			APPLE HORTICULTURE AND POSTHARVEST RESEARCH REVIEW	
			Wed 28 Jan 2015	**Pr**
Time	**Page**	**PI**		**Yrs**
8:00		Schmidt	Welcome and introduction	
8:05		McFerson	Research update	
			Final Reports	
8:30	1	Salazar	Development of apple bloom phenology and fruit growth models	12-14
8:45	13	Combs	Implementation/evaluation of apple pollen tube growth models	12-14
9:00	22	Killinger	Enhancing apple packing HACCP programs while ensuring fruit quality: ***Extension***	12-14
9:15	30	Wetherington	Apple microbial risk factors: ***Extension***	12-13
			Continuing Projects: 2:15 - 4:00	
1	34	Killinger	Assessment of overhead cooling practices for apple food safety	14-16
1	43	Wetherington	Analysis of packinghouse preventive controls for dump tank water	14-15
1	47	Hanrahan	Programs to increase packouts of apples	internal
1	53	Ganjyal	Improving food safety of fresh apples by hot air impingement drying: ***Extension***	14
2	60	Musacchi	WA 38: evaluation of rootstocks and training systems	14-16
2	68	Auvil	Apple rootstock and scion evaluation	internal
2	73	Peace	After RosBREED: Developing and deploying new apple DNA tests	14-16
2	81	Main	Adding apple map, marker and trait data to GDR	14-15
3		Kalcsits	Testing a portable, non-destructive measure of Ca, Mg and K in apple	14-15
3	86	Burke	Glyphosate fate in inland pacific northwest apple orchards	13-15
3	93	Mattheis	Identification of procedures to extend Honeycrisp storage life	13-15
3	100	Rudell	Commercial testing of early scald risk assessment tools	13-15
4	106	Kim	Effect of crabapple pruning on Sphaeropsis and speck rot incidence	13-15
4	113	Schmidt	Crop load and canopy management: ***internal***	internal

FINAL PROJECT REPORT **WTFRC Project Number: AP-12-104156**

Project Title: Development of apple bloom phenology and fruit growth models

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Total Project Request: **Year 1:** $70,000 **Year 2:** $82,500 **Year 3:** $85,000

Other funding sources

Indirect support through the existing infrastructure of AgWeatherNet and its network of 158 weather stations

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries	3,000	3,500	4,000
Benefits	1,200	1,400	1,600
Wages[1]	7,500	7,500	7,500
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel[2]	2,400	2,700	3,000
Plot Fees			
Miscellaneous			
Total	$14,100	$15,100	$16,100

Footnotes:

[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

Budget

Organization Name: ARC-WSU **Contract Administrator:** Carrie Johnston
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Item	2012	2013	2014
Salaries	53,936	65,536	67,496
Benefits	12,564	13,464	14,004
Wages			
Benefits			
Equipment			
Supplies	1,000	1,000	1,000
Travel	2,500	2,500	2,500
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	$70,000	$82,500	$85,000

Footnotes: The budget that is requested through this proposal includes partial support for an Assistant Research Professor (Dr. Melba Salazar) who will be responsible for the overall evaluation and implementation of the various growing degree models that are applicable for conditions in the Pacific Northwest and partial support for an Application Programmer (Sean Hill) for integration of the model on the web portal of AgWeatherNet (www.weather.wsu.edu). We also have budgeted for a Graduate Student (to be hired) who will be responsible for the development of a physiological fruit growth model. The proposal includes a request for a computer for the graduate student during the first year of the project. Additional budget items include operating expenses for computer software and related costs and travel to participate in field data collection. Finally, this proposal includes support for Professor Dasgupta in the Department of Statistics to complete her statistical model development and evaluation (objective 2).

OBJECTIVES

1. Continue data collection on bloom phenology and fruit growth for selected sites and cultivars to enhance model accuracy and vigor. (Schmidt in collaboration with Castillo)
2. Continue refinement of statistical models for bloom phenology and fruit growth. (Dasgupta)
3. Develop physiological-based models for bloom phenology and fruit growth of apples. (Hoogenboom, Salazar)
4. Implement and evaluate models as decision support aids on the AgWeatherNet portal using industry beta-testers. (Hoogenboom, Salazar and Dasgupta in collaboration with Lewis)
5. Improve model/portal user interface based on feedback from beta-testers and other stakeholders. (Hoogenboom, Salazar in collaboration with Lewis)

SIGNIFICANT FINDINGS

- Differences among locations and among cultivars for the different duration of the phenological stages where found in terms of Growing Degree Days.
- An overlap of consecutive phonological stages was found for each location, year and cultivar.
- A phenological mathematical modeling approach using the duration of each stage in terms of Growing Degree Days was proposed.
- A statistical model was developed to predict final fruit size based on observational data.
- Fruit growth was modeled mathematically using Growing Degree Days. Differences among cultivars were found for each location.
- Red Delicious and Gala had significantly larger diameters than Cripps Pink. Final fruit varied from year to year and location to location.

METHODS

1. Data collection

For the development of robust models, high quality data were collected for a diverse range of environments and annual weather conditions. WTFRC staff collected bloom phenology and fruit growth data from established sites to augment data sets from the previous project.

2. Continue refinement of statistical models for bloom phenology and fruit growth

For the bloom models, data were compiled for 2010 and an ordinal logit model was used to fit the data. All data for phenology, growth and temperature were compiled for 2011 through 2013. For the growth models, data for 2010 through 2013 were combined and new parameters were estimated. For the bloom model similar procedures were followed. Following a successful development of both the statistical bloom phenology model and the statistical fruit growth model, they were evaluated with the data that were collected during the 2012 and 2013 growing seasons.

3. *Develop physiological models for bloom phenology and fruit growth*

An analysis was performed for phenological data recorded at regular intervals for Gala, Red Delicious and Cripps Pink during the 2010, 2011, 2012 and 2013 growing seasons for 10 locations from bud break until full bloom in order to identify the dynamics of each phenological stage.

A descriptive analysis was conducted and trends were calculated for each phenological event as well as the duration for each year and location. Physiological time was used as input of the model for the different phenological stages for apple, referred to as Growing Degree Days (GDD) or Thermal time. The requirements for the different phenological stages of the most important apple cultivars using daily temperature records from the AgWeatherNet were summarized.

The phenological model was based on the duration of each stage in terms of GDD for a threshold temperature Tb = 43°F. The lower limit or beginning of a stage was determined as: Lower limit = $\sum_{i=1}^{10}\sum_{j=1}^{3} Min(GDD_{ij})/n$ and upper limit or the end of a stage was calculated as: Upper limit $=\sum_{i=1}^{10}\sum_{j=1}^{3} Max(GDD_{ij})/n$, where i = 1, 2, .., 10 are the locations and j = 1, 2, 3 the years 2011, 2012 and 2013. The data collected in 2010 and 2014 were used for model evaluation.

The performance of the model was compared using the weather data collected with the Hobo data loggers that were part of the data collected by WTFRC. To identify if there were significant differences between locations, a procedure GLIMMIX of SAS9.3 was used. The categorical variable stage was the dependent variable and was linked to a multinomial distribution.

The diameter of 50 apples of every cultivar in each location was measured weekly for each year. As a function of the GDD, a growth diameter mathematical model was proposed; the rate of the diameter growth was an exponential function derived from the Von Bertalanfy growth curve of the diameter:

$$\partial D = ake^{-kGDD}$$

Where D is the apple diameter, *a* is the upper asymptote (maximum diameter) and *k* is a shape parameter of the curve.

To simulate the diameter for each day *t*, an Euler method was used:

$$Diameter_t = Diameter_{t-1} + \partial D\Delta t$$

Where Δt is the time step equal to one day. Thus the estimation of the apple diameter for the day t depends on the day t-1 plus the rate of growth time the time step. An evaluation is planned to determine the most accurate growth model. Data collected in 2010 and 2014 will be used for model evaluation.

4. Implement and evaluate models as decision support aids on the AgWeatherNet portal

To assist the growers for making decisions, an information delivery system and media tool will be posted in the web page using the models developed. This tool will provide, in an easy and user-friendly way, thermal time in real-time (current) for different environmental conditions where local weather data are available through tables and graphs as well as information about the current phenological and development stages and the climatic requirements to complete the next stage as well as the current apple diameter. The system will be available through a link created on the AgWeatherNet web portal and other web portals where information for apples is provided

5. Improve model/portal user interface and release for general use

The overall goal is to develop a web portal that will provide a guideline and advisory for the growers who are monitoring their individual apple orchards in terms of weather conditions and weather predictions. That will ultimately allow for better planning to improve fruit quality, increase yield, more efficient marketing and ultimately result in an increase in net returns. We will work closely with WSU Extension and industry representatives as beta testers. We will try to incorporate all comments to help improve the tool and decision aid to the benefit of the local apple growers.

RESULTS & DISCUSSION

1. Data collection

Observations of bloom phenology were recorded from 2010 to 2014 by WTFRC internal staff every Monday, Wednesday, and Friday in 29 blocks clustered around 10 location nodes. Current varieties include Red Delicious, Cripps Pink and Gala. WTFRC staff also collected fruit size data starting at petal fall until final harvest with a brief break during thinning.

2. Continue refinement of statistical models for bloom phenology and fruit growth
In 2014 we focused on developing a predictive model for the growth data. Based on previous work we found that the weather and location related variables played an important role in the prediction of diameter close to harvest. We observed that the date of Full Bloom (FB) was a good proxy variable for the weather variables (high R-square when regressing weather variables on FB). Hence we tried to do our predictive model with predictors FB and days after full bloom (DAFB) to account for location and year. The model for Gala is shown here as an example. It is a mixture of the Richard's curve and a linear model given as:

$$y = \frac{\beta_0}{(1 + \exp(-\beta_1 (DAFB - \beta_2)} + \beta_3 FB + \beta_4 M_{40} + \beta_5 M_{50} + \beta_6 M_{60} + \beta_7 M_{70} + \beta_8 M_{80}$$

where y represents the predicted mean diameter at day 100, M40, M50, M60, M70, M80 represent mean diameter at day 40, 50, 60, 70, 80 respectively. Hence size at a particular time point was predicted using past data and the mean of diameter 20 days before the date we are predicting. An initial implementation of the model is discussed in section 4 below.

