINUING PROJECT REPORT **YEAR: 2 of 2**
(No-cost extension applied)

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

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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	**Year 1: 2010-2011**	**Year 2: 2011-2012**
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 the roles of candidate genes of ethylene, auxin 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. **Data analysis on ripening and quality phenotypes for 'Honeycrisp' (HC) x 'Cripps Pink' (CP) population:** The phenotypes of fruit ripening and quality, including fruit firmness, crispness, and fruit size, for each of 170 siblings in HC X CP cross population have been collected in 2008 and 2009 seasons. Fruit tissues were collected for continuous three weeks around physiological maturity (arbitrarily set at starch level of 3.5) if fruit number per tree allowed. The two parents 'Cripps Pink' (or Pink Lady) and "Honeycrisp" of this population have unique and distinct ripening behaviors (ripening season) and (texture attributes); and a wide-spectrum of phenotype variations are observed among siblings within HC x CP population for ripening times (seasons), fruit texture and fruit size.

2. **Systematically characterizing the fruit maturation and ripening process for 6 commercial cultivars:** The phenotypes of six commercial cultivars with distinct ripening seasons were subjected to detailed analysis with weekly sampling for both maturity data and fruit samples for duration of 2-3 months before harvest and after. These six cultivars represented two sets of ripening-time series, i.e. 1st set of SweeTango (early-season), Jazz (mid-season) and Pink Lady (late season); and 2nd set of Gala (early), Golden Delicious (mid-season) and Fuji (late-season). These weekly tissues represented whole developmental stages of apple fruit maturation and ripening and were used for investigating the behaviors of previously identified candidate genes and their potential roles regulating apple fruit ripening patterns.

3. **Quantitative gene expression analysis:** Candidate genes in auxin metabolism and transport with the implication of regulating fruit ripening and quality have been identified by a parallel transcriptome analysis. These candidate genes will be examine for their expression features (behaviors) and their association with specific phenotypes within HC x CP population, commercial cultivars and breeding selections. Sequence analysis and gene-specific primer design have been conducted, then gene expression patterns were characterized using Quantitative Reverse Transcription Real-Time PCR (qRT-PCR). Selections with defined phenotype from this cross population are being analyzed against the gene expression patterns of selected candidate genes for "associated segregation" between specific phenotypes and gene expression patterns.

SIGNIFICANT FINDINGS

1. Expression patterns for several genes including ACS3 (1-aminocyclopropane carboxylate synthase 3, a pre-climacteric ethylene biosynthesis gene), JOM (jasmonate O-methyl transferase gene) and AIP (a gene coding an auxin induced protein) showed strong correlation with fruit ripening season.

2. The expression patterns of an AUTR (auxin transporter) encoding gene showed good correlation with fruit firmness.
3. Auxin transporter genes PIN1-1 (Pin-formed 1) and AECFP (auxin efflux carrier family protein) have distinguishable expression patterns among three cultivars with early-, mid- and late-ripening seasons.

RESULTS AND DISCUSSION

1. Fruit ripening and fruit firmness within a HC x CP cross population

Phenotypic data of fruit ripening date/season, fruit firmness, fruit size were collected weekly based on maturity data for all fruiting trees within HC x CP cross population in 2009 season. Phenotypic data of individual siblings were categorized based on ripening date, fruit firmness and fruit size at a starch pattern index close to average of 3.5. Fruit from 12 individual trees in each phenotypic group (early versus late-ripening; firm versus soft fruit) were selected for gene expression analysis.

Table 1. Fruit from individual siblings sorted by ripening dates.

Group of **early-ripening** siblings				
Sibling No.	Ethylene (ppm)	8/26	9/2	9/9
157	0.00	**3.7**		
97	31.8	**3.8**		
140	1.97		**3.0**	
56	4.20		**3.3**	
139	5.61		**3.3**	
162	0.03		**4.2**	
23	1.23		**4.5**	
124	0.28			**3.2**
123	5.27			**3.5**
14	0.00			**3.7**
136	1.07			**4.0**
142	0.10			**4.7**
Group of **late-ripening** siblings				
Sibling No.	Ethylene (ppm)	10/15		
70	n/d	**2.6**		
85	n/d	**2.8**		
74	n/d	**3.0**		
171	n/d	**3.2**		
3	0.22	**3.3**		
46	n/d	**3.3**		
137	n/d	**3.3**		
84	n/d	**3.5**		
86	n/d	**3.5**		
168	n/d	**3.5**		
41	n/d	**3.7**		
173	0.01	**2.2**		

According to the number of fruit available from each sibling tree, three or more weekly harvests were sampled trying to capture the fruit with starch level close to a value of average 3.5. Starch pattern index is the average of three apples for each weekly sample. Due to the early freezing in 2009, fruit harvested after Oct. 15 cannot be used for evaluation. n/d = not detectable.

Table 2. Fruit from individual siblings sorted by firmness

Groupof siblings with **soft** fruit									
Sibling No.	Ethylene	Firmness (lb)	Starch values at harvest						
			9/2	9/9	9/16	9/23	9/30	10/7	10/14
174	0.07	**10.73**			3.5				
13	0.13	**10.91**						3.3	
138	0.52	**12.03**		2.2					
86	n/d	**12.09**							3.5
121	n/d	**13.32**					2.7		
70	n/d	**13.59**							2.7
159	0.97	**13.59**			3.2				
85	n/d	**13.65**							2.8
47	0.20	**14.61**			3.7				
133	0.03	**14.68**						3.3	
56	4.20	**14.72**	3.33						
46	n/d	**14.90**							3.3
17	n/d	**12.15**			4.33				

Group of siblings with **firm** fruit									
Sibling No.	Ethylene	Firmness (lb)	Starch values at harvest						
				9/9	9/16	9/23	9/30	10/7	
5	n/d	**18.23**				4.3			
14	n/d	**18.25**		3.7					
104	0.06	**18.52**				4.0			
88	n/d	**19.87**					3.5		
18	n/d	**20.79**			4.0				
122	0.30	**21.09**						3.5	
89	n/d	**21.19**			3.7				
129	n/d	**21.24**				4.5			
37	0.33	**21.55**						3.8	
92	0.07	**21.64**					3.5		
115	n/d	**22.11**			3.7				
60	0.44	**22.99**			3.5				

According to the number of fruit available from each sibling tree, three or more weekly harvests were sampled trying to capture the fruit with starch level at average 3.5. Starch pattern index is the average of three apple for each weekly sample. Due to the early freezing in 2009, fruit harvested after Oct 15 cannot be used for evaluation. n/d = not detectable.