3. Develop physiological models for bloom phenology and fruit growth
The percentage of buds for each phenological stage was determined for each sampling date, year, location and cultivar. Differences in the phenological stages distribution through time by cultivar year, and location were observed i.e. the duration of the stages for Cripps Pink, Gala and Red Delicious were not the same through the years and location. Thus the starting and final day of each stage varied among cultivars, years and localities. As a result there was an overlap of the consecutive stages (Fig. 1). There was significant effect for year, cultivar, location and their interactions as an indication of the dependence among factors. In other words, the pattern of the stage distribution for years depended on cultivar, year and location.

The trend of the distribution for each phenological stage was determined for each cultivar and each location. An example for Gala for each stage is presented for 2010 -2013 for blocks located in Brays Landing, Chelan, Prosser and Royal (Fig. 2). The dynamics of the different phenological stages were analyzed using Growing Degree Days (GDD). The base temperature for heat accumulation was 43 ºF for each location and each cultivar for the 2011, 2012 and 2013 growing season. The analysis showed different durations among locations and among cultivars for the different phenological stages (Table 2). Gala was the cultivar that started and ended later than Cripps Pink and Red Delicious and the duration depended on the cultivar and stage (Figure 3). More than 70% of the variability in the duration of the stages duration was explained by the model; the error (RMSE) varied from 40 to 56 DGG and the coefficient of variation from 23 to 25%

For all locations Red Delicious and Gala had significantly larger diameters than Cripps Pink. The final size of the fruit varied from year to year and location to location. Cultivar differences in fruit diameter reflected differences in mean fruit diameter as well as fruit growth period (Fig 4). The proposed model estimates the apple diameter as functions of the GDD. If local weather data are available from a meteorological station the model can provide an estimate for fruit size diameter without having to take samples from the field.

4 Implement and evaluate models as decision support aids on the AgWeatherNet portal
During the final year of the project the initial models were implemented on the AgWeatherNet portal for in-house testing only. An example of the phenology model is shown in Figure 5. Further

development in cooperation with stakeholders is required with expected Beta testing during the 2015 growing season.

An initial version of the model developed under Objective 2 is shown in Figure 6. Based on the observed bloom data and measured early fruit diameter the model provides a projection of fruit diameter change as the fruit grows and final fruit diameter. The different lines in the figure depict the different model parameters that are used during the growing season. Inputs for this model are local measurements only and are not site dependent.

5 *Improve model/portal user interface and release for general use*

Due to the delay in the development of the models for implementation on the AgWeatherNet portal, the models were not released to the general public.

Table 1. Locations and associated elevation for each orchard and cultivars sampled.

Location	Elevation (ft)
Brays Landing	900 (RD,CP,G)*
Chelan	1120 (CP), 1450 (RD,G)
East Wenatchee	910 (RD, CP), 1025 (G)
Konnowac Pass	870 (RD,CP,G)
Naches	1580 (RD,G)
Omak	1250 (RD,G)
Prosser	681 (RD,CP,G)
Royal City	1095 (CP), 1055 (RD,G)
Orondo	755 (RD,CP,G)
Sunrise Orchards	910 (RD), 880 (G), 775 (CP)
Wapato	879 (RD,CP,G)

* RD = Red Delicious, CP = Cripps Pink, G = Gala

Table 2. Degree days for the start and end of each stage and the standard deviation for Cripps Pink, Gala and Red Delicious.

Cultivar	Stage	Stage Start GDD	Stage End GDD	Standard Deviation of the Start	Standard Deviation of the End
Cripps Pink	A	34.81	71.81	16.48	20.78
Cripps Pink	B	48.53	106.28	14.23	24.23
Cripps Pink	C	90.07	149.24	21.17	35.26
Cripps Pink	D	117.21	170.49	24.70	38.37
Cripps Pink	E	148.26	224.14	30.91	44.36
Cripps Pink	F	187.42	255.90	37.55	48.42
Cripps Pink	G	216.72	326.19	48.02	52.49
Cripps Pink	H	262.71	332.98	57.89	77.51
Gala	A	35.47	84.28	15.88	26.96
Gala	B	58.31	122.51	18.44	34.42
Gala	C	111.23	175.38	24.38	48.12
Gala	D	148.29	205.14	29.48	45.59
Gala	E	182.63	246.28	40.02	52.89
Gala	F	220.95	273.88	45.59	53.52
Gala	G	243.19	328.51	50.22	70.90
Gala	H	297.69	365.39	51.55	80.94
Red Delicious	A	37.32	87.61	14.84	28.15
Red Delicious	B	59.33	122.94	17.87	30.27
Red Delicious	C	112.13	177.01	25.88	53.15
Red Delicious	D	142.41	201.44	28.99	46.62
Red Delicious	E	171.14	243.67	41.34	50.43
Red Delicious	F	207.00	266.50	43.53	54.78
Red Delicious	G	234.47	332.74	46.67	76.68
Red Delicious	H	291.06	360.55	53.95	85.25

A = Green Tip; B = ½ inch green; C= Tight Cluster; D = First Pink; E = Full Pink; F = First Bloom; G = Full Bloom; H = Petal Fall

Table 3. Evaluation of the phenological model based on Growing Degree Days.

Cultivar	RMSE (GDD)	R^2 (%)	CV (%)
Cripps Pink	40.60	82.82	23.76
Gala	46.39	79.78	24.20
Red Delicious	55.21	72.18	24.79

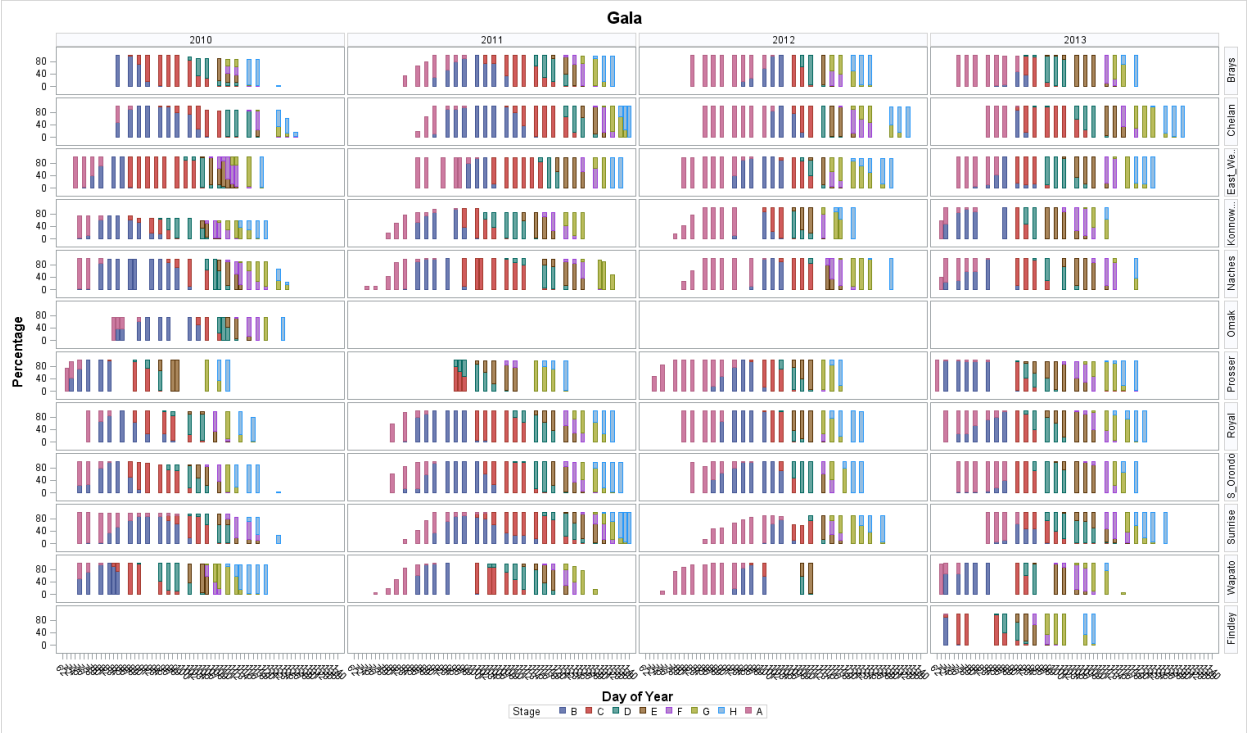

Figure 1. Phenological stage distribution for each sampling date for Gala for 2010 through 2014 (columns) for each location (rows). Each color represents a different phenological/growth stage; see Table 2 for Growth Stage Definitions.