2. Transcript levels of identified candidate genes for fruit ripening season, firmness, and size phenotypes

Table 3. Relative gene expression level compared between phenotypes

Ripening time	Early	Late	P value
ACS3 (1-aminocyclopropane carboxylate synthase 3)	20.8 ± 3.5	28.7 ± 3.3	0.00003
JOM (jasmonate O-methyl transferase)	25.6 ± 2.3	31.9 ± 2.3	0.000004
AIP (auxin induced protein)	24.3 ± 1.2	28.3 ± 1.5	0.000002
AUTR (auxin transporter)	24.5 ± 1.1	22.0 ± 1.2	0.00005
Firmness	Soft	Firm	
JOM (jasmonate O-methyl transferase)	25.6 ± 2.8	28.8 ± 3.5	0.0467
AUTR (auxin transporter)	23.0 ± 0.9	24.8 ± 1.2	0.0023
XTH7 (Xyloglucan endotransglycosylase/hydrolase 7)	24.4 ± 2.4	26.2 ± 2.8	0.1092
BRIP (Brassnosteroid induced protein)	26.1 ± 3.0	28.4 ± 3.7	0.1495
Aquaporin PIP	29.3 ± 3.4	29.4 ± 2.1	0.9505
Size	Small	Large	
JOM (jasmonate O-methyl transferase)	31.2 ± 2.3	27.9 ± 2.5	0.003
AUTR (auxin transporter)	23.9 ± 1.1	24.2 ± 1.3	0.4477

Values in the 2nd and 3rd columns are normalized Ct values (Ct = qPCR cycle number where a significant increase in gene transcript amplification occurs) for tested candidate genes. Each value is the average based on the gene expression data from fruit on 10-12 individual trees within the same phenotypic group. Fruit cortex tissues of 3 apples were pooled for RNA isolation and qPCR was performed in triplicate. $P<0.01$ is statistically significant and $P<0.001$ highly significant.

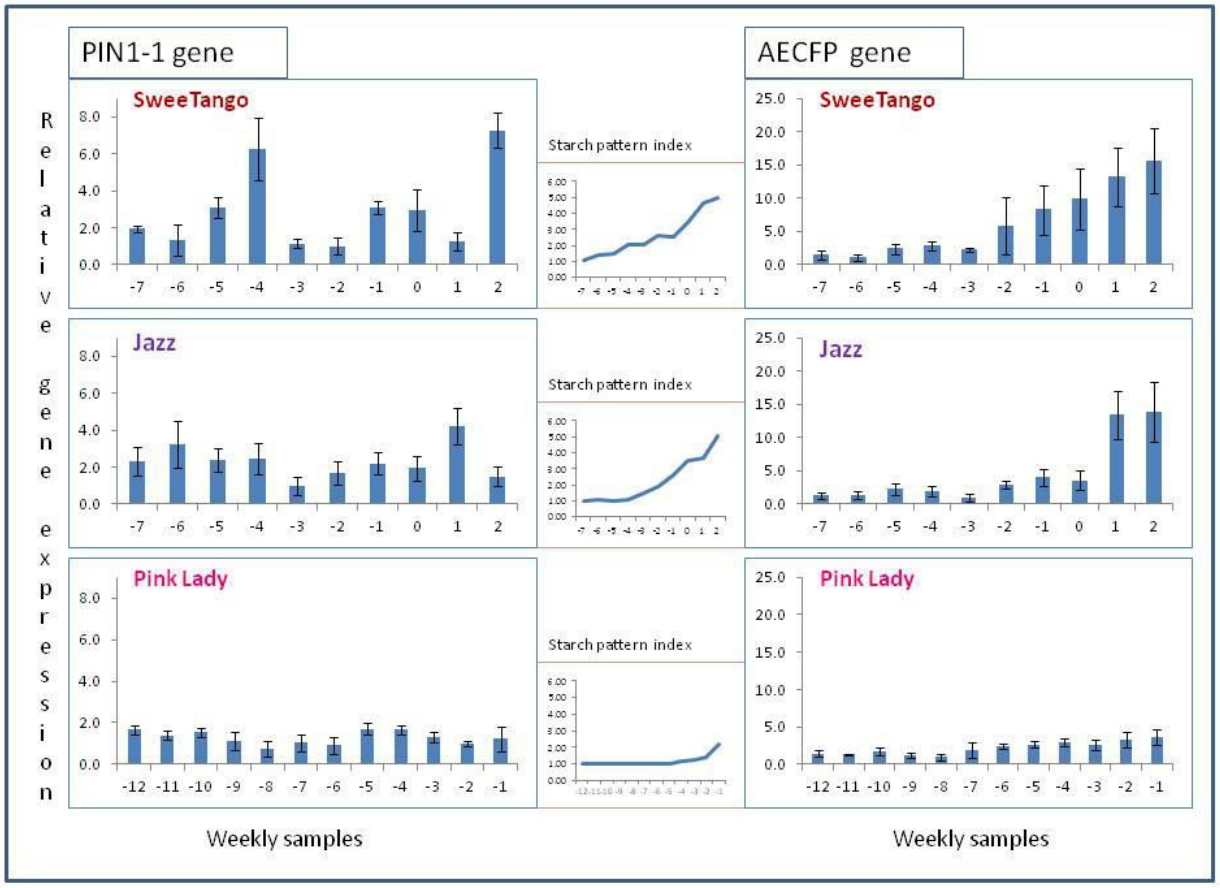
The results indicate, all four candidate genes (ACS3, a pre-climacteric ethylene biosynthesis gene; JOM, a jasmonate O-methyl transferase encoding gene; AIP, an auxin induced protein; and AUTR, an auxin transporter component) have significant correlations with fruit ripening date (or early/late-ripening phenotypes). Only AUTR has a significant difference in expression level between the two groups of soft and firm phenotypes.

3. The expression profiles of auxin transporter genes correlate apple fruit ripening patterns in three cultivars with distinguishable ripening seasons

Among four auxin transporter genes identified by microarray analysis, PIN1-1 and AECFP encoding genes showed distinguishable patterns between early-, mid- and late-season ripening cultivars (see Figure 1. below). In general, expression of both genes decreases toward later-season cultivar, i.e. the early ripening cultivar 'SweeTango' has highest expression and 'Pink Lady' lowest expression; mid-season 'Jazz' being somewhere in between. This is an indication of a good association between the behavior of these genes and the phenotype of ripening season.

Another interesting feature of PIN1-1 are the "double-peaks" for the early-ripening 'SweeTango', at 4 weeks prior and 2 weeks after the starch pattern index reached 3.5. If these results are reproducible for 'SweeTango' and other early-ripening cultivars, the sequence features of PIN1-1 could be useful for devising markers for fruit ripening season.

Figure 1. The gene expression profiles of two auxin transporter encoding genes in three cultivars with distinct ripening seasons.



X axis denotes the weekly sample for each cultivar, with the number representing weeks in related to physiological maturity at starch level of 3.5 or week 0. Y axis denotes the relative gene expression level. The middle inserts show the change of starch pattern index in each cultivar.

4. Future directions

1) Complete gene expression analysis in another set of three cultivars: Gala, Golden and Fuji.
2) Clone and analyze the regulatory genomic sequences of PIN1-1 and AECFP genes from different cultivars.
3) Summarize the overall data for the genotype-phenotype association.

5. What this study means to the tree fruit industry?

The fruit ripening time apple can span almost three months among elite commercial apple cultivars under the same climatic condition, from early August to late October. Although ripening time is an easy-to-define horticultural trait, there is surprisingly limited knowledge on its genetic controls or commanding genes/pathways. At the same time, fruit ripening patterns intimately influence cultivar-specific fruit quality. The early-ripening cultivars are with less-firm fruit with less-than-perfect storability due to seemingly "fast-revolving" ripening machinery which speeds up the whole process of maturation and ripening; while late-ripening cultivars commonly correlated with firm fruit with better storability as the ripening process was severely "slowed down" yet risk freeze damage in regions like north central Washington State. In any given cross population up to 10% of seedlings

may never reach commercial maturity due to the “mix and match” of “bad” alleles. With the goal of generating a portfolio of apple cultivars with different ripening characters and quality attributes in Washington State Apple Breeding Program, Elucidating the genetic control of apple fruit ripening and designing the predicative marker should assist targeted and precise breeding of new apple cultivars. Better-aligning apple cultivars with defined ripening season could also be part of the solution for inevitable and ever increasing pressure of labor availability at harvest time. Following the path of functional genomics, the results from current research and our earlier study on *MdACS3* characterization, the markers for predicting apple fruit ripening patterns (ripening season) are quite possible in near future.

From a more practical point of view, the elucidation of the mechanism of plant hormone function in regulating apple ripening process could open up a whole new avenue for fruit quality management. Taking auxin as an example, variable binding specificity was observed for different combinations of auxin derivatives and receptors. Indeed, the usefulness of incompetent auxin analogs to decoy auxin receptors are being tested in the lab, the implication for practical application cannot be under-estimated, just like 1-MCP interferes with ethylene receptors. Based on our current understanding, auxin likely functions in the upper-stream of ethylene, or during the earlier stages of fruit development, so manipulation of auxin may potentially adjust ripening time and associated quality attributes. Both DNA marker assisted selection tools in apple breeding and exploration of potential new technology in managing pre- and postharvest fruit quality will be the critical components for the sustainability and profitability of the apple industry.

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

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

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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, SCRI, proposal in preparation.
Amt. requested: $250,000

WTFRC Collaborative expenses: None

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Chuck Meyer
Telephone: 510-559-5769 **Email address**: chuck.myers@ars.usda.gov

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	4,000
Travel			500
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 2011 apple research priority concerning soil health and productivity. Specifically, elements of this program seek to identify relationships between soil indices, such as mineral nutrition and plant response, and to better understand interactions between rhizosphere biology and rootstock performance/development. 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.

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.

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.

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

SIGNIFICANT FINDINGS:

- Type of nitrogen amendment significantly altered microbial N cycling gene abundance, and therefore potential for retention or loss of N from soils

- In certain soils archaeal ammonia monooxygenase gene (*amoA*) abundance dominated over the bacterial ammonia monooxygenase gene; this is important as amoA which initiates the process of nitrification does not respond to mineral fertilizers

- The effect of nitrogen amendment on microbial cycling gene abundance was influenced by the long-term soil management system

- In extension of initial year findings, the effect of nitrogen amendments on rootstock lateral root development were examined in an additional orchard soil and were similarly shown to be indirect, apparently being determined by the orchard soil biology

- Soil pasteurization, as a proxy for fumigation, abolished nitrification over the study period.

- Through examination of a larger population, root stimulation induced by *Streptomyces* bacteria was found not to be linked directly to the ability of the bacterium to produce nitric oxide, and could be replicated using other bacterial species.