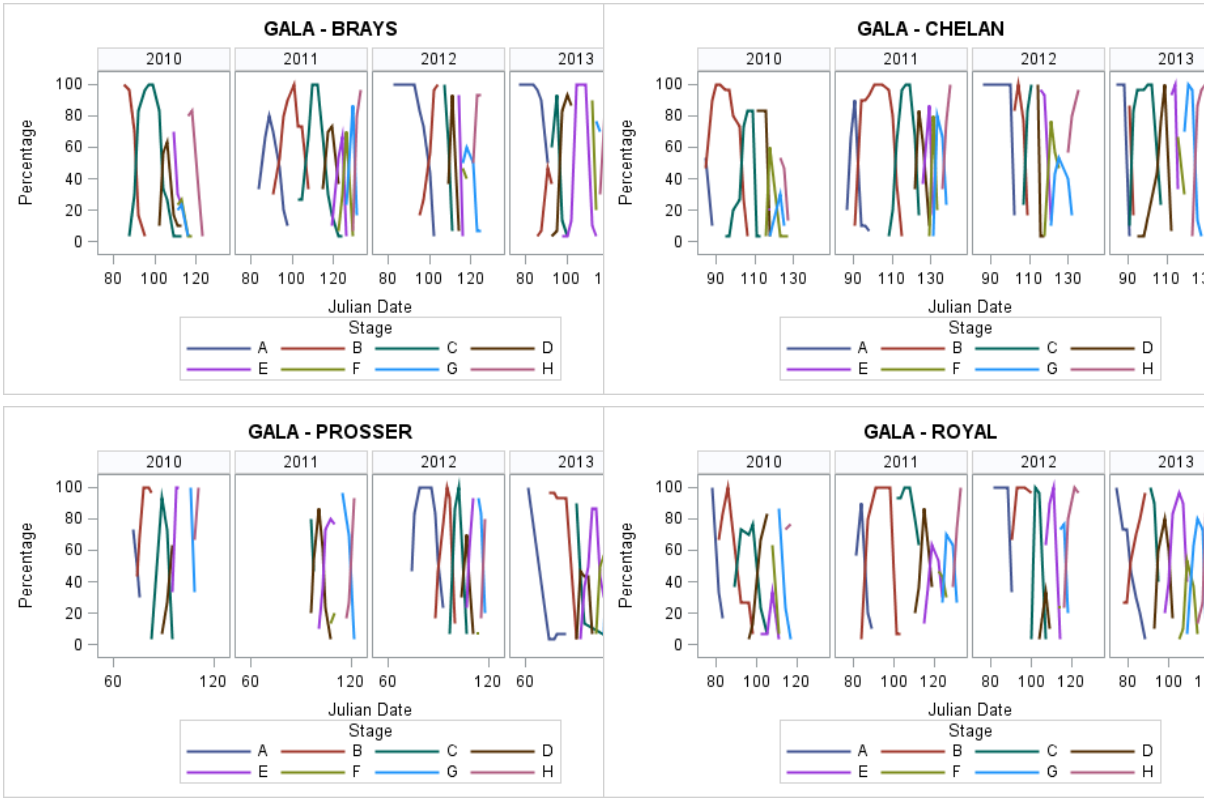

Figure 2. Trend of the distribution for each phenological stage for Gala for four study locations (Brays Landing, Chelan, Prosser, and Royal City). See Table 2 for growth stage definitions.

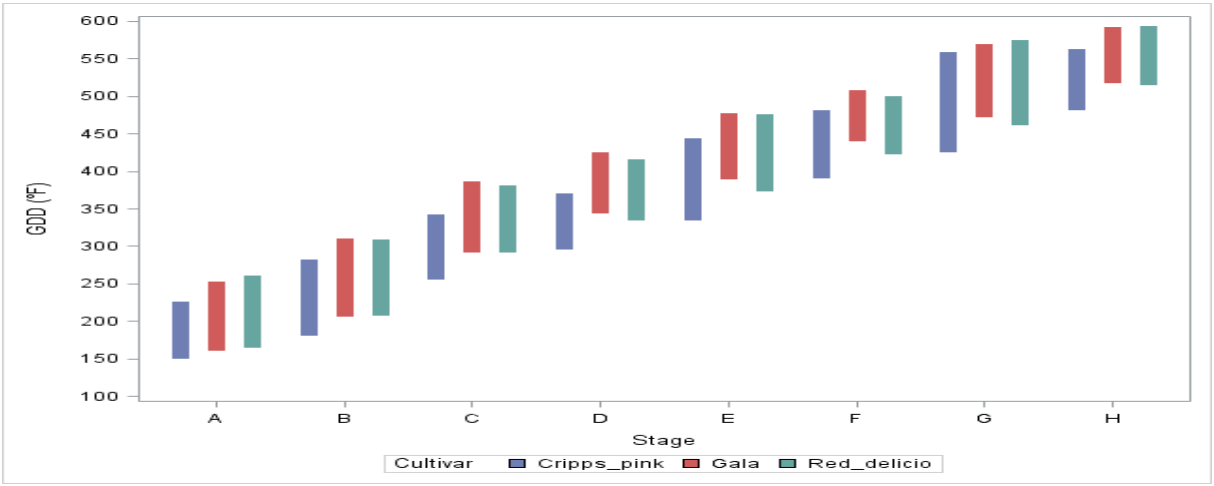

Figure 3. Duration of the phenological stages for Cripps Pink, Gala and Red Delicious. See Table 2 for growth stage definitions.

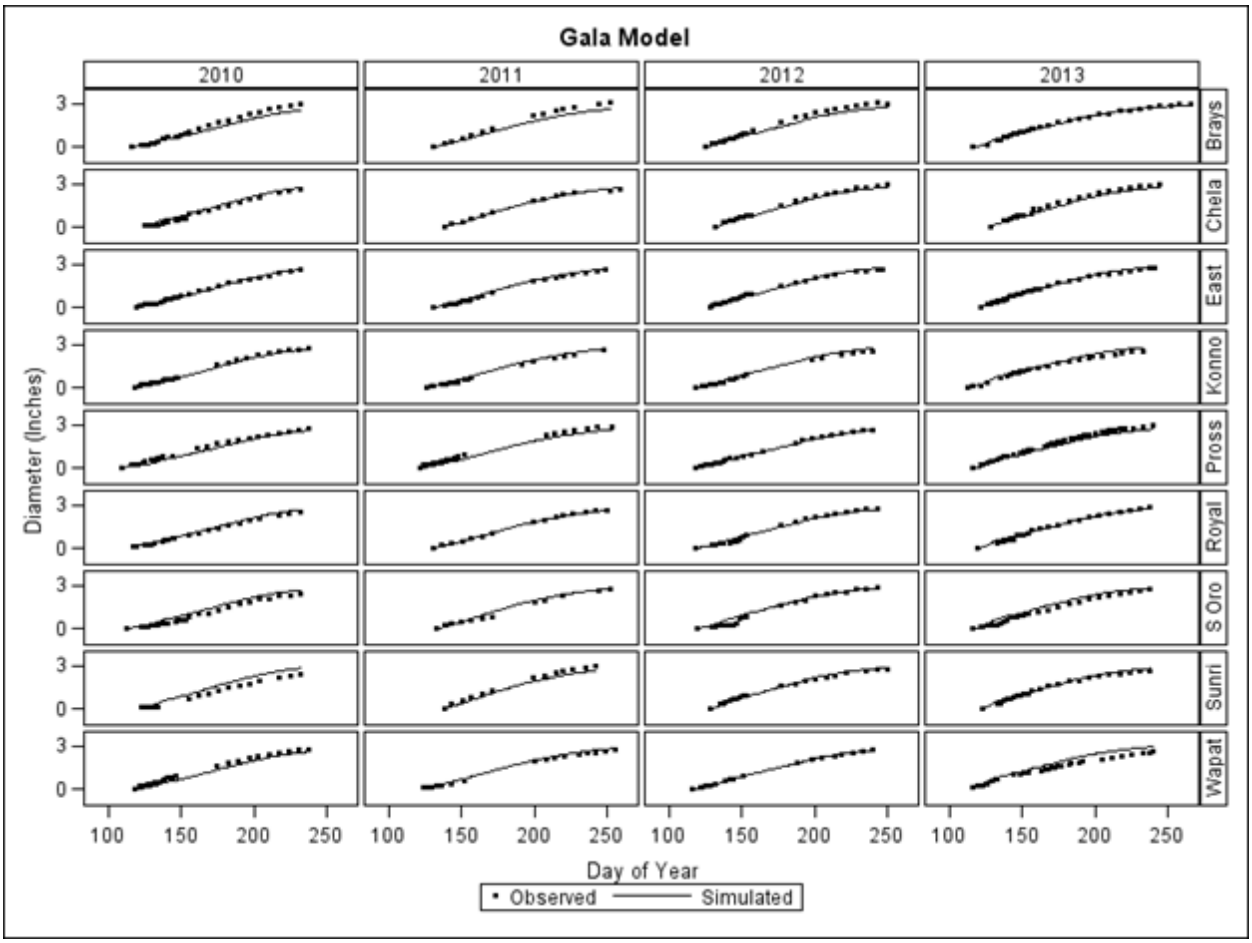

Figure 4. Observed and estimated diameter for each cultivar, year and location for Gala.

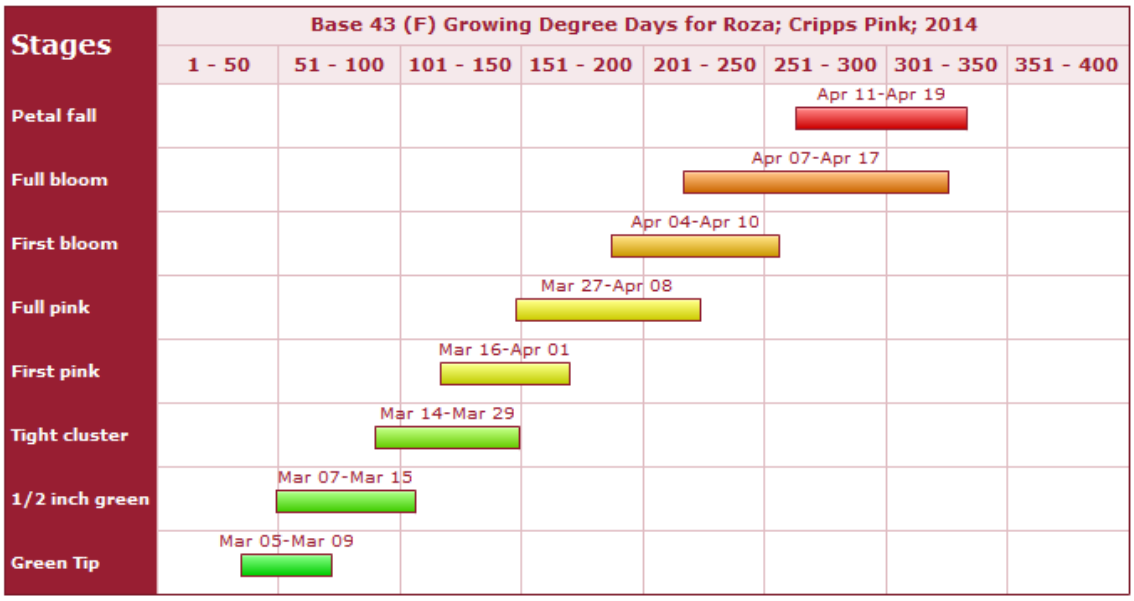

Figure 5. Example of the interface for the phenological model for Cripps Pink for 2014 based on weather data from the Roza farm at Prosser as implemented on the AgWeatherNet portal.

Figure 6. A prototype example of the statistical diameter prediction model.

EXECUTIVE SUMMARY

The overall goal of this project is to be able to predict bloom phenology and final fruit size of apples. During the first phase of the project from 2009 through 2011 protocols were established to measure apple bloom phenology, starting at green tip until petal fall as well as non-destructive measurements of fruit size. Data were collected for Red Delicious, Cripps Pink, and Gala for 11 locations. During the second phase of the project from 2012 through 2014 data collection was continued, while at the same time a range of modeling approaches was tested to determine the relationship between apple bloom phenology and local weather conditions as well as fruit diameter. A simple Growing Degree Day model has been developed using a base temperature of 43 F that can predict the beginning and ending for all eight successive apple stages based on local weather conditions. Two fruit diameter models were also developed. One is based on local measurements and can provide a prediction for final fruit diameter. The second model predicts the actual average fruit size based on local weather conditions. Initial versions of all models have been implemented for demonstration on the AgWeatherNet portal and were demonstrated to a select group of stakeholders during a meeting held in Prosser in October, 2014. Further work is needed for testing of all models, possibly with an integration of both fruit size models as a combined hybrid model. The interface for the models on the AgWeatherNet portal will be shared with a select group of growers for initial evaluation and improvement, followed by release to the general public. During the past six years this project has made great progress in not only understanding the impact of weather on apple development and fruit growth, but also developing sound scientific models that can be used by growers through the AgWeatherNet portal.

FINAL PROJECT REPORT

Project Title: Implementation and evaluation of apple pollen tube growth models

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Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA

Other funding sources: None

Total Project Funding: **$263,014**

Budget History:
Notes: **Virginia Tech and Washington State University submitted separate budgets as collaborative institutions.**

Budget 1
Organization Name: Virginia Polytechnic Institute and State University (Va. Tech)

Item	2012	2013	2014
Salaries*	35,762	37,192	38,680
Benefits	10,282	10,693	11,121
Equipment (laptop-field work)	1,500		
Supplies (lab &field)	1,500	1,500	1,500
Travel (to Wash. St. orchards)	5,000	6,000	6,500
Contractual services & repairs	1,250	1,250	1,250
Total	**$55,294**	**$56,635**	**$59,051**

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

Budget 2
Organization Name: Washington State University ARC

Item	2012	2013	2014
Salaries	24,699	16,674	17,341
Benefits	9,490	6,534	6,796
Wages			
Benefits			
Equipment			
Supplies	1,000	1,000	1,000
Travel	2,500	2,500	2,500
Miscellaneous			
Total	**$37,689**	**$26,708**	**$27,637**

Footnotes: Partial salary support for Research Associate (Dr. Melba Salazar) and for Application Development Programmer (Mr. Sean Hill).

RECAP ORIGINAL OBJECTIVES

Our overall goal for 2012-14 was to collaborate with Washington State University and Washington Tree Fruit Research Commission to validate and implement pollen tube growth models for the most important commercial apple cultivars and to have those models generated in real-time through the AgWeatherNet portal (www.weather.wsu.edu). The specific objectives included:

1) Complete model parameters for Red Delicious, Honeycrisp, and Granny Smith (in 2013).
2) Guide collaborative effort to validate the models in commercial orchards and to incorporate the models into the AgWeatherNet website (Gerrit Hoogenboom, Melba Salazar, and Sean Hill).
3) Provide training to commercial apple growers on how to determine the desirable amount of king bloom open before "starting the model clock" (Combs, Virginia Tech).
4) Continue beta-testing of models for Gala, Fuji, Golden Delicious and Cripps Pink and begin beta-testing on Red Delicious and Honeycrisp (Combs, Virginia Tech).
5) Add plantings of Aztec and September Wonder Fuji and other new cultivars and strains for temperature testing (Combs, Virginia Tech).
6) Further develop reliable techniques for the study of a range of constant and variable temperatures and light conditions on pollen germination and pollen tube growth (Virginia Tech).

SIGNIFICANT FINDINGS

- Using real-time weather data we showed the pollen tube growth models to be a robust tool that can assist Washington apple growers in making more reliable bloom thinning decisions.
- Cultivar-specific models for Gala, Fuji, Golden Delicious and Cripps Pink were developed for pollen tube growth and were added to the AgWeatherNet website.
- The AgWeatherNet interface and output was found to be intuitive by the beta-test participants.
- Site-specific temperature data from AgWeatherNet's large network of weather stations allowed the model to be tested in many different microclimates.
- Integrating 48-hours of forecasted hourly temperature data into the pollen tube growth model algorithms allowed growers to schedule bloom-thinning sprays in advance.
- Validation of the models included sampling flowers from the field to determine the percent of flowers that had been fertilized.
- When compared with lab measurements, beta-test participants were very capable of measuring style length in the field.
- Comparisons between field evaluations of desired bins per acre and the actual harvested bins per acre showed that beta-test participants often achieved their targeted crop load. The beta-testers also reported to have improved return bloom the following year.
- Comparing the desired yield with the actual harvested yield demonstrated that the beta-test participants were able to understand the principles of the model, as well as access the models through the AgWeatherNet website.
- Comparisons between temperature sensors in commercial orchards and the nearest AgWeatherNet weather station showed nominal variation in model outputs, thus giving a high level of confidence in using the AgWeatherNet systems with the pollen tube growth model.

RESULTS AND DISCUSSION

In 2012, the Excel spreadsheets (Figure 1) that were previously used for tracking pollen tube growth, were incorporated into the WSU's AgWeatherNet website (Figure 2). To date, we have developed models for Gala, Golden Delicious, Fuji and Cripps Pink (Pink Lady). The AgWeatherNet interface was presented to industry representatives and beta-testers through a series of training sessions held in Naches, WA and Chelan, WA in early April 2012. Approximately 60 orchards amounting to several hundred acres of apples were used as beta-test field sites in 2012. In addition to the implementation of the pollen tube growth models on the AgWeatherNet, field testing continued to validate and expand the effectiveness of the modeling program. Validation included checking whether flower samples collected in Washington orchards were fertilized after thinning chemicals were applied by comparing model-predicted pollen tube growth versus actual growth in flowers (visualized with the use of florescence microscopy). In addition, yield data was recorded for the 2012 season (Figure 3).

Through growth chamber work conducted at Virginia Tech's Alson H. Smith, Jr. AREC (Winchester, VA) new pollen tube growth models were developed. Our work in 2012 focused on Honeycrisp, Red Delicious, and Granny Smith. The Honeycrisp was beta-tested through the AgWeatherNet site by a select group of growers during the 2013 growing season, and then by a larger beta-test group in 2014. Growth chamber tests in 2013 on Granny Smith and Red Delicious allowed us to release those models to select beta-testers in 2014. In 2014, Gala, Golden Delicious, Fuji, and Cripps Pink models were available to all registered users of the AgWeatherNet website. In 2014, we also started beta-testing the Red Delicious and Granny Smith models in commercial orchards. We highly recommend another two years of beta-testing the Red Delicious and Granny Smith models before these models are publicly released.

Temperature data collected by weather stations in the AgWeatherNet system showed nominal variation for the pollen tube growth model compared with temperature data-loggers placed in commercial orchards (Figure 4). Field sample tests were conducted in orchards on hand-pollinated flowers that were harvested at mid growth range intervals (48 and 72 hours after pollination) and evaluated in the laboratory to track actual growth versus predicted model growth (Figure 5).

A total of 145 models beta-test sites were used for validation and verification of the pollen tube growth models in 2014. These test sites spanned Washington's apple growing regions and gave valuable data and feedback concerning model-predicted timing of bloom applications versus grower projected timing. As seen in Figures 6, 7, and 8, which show the average style lengths (determines the time point when the model recommends application of first bloom thinning), the actual application timing can be altered by the model user to adhere to more specific conditions in the field. The final decision for application timing always rests with the user in the field.

Grower/beta-tester feedback has been given throughout the development of the models. Surveys sent to beta-testers regarding models have been useful in identifying problem areas of understanding how and what to do to when setting up and maintaining grower model data sets. Retrievable historical model data will give growers access to previous years' model application timings for comparison to present day environments. Additionally at fall 2014 model evaluation meetings, beta-besters expressed concerns regarding pollen tube growth rates at lower temperatures and requested additional testing in that area be conducted. This feedback is vital to the development of modeling programs.