GOALS & ACTIVITIES

During year three of this project a primary objective will be to further examine the amendment-based but biology driven modulation of rootstock root development, as observed in WSU-Sunrise orchard soil. Further characterization of the functional biology responsible for enhanced root development will be conducted at the WSU-Sunrise orchard and will utilize a high throughput DNA sequencing

approach with the goal of a prescriptive basis for use of inputs to enhance root proliferation. 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. Specifically the goal will be to link gene activity to resulting N form and concentrations in different orchard soils as a means of characterizing the relative dominance of edaphic or genetic factors in determining N availability. Thirdly, the effect of fertility management options on the dynamics of nematode communities, which are important factors in N cycling and consistently have been correlated with "soil health" and biological resilience of soil systems, will be characterized through application of DNA-based studies including T-RFLP, real-time quantitative PCR and sequencing of clone libraries. It is anticipated that these findings will be used to identify appropriate fertility treatments to be applied at the Sunrise orchard in fall of 2012 to address objective 4 of this project plan.

METHODS:

Soil will be collected from the rhizosphere of trees grown in fumigated and control soil, and a root-stimulating (brassicaceae seed meal) soil amendment at the WSU-Sunrise orchard (sandy texture; 1.2% organic matter [OM]). Soil will be collected from two trees in each 10 tree block with five replicate blocks per soil treatment and two rootstocks; M9 and G11. DNA will be isolated and purified to standardized concentration. DNA will be amplified using primers specific from bacteria/archaea (926F-1392R) and fungi (ITS1F-ITS4R) in pyrosequencing reactions. Reactions will be conducted to obtain an average of 3000 sequences/microbial group/sample (72 samples). Community profiles among treatments will be compared and contrasted, and analyzed in the context of relative root development.

The link between DNA contents and activity in the field is not always conclusive and therefore, increased gene copies do not always mean that activity will be increased. Studies will be conducted to assess the relative dominance of edaphic factors and genetic factors in the efficient retention of N in soil systems. To date, we have completed analysis of the abundance and activity of genetic factors involved in N cycling for two orchard soils as influenced by nitrogen amendments (WSU Sunrise, sandy texture; 1.2% organic matter[OM]; RF, sandy loam, 4.2% OM). Analysis of the third orchard soil (GC, gravelly sandy loam, 3.3% OM is currently in progress. In addition, rhizosphere soils from these treatments have been collected and determination of ammonium (NH^{+4}) and nitrate (NO_{-3}) concentrations is currently in progress. To assess the relative correspondence between gene abundance and function in these soils, potential ammonia oxidation, an estimation of the production of nitrite in soil, will be determined. These data will provide guidance on the relative activity of the bacterial and archaeal ammonia oxidation genes (*amoB* and *amoA*), which have been quantified in these soils.

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 that lead to a more resilient ecosystem and indirectly via mineralized nitrogen production. The effect of different N inputs on orchard soil nematode communities will be examined. Orchard soils will be treated with mineral ($CaNO_3$ or urea) or organic (chicken manure, canola meal or mustard meal) nitrogen inputs at the rate of 70 lbs per acre. Soils will be planted to apple and sampled every three weeks for a period of 18 weeks with three replicates per soil treatment. DNA will be isolated from each soil sample and amplified using the nematode specific primer pair. Nematode communities will be analyzed by T-RFLP analysis to determine whether significant qualitative

changes result from these treatments. If significant differences are observed the specific nature of these changes will be determined by sequencing of a clone library generated using the amplified nematode DNA from each soil.

RESULTS & DISCUSSION:

Effect of amendment type on N cycling gene abundance

Studies were conducted in multiple orchard soils to monitor the presence of specific microbial genes that function in the cycling of nitrogen. The goals are to gain an understanding of the active microbial populations responsible for nitrogen cycling in orchard soils and 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. Studies were conducted in three orchard soils of diverse physical character including a low organic matter content (WSU Sunrise [SR] orchard; 1.2%) sandy soil, a sandy loam (RF orchard; 4.5% OM) and a gravelly loam (GC orchards; OM 3.2%). Correspondingly, these soils yielded diverse responses in terms of N-cycling gene abundance; outcomes which appear to have a greater relationship with long-term management practices/soil type rather than the consequence of short-term fertility amendments. Of particular note were observations concerning the size of the denitrifying bacterial communities based upon abundance of the *nirK* gene; this gene encodes an important step in the denitrification process leading to loss of nitrogen from orchard soils through volatilization. Abundance of bacterial *nirK* was 1 to 2 orders of magnitude lower in the high OM content RF orchard soil than in the low OM content SR orchard soil (**Fig 1A and B**). In addition, fertility amendments had no significant impact on bacterial *nirK* gene abundance detected in the RF soil but urea amendment resulted in a significant (two orders of magnitude) increase in *nirK* abundance in the SR soil. Co-application of either urea or *B. napus* N with compost significantly reduced *nirK* abundance but only in the SR orchard soil. In terms of orchard soil and inputs, fungal *nirK* gene abundance exhibited the same pattern. Thus, adding carbon as a strategy to retain N thru decreased volatilization may be of significant benefit at the SR orchard but not in RF soil.

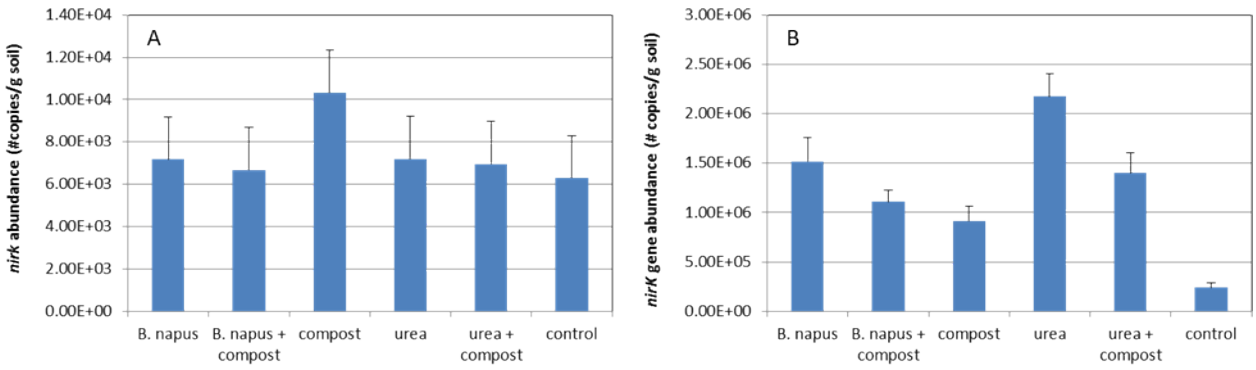

Fig. 1. Abundance of bacterial denitrifiers in two orchard soils based upon the presence of the gene *nirK*, and the effect of fertility inputs on bacterial *nirK* gene abundance in RF (A) and SR (B) orchard soils.