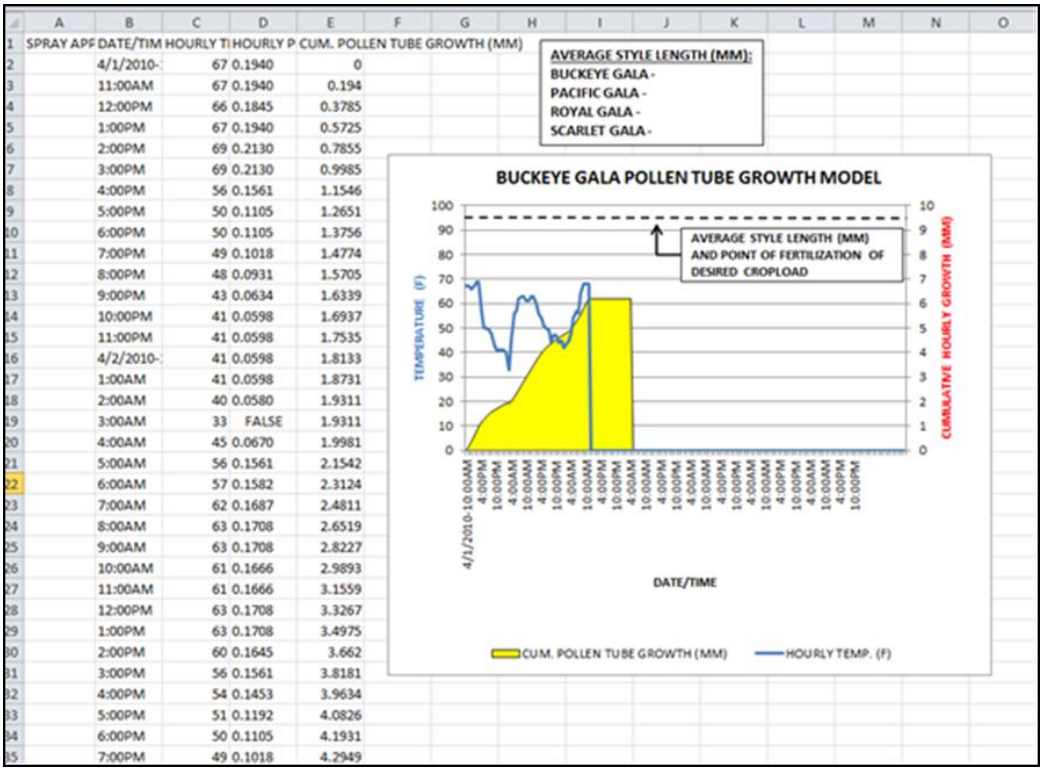

Figure 1. The pollen tube growth model in the Excel worksheet format.

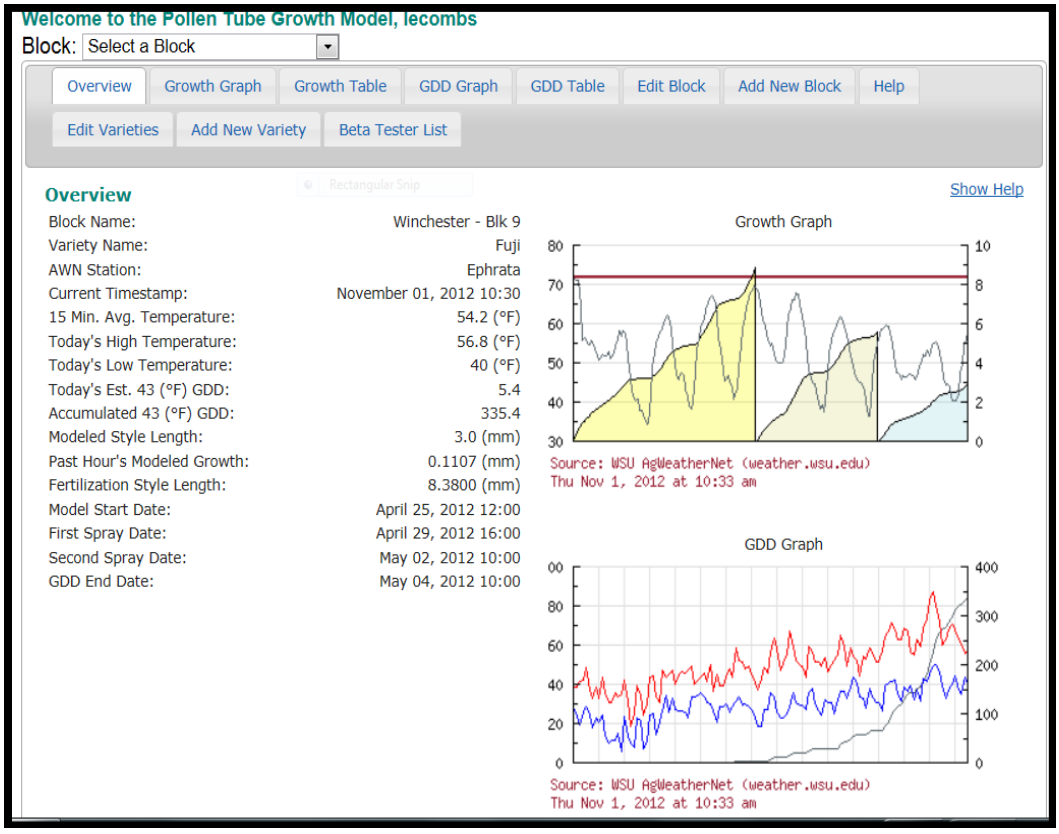

Figure 2. The pollen tube growth model in the WSU AgWeatherNet format.

POLLEN TUBE MODEL HARVEST DATA FOR BETA-TEST SITES IN QUINCY, WA (2012)			
CULTIVAR / STRAIN	**DESIRED YIELD (BINS / ACRE)**	**ACTUAL YIELD (BINS / ACRE)**	**% DESIRED YIELD (BINS / ACRE)**
Gala (Pacific)	**50**	**55.7**	**111**
Fuji (Nagafu 6)	**35**	**23.5**	**67**
G. Del. (Standard)	**55-60**	**61.8**	**103**
G. Del. (Standard)	**55-60**	**50.3**	**84**
Fuji (TAC114)	**35**	**27.4**	**78**
Fuji (TAC114)	**35**	**22.4**	**64**
FUJI (Early)	**40**	**38.7**	**97**
Cripps Pink Lady	**45**	**40.3**	**90**
Gala (Pacific)	**45**	**44.0**	**98**
G. Del. (Smoothie)	**55**	**32.1**	**58**

Figure 3. Harvest totals for 2012 comparing desired crop load versus actual harvest totals at beta-test sites in Quincy, WA.

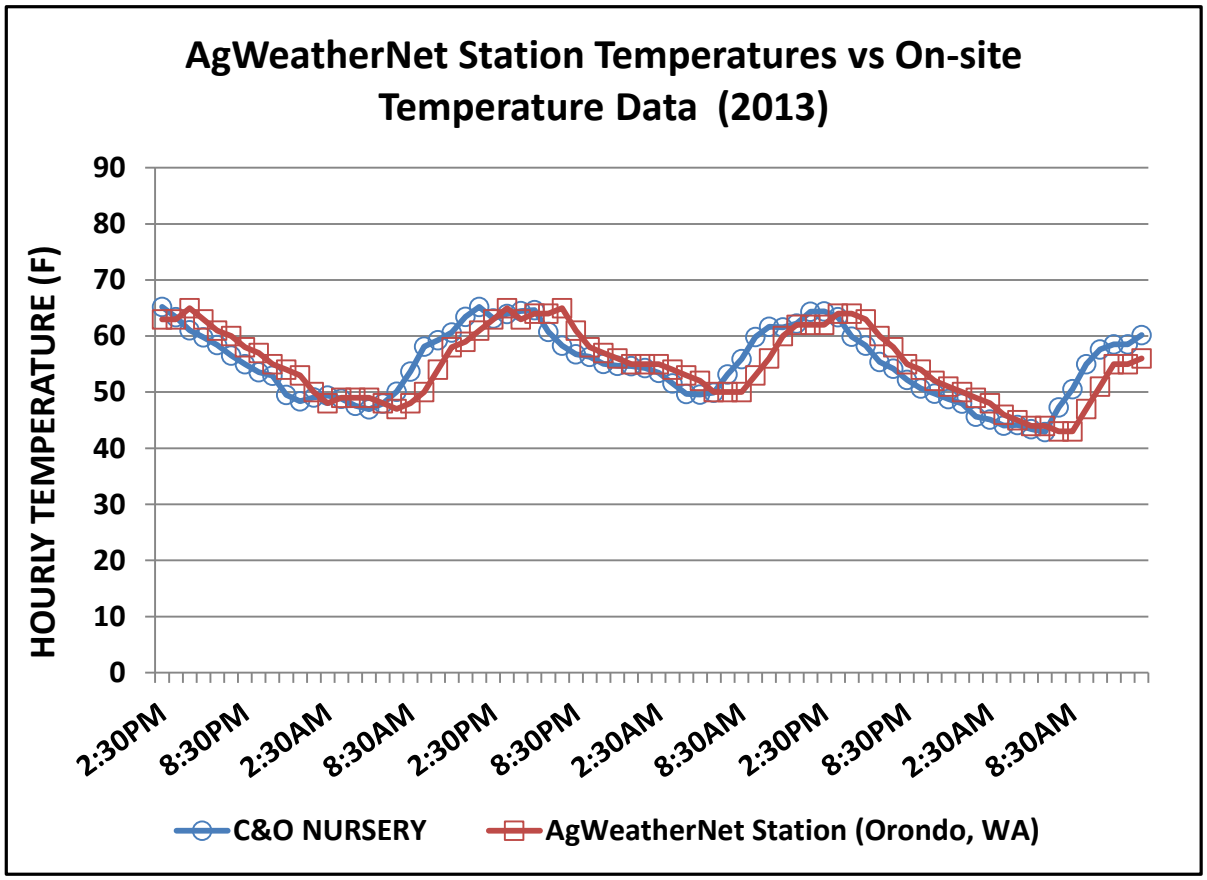

Figure 4. AgWeatherNet weather station data versus actual on-site temperature data-loggers.