Despite the large additions of N to these soils, bacterial N_2-fixation genes (*nifH*) were present in both soils, though regardless of amendment, they were significantly more abundant in the RF than SR orchard soils (**Fig. 2**).

Soil amendments had significant effects on the abundance of ammonia monooxygenase gene (*amo*) detected in the apple rhizosphere. This gene encodes the enzyme involved in the first step in the process of nitrification, resulting in the conversion of ammonia to nitrate. Interestingly, the relative contribution of ammonia oxidizing bacteria (AOB) and archaea (AOA) to the process of nitrification differed dramatically between the RF and SR soils. While ammonia oxidizing archaea (AOA) and bacteria (AOB) were nearly equivalent in the SR soil, AOA abundance was over 10 times greater than AOB in the RF soil system (**Fig. 3**).

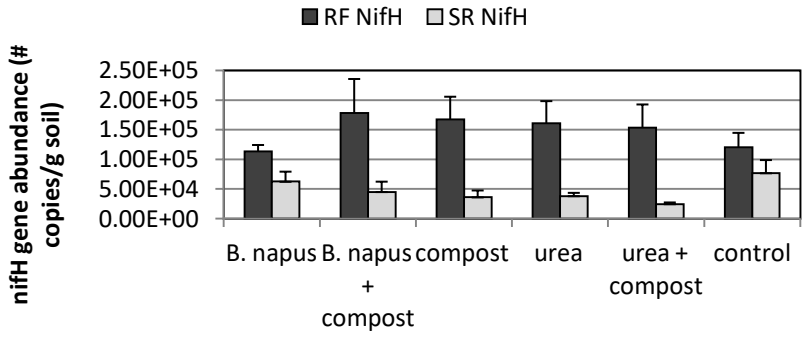

Figure 2. Abundance of bacterial nitrifiers in two orchard soils based upon the presence of the gene *nifH* and the effect of fertility inputs on bacterial *nifH*gene abundance in two orchard soils.

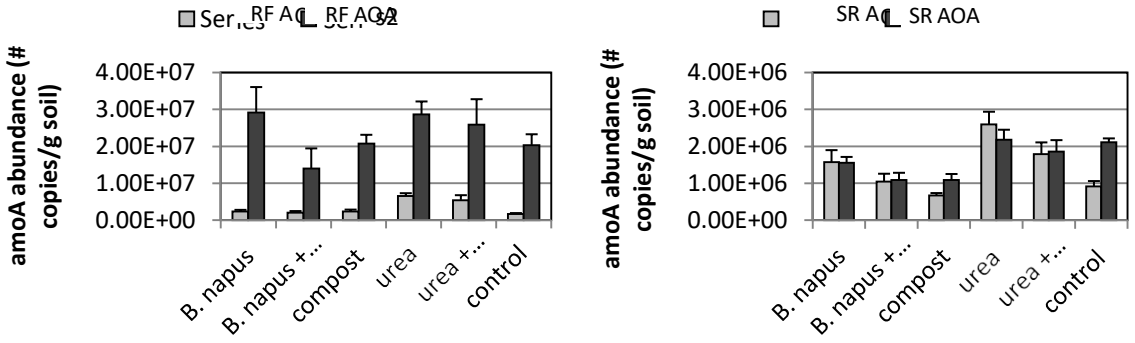

Figure 3. Abundance of ammonia oxidizers in two orchard soils based upon the presence of the bacterial (AOB) and archaeal (AOA) amoA gene abundance in the RF (left) and SR (right) orchard soils.

Preliminary examination of potential ammonia-oxidation activity in these soils found significant effects of pasteurization. After 21 days, pasteurized soils with fertility amendments had negligible rates of ammonia-oxidation, indicating a removal of that portion of the microbial community involved in this process (Fig. 4). While pasteurization, a proxy for the practice of soil fumigation, may prevent plant pathogen growth, it also appears to prevent any nitrification, potentially resulting in significant loss of added N fertilizers.

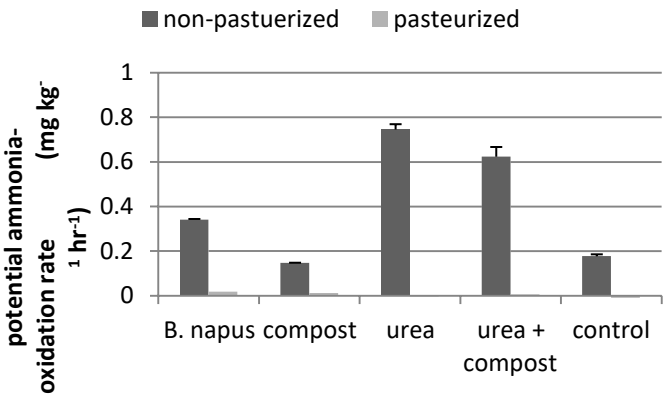

Figure 4. Potential ammonia-oxidation activity in GC pasteurized and non-pasteurized soils 21 days post-amendment application.

Significance: Overall, the relative abundance of the NifH, AOB, and AOA genes compared to the NirK genes indicates a potentially greater capacity for N retention, and thus plant availability, in the RF than SR orchard soil system. The relative differential abundance of AOB and AOA in soil systems is of significance because AOA are only responsive to organic nitrogen and do not respond to inorganic N fertilizer. Fumigation will adversely affect the process of nitrification in soil systems and could lead to significant N losses.