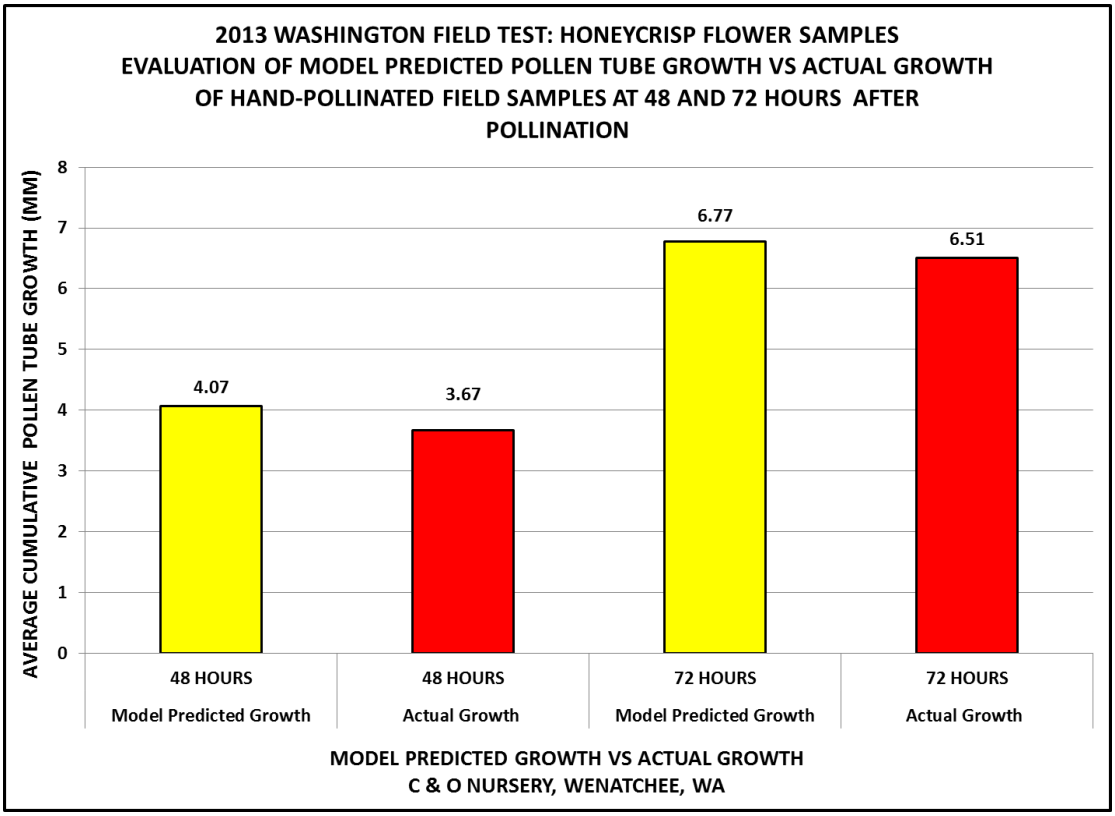

Figure 5. Model predicted growth versus actual growth.

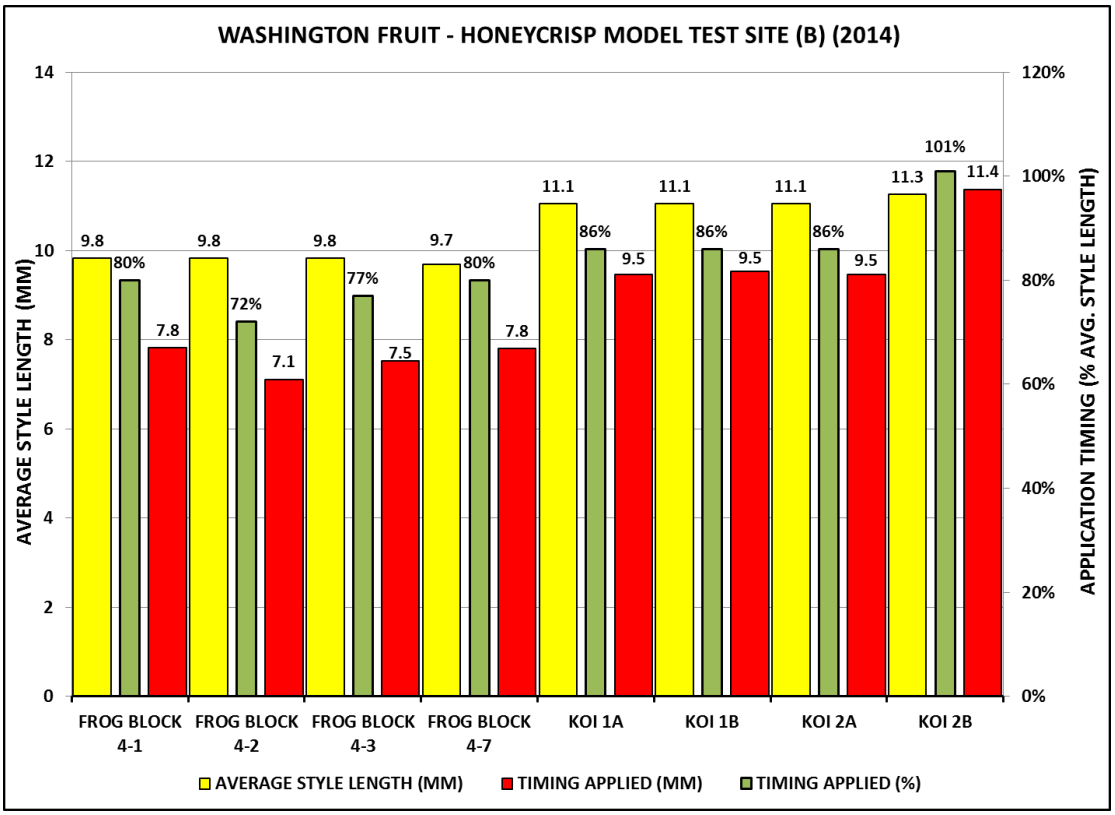

Figure 6. Model predicted timing versus actual application timing by grower.

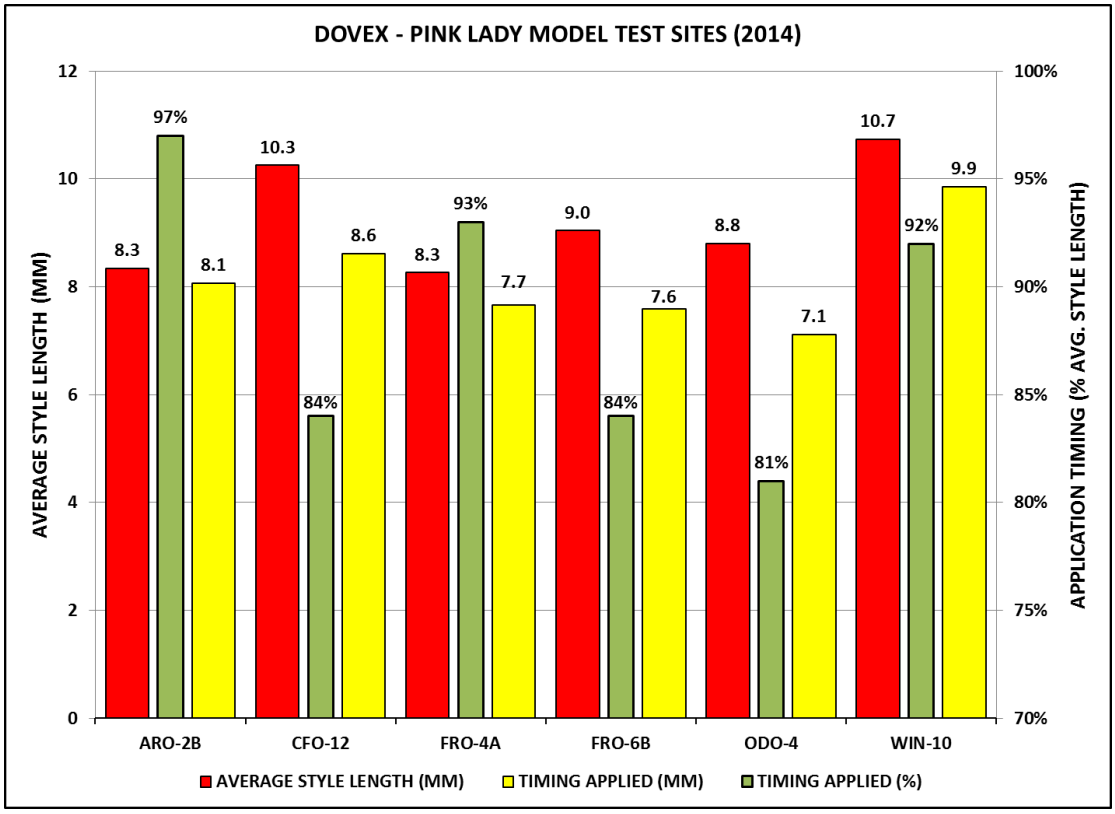

Figure 7. Model predicted timing versus actual application timing by grower.

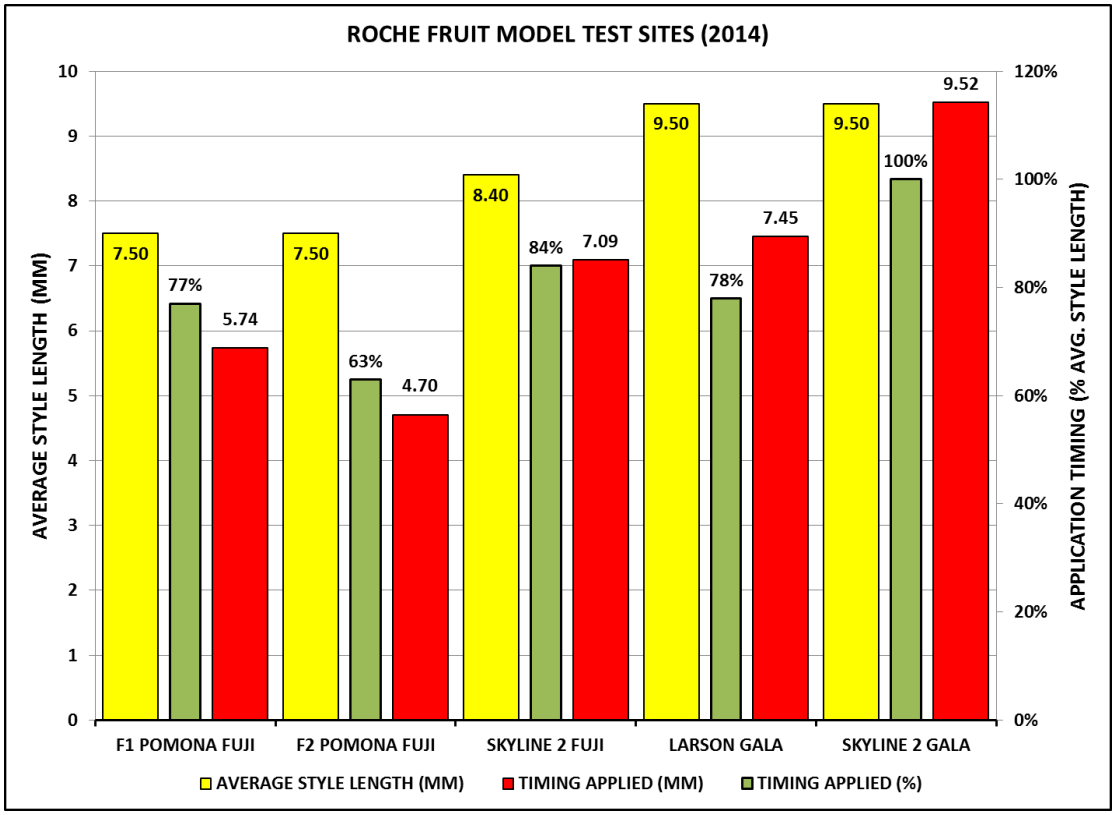

Figure 8. Model predicted timing versus actual application timing by grower.

EXECUTIVE SUMMARY

In apple (*Malus Xdomestica* Borkh.) production, crop thinning during bloom produces the largest fruit, the greatest return bloom in the following year, and reduces biennial bearing. The application timing for this spray has been subjective, and in the past was usually based upon the percent of full bloom open (e.g., applications at 20 and 80% full bloom). While this approach became a standard practice in some growing regions, more precise application timing can be achieved through modeling the fertilization of the desired percent of king bloom needed to achieve a full crop at the desired fruit size. When this target is achieved, a bloom thinner can be applied so that later blooming flowers are prevented from setting fruit. By measuring pollen tube growth rates under controlled atmospheric conditions using growth chambers, we have developed a model that calculates the time required to fertilize the king bloom after pollination.

Using real-time weather data we are evaluating the model as an important bloom-thinning tool for Washington apple growers and this allows them to make immediate bloom thinning decisions. Cultivar-specific equations that we have developed for pollen tube growth have been built into the AgWeatherNet website. The web-based interface makes these models straightforward to use and the output results easy to understand. The generated information allows growers to schedule bloom-thinning sprays in advance by using a 48 hour projected temperature feature.

Properly timed bloom-thinning gives the grower the optimum advantage for producing the best quality fruit. Understanding the progression of pollen tube growth after pollination is critical in applying bloom thinners at the proper time. In addition to optimal sizing benefits, crop loads not sufficiently thinned could result in trees being thrown into biennial bearing with little or no crop in the 'off' year. The primary focus is to evaluate the pollen tube growth model for the wide range of growing conditions in Washington. Real-time weather station data specific to that growing region will be downloaded to the AgWeatherNet model interface for program assimilation.

We thank the Washington Tree Fruit Research Commission for their continuing support of this project. We would also like to thank the following Washington growers who have supported this research project as beta-testers and/or allowed us access to their orchards to conduct research: Dovex Fruit, Stemilt Growers, Washington Fruit & Produce Co., Roche Fruit, Chelan Fruit Company, C & O Nursery, and Columbia Basin Nursery. In addition we would like to thank the support staff at Washington Tree Fruit Research Commission for their help with this project.

CONTINUING PROJECT REPORT: ***Extension*** **YEAR**: 3 of 4
WTFRC Project Number: AP-12-102

Project Title: Enhancing apple packing HACCP programs while ensuring fruit quality

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Cooperators:

Richard Kim, Ph.D., Pace International. Dr. Kim designed and conducted experiments related to fruit quality during a commercial trial. He also reported results related to those experiments in Year 1.

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 are participating in packing plant studies to validate interventions to ensure that laboratory results relate to large scale production treatments.

Total Project Request: Year 1: 80,089 (funded) **Year 2:** 81,034 (funded) **Year 3:** 85,571 (funded) **Year 4:** None Requested

Other funding sources

Agency Name: Center for Produce Safety
Amt. requested: $199,784.60
Notes: A proposal requested funding to:

1) Evaluate the potential influence of wax applications and drying temperatures on microbial levels in laboratory and pilot line studies and

2) Evaluate a chlorine stabilizer to enhance apple dump tank chlorine applications in laboratory and commercial settings.

The proposal was not selected for funding.

WTFRC Collaborative expenses:

Item	2012	2013	2014	2015
Salaries				
Benefits				
Wages[1]	6,221	6,470	6,728	0
Benefits	1,555	1,617	1,682	0
RCA Room Rental				
Shipping				
Supplies				
Travel				
Plot Fees				
Miscellaneous				
Total	7,776	8,087	8,410	0

[1] Wages and benefits for assistance from WTFRC crew.

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

Item	2012	2013	2014	2015
Salaries[1]	44,856	38,051	41,324	0
Benefits	6,457	7,966	16,843	0
Wages		4,080	5,220	0
Benefits		82	506	0
Equipment		3,000	0	0
Supplies[2]	19,000	17,500	11,188	0
Travel[3]	2,000	2,268	2,080	0
Plot Fees				
Miscellaneous				
Total	72,313	72,947	77,161	0

Footnotes:
[1] Technical support and undergraduate students in Pullman.
[2] Fruit, chemicals, measurement devices, microbial supplies and analysis fees.
[3] Travel to Wenatchee and Yakima for commercial studies and fruit collection.

Objectives:

1) Evaluate the most effective microbial controls for HACCP systems individually and in combination using indicator organisms under commercial conditions
2) Identify and evaluate potential alternative approaches or novel compounds to enhance microbial control
3) Perform laboratory studies to examine pathogen and fungicide adherence to different apple varieties and examine the effects of storage over several harvest seasons
4) Conduct appropriate food safety extension and outreach with the apple packing industry

Significant Findings (Year 3 only; see previous reports for additional significant findings):

- Commercial scale PAA spray bar applications resulted in a >90% reduction of generic *E. coli* (1.4 to 1.5 $\log_{10}$ reduction) when 60-80ppm was directly applied to apples for at least 30 seconds with or without soap application.
- Data associated with commercial dump tank systems indicated that some facilities at best achieve microbial control in their post-harvest water systems while other facilities may at times during production (under low organic load conditions coupled with specific line design) achieve some microbial reduction on apple surfaces (90 – 99%).
- **The use of antimicrobials to ensure microbial control (limitation of cross-contamination) in any water tank or flume system is essential.**
- Several factors influence microbial control and the potential for microbial reduction in apple packing systems, including but not limited to:
    - Order of steps within a packing system (dump tanks, hyperwashes, spray bars)
    - Separation of dump tank (and associated water systems) from other flumes
    - antimicrobial concentration or activity (for chlorine, in association with pH)
    - organic load
    - water source or use of recycled water
    - water change schedules
    - agitation of the solution
    - cleaning and sanitation programs
- For measurement of chlorine activity using oxidation/reduction potential (ORP), factors for consideration are placement of the in-line probe and proximity to chlorine injection systems, frequency of calibration, and accounting for the level of accuracy of the instrument.
    - The ability to verify ORP probes using portable meters was a challenge in this study. Additional factors such as free chlorine (not total chlorine), organic load or other chemical parameters are likely needed to supplement verification activities in HACCP systems and could be used in combination with ORP values.
- Methods for examination of bacterial attachment to apple surfaces were optimized.

Methods:

Year 3, Objective 1: Data related to ORP, pH, turbidity, total suspended solids (TSS) and chemical oxygen demand (COD) were organized and evaluated from Years 1-2. To evaluate ORP probes used in industrial settings, several packinghouses were visited. ORP and pH readings from in-line probes and a portable meter were taken to compare readings.

A preliminary study was performed with a single facility to evaluate microbial distribution in packingline water systems and the potential for cross-contamination on apples. The purpose of this experiment was to understand the influence of specific packing steps on microbial samples; it should be noted that each packing facility is different so these results are limited to the specific packinghouse where the study was performed. Water samples and apples were examined at the four points in the pre-size operation and five points during final packing for a total of 649 apples. Water and apples were quantified for total coliforms and generic *E. coli* using 3M Petrifilm™. ORP and pH values were measured using a portable meter; the facility did not monitor ORP.

Year 3, Objective 3.

Methods for assessment of bacterial attachment and recovery were optimized as part of a project through the Western Center for Food Safety. Six preliminary experiments were conducted. Drying the bacterial inoculum under several conditions were compared.

A preliminary longitudinal study of Fuji apples from orchards in two regions was performed to examine levels of total coliforms and generic *E. coli* present on apples at harvest continuing through storage, see below. Orchards differed in water quality based on levels of generic *E. coli* in irrigation water used throughout the season. At harvest, samples were collected from fruit known to have been touched by irrigation water, and apples that had not been directly contacted with irrigation or overhead cooling water. Apples (approximately 600) were placed in refrigerated and controlled atmosphere storage. The controlled atmosphere storage included treatment with and without ozone.

Year 4, Objectives 1-4.

Data analysis from laboratory studies of bacterial attachment and recovery will be performed. Additionally, harvest data from Fuji apples harvested in two regions will also be performed. The Fuji apples stored in refrigerated storage for approximately three months will be examined for total coliform and generic *E. coli* levels. Additionally, Fuji apples stored in controlled atmosphere with and without ozone for approximately six and eight months will be examined for total coliform and generic *E. coli* levels. To examine the potential influence of factors associated with bacterial attachment to fruit, common maturity testing and disorder evaluation will be performed at harvest and after storage. Measurements of natural wax levels (Belding et al., 1998; Schreiber and Riederer, 1996) and lenticel size and density will be performed (Turketti et al., 2011). Depending on quality of the fruit upon removal from storage, examination of bacterial attachment will be performed.