Effective of N amendment on lateral root development

In a follow-up to those reported last year, studies were repeated in additional orchard soils to examine the effect of nitrogen amendment type and soil biology on rootstock lateral root development. In the RF soil, root proliferation in responses to amendments in pasteurized and natural soils were similar to those observed in assays conducted in the SR orchard soil in 2010. In pasteurized orchard soils, amendments had no effect on rootstock root development and there were no significant differences in the number of lateral roots per length of primary root (Fig. 5A).

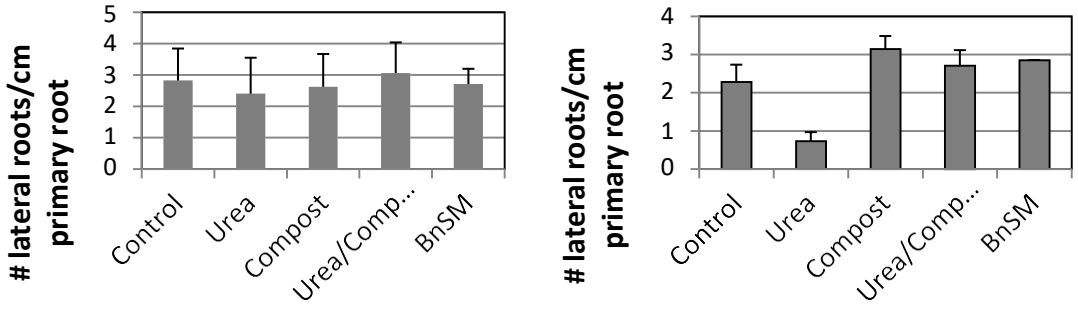

Figure 5. Effect of amendments on lateral rootstock lateral root development in pasteurized (A) and natural (B) RF orchard soil.

As was observed in Sunrise orchard soil, application of urea had a significant deleterious effect on lateral root development (5B). In contrast to the SR soil, where compost, urea/compost and canola (*B. napus*) seed meal all stimulated root production, only the compost amendment resulted in a statistically significant increase in lateral root number relative to the no treatment control.

Significance: Based upon the similar results in two disparate orchard soils, tree root development in response to organic/mineral amendments may be consistent across soil systems which would enable the use of such materials in a predictive manner to enhance tree establishment and long-term performance.

Effect of Streptomyces nitric oxide production on root development

We reported in 2010 that increased root development in response to these amendments was associated with a significant increase in the density of bacteria belonging to the genus *Streptomyces*. In addition we demonstrated that when inoculated with four individual *Streptomyces* isolates, only those producing nitric oxide (NO) stimulated apple root development in sterile orchard soil. In 2011 a range of *Streptomyces* isolates were screened for induction of lateral root development using the model plant *Arabidopsis thaliana* to enable the examination of a large number of bacterial strains. Although the four Streptomyces isolates used in 2010 elicited the same response in Arabidopsis as observed in apple, there was no association observed between an isolates capacity to produce NO and its ability to stimulate root development. In addition, specific isolates from other bacterial groups that were stimulated by the root inducing amendments (*Pseudomonas fluorescens*) also stimulated root development (Fig. 5). Isolates were screened for the production of the plant growth hormone indole acetic acid and preliminary results have not revealed an association between *Streptomyces* isolate production of IAA and root development.

Figure 6. Effect of bacterial isolates on proliferation of *Arabidopsis thaliana* root systems.

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

Project Title: Crop load and canopy management of apple

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Organization: WTFRC
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City: Wenatchee
State/Province/Zip WA 98801

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

Year	2011	2012	2013
Salaries	16,000	16,000	16,000
Benefits	4,500	4,500	4,500
Wages	12,000	15,000	15,000
Benefits	3,800	4,800	4,800
Equipment			
Supplies	1,000	1,000	1,000
Travel	2,000	2,500	2,500
Stemilt lab fees	2,000	2,000	2,000
Statistical consulting	0	1,000	1,000
Total gross costs	41,300	46,800	46,800
Reimbursements	(20,000)	?	?
Total net costs	$21,300		

Footnotes: Supply costs primarily covered by private industry cooperators
Travel includes fuel costs for driving to trial sites
Stemilt lab fees for use of single lane Aweta color grader
Statistical consulting for analysis of tree-to-tree variability for long-term cropping study on WSU Sunrise Granny Smiths

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

OBJECTIVES:

1) Continue screening new chemical and mechanical thinning technologies for apple
2) Develop practical PGR programs to manipulate floral initiation and promote annual bearing
3) Investigate horticultural effects of synthetic materials deployed as reflective ground covers or overhead shade/wind/bird protection
4) Profile natural tree-to-tree variation in long-term cropping patterns in a newly planted apple block
5) Expand collaborative efforts with other research programs working on crop load and canopy management

SIGNIFICANT FINDINGS:

Neither Sysstem CAL nor GC Ag Aide paired with lime sulfur thinned adequately in initial 2011 tests; further study may be required for robust evaluation (Table 2)

Endothall (ThinRite) has been effective in recent trials and may provide a viable alternative for chemical bloom thinning of apple (Tables 2, 3)

Postbloom chemical thinning programs of Sysstem CAL + BA and metamitron provided some modest reductions in fruit set, but were inferior to programs of BA + NAA, carbaryl + NAA, and carbaryl + BA (Table 4); metamitron may be more effective at higher rates

Crops may be effectively thinned chemically without use of carbaryl; BA + NAA programs demonstrate results equal or superior to programs using carbaryl (Tables 4, 5)

GA trials to inhibit return bloom show promise for mitigation of biennial bearing, but results are inconsistent (Tables 6, 7)

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 8)

BACKGROUND:

After years of robust efforts to evaluate various aspects of bloom and postbloom chemical thinning programs, our current focus is to screen new chemistries and provide collaborative support for external research programs working on crop load and canopy management. 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.

Programs in 2011 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 2011.

BLOOM THINNERS (applied 100 gal water/A at 20% & 80% bloom)
2% Crocker's Fish Oil (CFO) + 2-3% Lime Sulfur (LS)
24 oz ThinRite (TR)/A
24 oz ThinRite /A + 2-3% CFO
24 oz ThinRite /A + 2% LS
24 oz ThinRite /A + 16 oz Cuprofix (CF)/A
2% GC Ag Aide (GCAA)+ 1.5-3% LS
0.5-1% Sysstem CAL (SC) + 3% LS

POSTBLOOM THINNERS (applied 100 gal water/A at PF & 10mm)
48 oz Sevin (carbaryl) + 3 oz NAA/A
48 oz Sevin (carbaryl) + 128 oz BA/A
128 oz BA + 3 oz NAA/A
96 oz BA + 3-4.5 oz NAA/A
0.5-1% Sysstem CAL + 128 oz BA/A
250-1000 g metamitron/A (100-400 ppm)

BLOOM THINNING:
Reports from industry and research colleagues on the East Coast have suggested that use of phosphite materials with standard chemical thinners may increase their efficacy, presumably due to increased penetration and uptake by the plant. To test this program locally, we applied Sysstem CAL at rates reported successful by Duane Greene (UMass) in combination with standard rates of lime sulfur. Likewise, we also tested GC Ag Aide, a yucca-based plant extract solution, as a possible adjuvant for lime sulfur. None of these treatments increased thinning or fruit size on Fuji, but performed no worse than other programs in this trial (Table 2); the trial block used has a historic of poor chemical thinning results and we should test the products further before drawing firm conclusions.

We continue to see positive results with our renewed trials with endothall (ThinRite), a material currently registered as an aquatic herbicide. At relatively low concentrations, it has proven effective at reducing fruit set, but has yet to demonstrate any effect on fruit size or return bloom (Tables 2, 3). Our first evaluations of ThinRite applied in combination with Crocker's Fish Oil on Red Delicious showed strong thinning and fruit size responses (Table 2), and merits wider testing. According to the registrant, United Phosphorus, ThinRite should be fully labeled as a stand-alone chemical bloom thinner and available for the 2012 season.

Table 2. Crop load and fruit quality effects of bloom thinning programs. WTFRC 2011.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		%	%	g		%
Fuji/Sdlg - Manson						
2% CFO + 3% LS	114 ns	42 ns	23 ns	194 ns	94	41 bcd
2% GC Ag Aide + 1.5% LS	117	38	28	202	90	71 a
2% GC Ag Aide + 3% LS	115	40	25	189	96	65 ab
0.5% Sysstem CAL + 3% LS	109	43	24	212	86	37 cd
1% Sysstem CAL + 3% LS	103	40	29	204	89	67 a
24 oz ThinRite	98	40	32	196	93	25 d
24 oz ThinRite + 2% CFO	100	46	24	202	90	61 cd

24 oz ThinRite + 3% CFO	90	46	30	188	97	52 abc
24 oz ThinRite + 16 oz CF	105	44	23	206	88	71 a
Control	111	44	22	197	92	74 a
Red Del./M.9 - Royal Slope						
2% CFO + 2% LS	42 d	70 ab	20 bc	235 ab	77	43 a
24 oz ThinRite	77 b	51 de	27 ab	219 bc	83	25 b
24 oz ThinRite + 2% CFO	37 d	75 a	15 c	258 a	70	37 ab
24 oz ThinRite + 16 oz CF	72 bc	54 cd	27 ab	196 c	93	41 ab
24 oz ThinRite + 2% LS	52 cd	65 bc	21 bc	213 bc	85	39 ab
Control	105 a	37 e	29 a	197 c	92	28 ab

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 1999-2011.

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	62 / 108 (57%)	27 / 99 (27%)	21 / 96 (22%)
JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	14 / 27 (52%)	4 / 26 (15%)	4 / 26 (15%)
ThinRite	7 / 18 (39%)	0 / 19 (0%)	0 / 6

[1]Does not include data from 2011 trials.

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

POSTBLOOM THINNING:

We continue to seek out efficacious postbloom thinning programs that do not rely on the use of carbaryl, which faces an increasingly uncertain future. As with bloom thinning, we tested the phosphite product Sysstem CAL as an adjuvant for BA. While these programs did induce some mild thinning in two trials (Table 4), they did not improve fruit size and were generally less efficacious than other BA treatments that included carbaryl or NAA.

After considerable wrangling with the registrant, we successfully acquired samples of metamitron, a sugar beet herbicide which has shown promise in initial East Coast studies and is nearing registration as a postbloom thinner in the European Union. Research experience in those regions has suggested that single applications of 150-350 ppm caused substantial thinning. Assuming that WA apple trees require more aggressive programs for adequate thinning, we tested 2 applications of 100-400 ppm, but still found them to be too timid to produce the desired effects in our conditions (Table 4). We were pleased to find none of the phytotoxicity reported in Europe or the Eastern US associated with higher rates and hope to have enough material left to try more aggressive programs with metamitron in 2012.

Our 2011 results further bolster our confidence that Washington growers can have postbloom thinning success without carbaryl, namely in the form of BA + NAA programs (Table 4). Over the last several years, these treatments have been even more effective as standard carbaryl + NAA programs (Table 5) and we feel they merit wider adoption by industry.

Table 4. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2011.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		%	%	g		%
Gala/M.9 337 - Manson						
96 oz BA + 3 oz NAA	68 bcd	53 abc	30 ns	191 bcd	95	36 ab
96 oz BA + 4.5 oz NAA	73 abcd	51 abc	30	194 abc	94	29 ab
128 oz BA + 3 oz NAA	52 d	61 a	28	210 a	86	31 ab
48 oz carbaryl + 128 oz BA	62 cd	56 ab	29	193 abcd	94	30 ab
48 oz carbaryl + 3 oz NAA	63 cd	54 abc	32	200 ab	91	30 ab
200 ppm metamitron	95 a	42 c	29	185 bcd	98	21 b
400 ppm metamitron	76 abcd	48 abc	33	178 cde	102	42 a
0.5% SC + 128 oz BA	81 abc	46 bc	32	176 de	103	41 a
1% SC + 128 oz BA	72 abcd	53 abc	28	180 cde	101	46 a
Control	91 ab	41 c	33	168 e	108	37 ab
Gold Del./M.26 - Manson						
128 oz BA + 3 oz NAA	59 b	59 a	27 ns	225 ab	81	40 ns
48 oz carbaryl + 128 oz BA	74 ab	51 ab	29	222 abc	82	39
48 oz carbaryl + 3 oz NAA	80 ab	47 ab	33	229 a	79	52
100 ppm metamitron	83 ab	47 ab	31	204 c	89	36
200 ppm metamitron	79 ab	48 ab	30	209 abc	87	26
300 ppm metamitron	96 a	41 b	31	212 abc	86	45
400 ppm metamitron	93 a	43 ab	30	215 abc	84	45
0.5% SC + 128 oz BA	102 a	39 b	29	204 c	89	36
1% SC + 128 oz BA	95 a	44 ab	26	206 bc	88	41
Control	106 a	38 b	32	203 c	89	36

Table 5. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2011.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	2 / 18 (11%)	0 / 19 (0%)	0 / 19 (0%)
Carb + BA	30 / 80 (38%)	10 / 79 (13%)	10 / 74 (14%)
Carb + NAA	13 / 54 (24%)	9 / 54 (17%)	5 / 51 (10%)
BA + NAA	7 / 17 (41%)	5 / 17 (29%)	1 / 12 (8%)
Carb + NAA + Ethephon	0 / 5	0 / 5	2 / 5
Carb + NAA + BA	0 / 8	0 / 8	3 / 8

[1]Does not include data from 2011 trials.

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

RETURN BLOOM PROGRAMS:
Our ongoing work with gibberellic acid (GA) products to inhibit return bloom as a tool to mitigate biennial bearing continues to be simultaneously encouraging and frustrating. We are convinced that relatively inexpensive rates of GA_3 can indeed temper a snowball bloom, but our trials results demonstrate the inconsistency that plagues most plant growth regulator and crop load management research. Further, even when we seemingly achieve desirable treatment effects, plot-to-plot variability frequently renders statistical certainty elusive (Table 6).

Table 6. Key effects of WTFRC 2010 GA_3 return bloom trials.

GA_3 concentration	**2010 shoot length**	**2010 fruit weight**	**2011 return bloom**
ppm	cm	g	flower clusters/cm^2 LCSA
Red Del. – Rock Island			
10	43.7 ns	226 ns	3.1 ns
50	38.9	225	3.2
100	43.7	214	3.0
500	41.9	212	3.3
1000	38.5	217	3.5
Control	39.5	220	3.9
Fuji/M.26 - Orondo			
2 x 100 (weekly apps)	32.8 ns	244 ns	0.44 ns
4 x 100	37.7	225	0.04
2 x 200	39.4	no data	0.10
4 x 200	39.3	235	0.34
Control	32.8	257	0.41

Nonetheless, we often see treatment effects which may be horticulturally significant both in the year after treatment (when reduced bloom is desired) and again in the second year after treatment (when increased bloom is desired. Table 7 details the carryover effects of 2009 treatments on lightly cropped trees on 2011 bloom.

Table 7. Second year effects of WTFRC 2009 GA_3 return bloom trials.

GA_3 concentration	**2010 return bloom**	**2011 return bloom**	**% improvement**
ppm	flower clusters/cm^2 LCSA	flower clusters/cm^2 LCSA	2009 flower clusters/ 2011 flower clusters
Gala/M.26 – George			
200	4.3 ab	0.8 ns	380 ab
4 x 200 (weekly apps)	3.2 b	1.2	572 a
400	4.4 ab	0.4	401 ab
800	4.0 ab	0.7	226 b
Control	4.7 a	0.8	271 ab
Jonagold/M.26 – Royal Slope			
200	2.6 ns	0.6 b	427 ab
4 x 200 (weekly apps)	2.5	0.3 b	330 b
400	1.7	1.1 a	753 a
800	2.8	0.4 b	279 b
Control	2.9	0.5 b	353 b

REFLECTIVE MATERIAL TRIALS:
As with chemical thinning trials, our activity in reflective material studies has been significantly scaled back in the recent seasons. Our lone 2012 project was to help evaluate and contrast four Extenday ground cover products and was fully compensated by the company (data not shown). Our previous work with Extenday has clearly demonstrated its ability to increase yields of target fruit in apple, pear, cherry, peach, and nectarine by simultaneously increasing fruit set and/or size, as well as color in red or partially red fruits.

We are preparing for a new research venture with Extenday to evaluate the horticultural effects of enclosing apple trees in pods constructed of protective wind/shade cloth. The working hypothesis is that reduction of heat, light, and wind stress may improve tree performance and fruit yields and quality, as well as helping exclude bird pests. A prototype pod was constructed at the WSU Sunrise research orchard near Rock Island this fall (Figure 1) and will serve as a model for replicate pods to be constructed for the 2012 season.

Figure 1. Prototype protective pod at WSU Sunrise orchard (l) and mesh material sample (r).

COLLABORATIVE RESEARCH:
We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to crop load and canopy management (Table 8). These working relationships leverage 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 and industry.

Table 8. 2011 WTFRC collaborations on external crop load and canopy management projects.

COLLABORATOR(S)	PROJECT	COMMENTS
Yoder, Combs	Pollen tube growth model	WA field testing, flower style sampling
Dasgupta, Lewis	Apple 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
Brunner, MSU	Solid set spray system	Cooperator on newly approved SCRI project
McArtney, Greene	Chemical thinning trials	Sysstem CAL, metamitron evaluations
Elfving	PGRs for shoot growth	Field support for vegetative growth trials