Results and Discussion: *Year 3, Objective 1:*

Examination of chlorine, chlorine dioxide and peroxyacetic acid in low organic load dump tanks.

Industry partners identified that different points in a dump tank or flume system can vary in ORP readings. Portable meter readings from two points within the dump tank were used to calculate an average. The measurement range was also documented, taking into account the expected accuracy of the probe (±25mV). The averages and ranges for the portable meter and in-line probes were compared. Data related to ORP levels measured with in-line and portable meters indicated that in one replication for the low ORP treatment, the portable meter delivered readings that would not ensure control of cross-contamination in the water (minimum of 665 mV ORP) when the in-line meter indicated that microbial control was achieved (Table 1). Microbial results are reviewed in Figure 1.

Examination of high and low organic loads in a chlorinated dump tank, hyperwash with chlorine dioxide and chlorinated flume system.

In this study, the portable meter was used to take readings from multiple points within the dump tank and flume (3 points each) before and after each treatment, which were then used to calculate an average. Results indicated that oxidation-reduction potential meters (ORP) from in-line probes did not frequently align with ORP readings from a portable meter (Tables 2-3). The portable meter readings were typically lower than the in-line probe and frequently readings from the portable, and in-line probes

did not overlap when the accuracy of the probes were taken into account. Additionally, when a third probe similar to in-line probes was used, clarification in probe accuracy was not achieved (Table 3). Additional measures of organic load were also monitored, including turbidity, total suspended solids (TSS) and chemical oxygen demand (COD). **All of the parameters (turbidity, TSS or COD) clearly indicated the presence of increased organic load and would be useful to supplement observations related to water quality management.** Microbial results are reviewed in Figure 2.

Antimicrobial activity at different points in the packing system differed depending on organic load (Figure 2). For the low organic load treatments (fresh water with antimicrobials), apples treated in the chlorinated dump tank had significantly lower microbial levels (0.8 log reduction) compared to apples treated in the water control dump tank and a >90% reduction compared to the inoculated control apples. In the high organic load treatment, the dump tank chlorine application was similar to the reduction achieved in the dump tank water control treatment. However, a significant microbial reduction (almost a 99% reduction) was achieved for apples collected after the chlorine dioxide hyperwash and chlorine flume in the high organic load treatment. It should be noted that similar reductions were achieved in the high organic load treatment, but at different points in the packing system. Therefore, organic load in water tanks or flumes must be carefully monitored to ensure effective microbial control and to achieve microbial reduction.

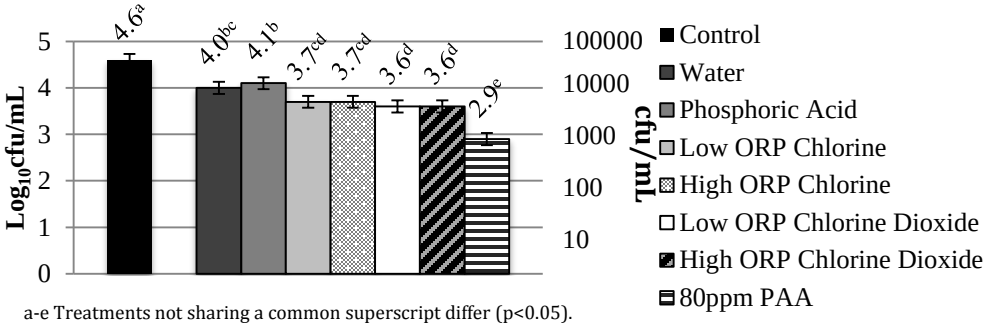
<u>Preliminary study of microbial distribution in packingline water system and the potential for cross-contamination on apples.</u>

Coordination with the packinghouse confirmed that the same grower lot would be packed in a pre-size and final packing line over a two day period. Water was evaluated for microbial and chemical parameters on the afternoon of day 1 and in the morning and afternoon of day 2 at the following points: dump tank (changed daily), transfer (schedule not available) and transition flumes (changed weekly ; changed the day prior to the first day of the study) and bin fillers (schedule not available; changed prior to day 2) in a pre-size system. Water from the dump tank of the final packing line was also examined. Apples were collected from bins prior to the pre-size line and after pre-sizing as well as at each point mentioned previously. On the final packing line, apples were collected prior to and after the dump tank, after soap and water spray bars, after fans and after waxing and drying.

In the pre-size dump tank system, water samples had no detectable total coliforms or generic *E. coli* (<10 cfu/ml). In the afternoon on both days, the distribution of apples with no detectable total coliforms increased after the dump tank; however, in the morning of day 2, the number of detectable total coliforms increased, more apples with 10 – 999 cfu/ml (1-2 log) total coliforms were observed.

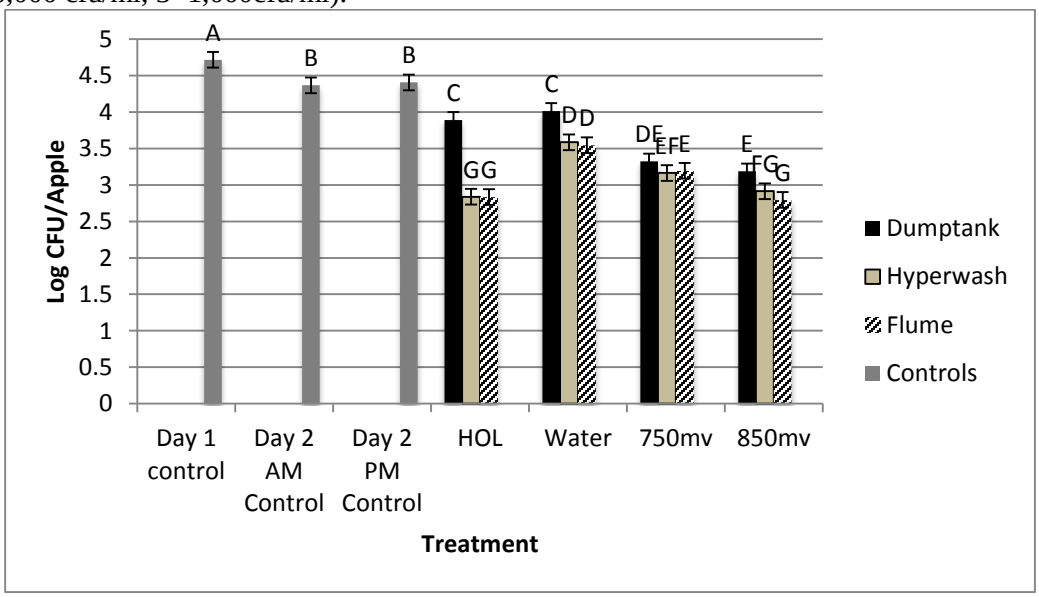
In the pre-size system beyond the dump tank, generic *E. coli* levels in the water were not detectable or were detectable at very low levels. However, total coliform levels indicated that microbial levels in the transfer and transition flumes increased between day 1 and day 2; a one log increase from 2 to 3 log cfu/ml in the transfer flume (450 to 1,952 cfu/ml). The bin fillers had consistent levels ranging from 117 – 230 cfu/ml (2 log). After the dump tank, shifts to greater levels of total coliforms on apples could be observed in the transfer and transition flumes as well as the bin filler. **The lack of an antimicrobial in the flume system and bin fillers appeared to contribute to an increased number of apples having a greater microbial load after pre-sizing.** On the morning of day 2, 18 apples had 100 – 999,999 cfu/apple (2-4 log cfu/apple). M**icrobial control must be maintained throughout the packing process; higher microbial levels on larger amounts of fruit could overwhelm current intervention strategies.** The final packing line included the following steps: dump tank, soap spray bar, hyperwash, water rinse spray bar, fans, wax application, dryer and packaging. On the final packing line, the step that appeared to cause the greatest shift in microbial reduction was drying.

Figure 1. Average generic *E. coli* levels on apples after inoculation and direct application of water, phosphoric acid (pH 3.5), chlorine (low and high ORP) and chlorine dioxide (low and high ORP) and peroxyacetic acid (80ppm) in a commercial study. Values reported in $\log_{10}$ colony forming units (cfu)/ml (5=100,000 cfu/ml, 3=1000cfu/ml).



a-e Treatments not sharing a common superscript differ (p<0.05).

Figure 2._Average generic *E. coli* levels on apples after inoculation and direct application of water at each collection point, or typical production levels of chlorine in the dump tank and chlorine dioxide in the hyperwash and chlorine (low and high ORP) in the flume system at low organic loads or high organic loads (HOL) in a commercial study. Values reported in $\log_{10}$ colony forming units (cfu)/ml (5=100,000 cfu/ml, 3=1,000cfu/ml).



A-G Treatments not sharing a common superscript differ (p<0.05)

Table 1. **Commercial packing house pH and ORP (mV) readings in a dump tank using portable pH/ORP meter and stationary pH/ORP meter for three replications of treatments: water, phosphoric acid, chlorine (low and high ORP) and chlorine dioxide (low and high ORP).**

Treatment	Rep	pH				ORP (mv)					
		Portable			Stationary	Portable				Stationary	
		Average Entry[bc]	*Average By Stationary*[c]	*Overall Portable Average*	*Average*[c]	*Average Entry*[bc]	*Average By Stationary*[c]	*Overall Portable Average*	*Range*	*Average*[c]	*Range*
Water	1	7.9	-	7.9	-						
	2	7.4	-	7.4	-						
	3	7.2	7.3	7.3	7.5						
Phosphoric Acid	1	3.2	3.4	3.3	-						
	2	3.2	3.2	3.2	3.3						
	3	3.4	3.4	3.4	3.1						
Low ORP Chlorine	1	6.8	7.1	7.0	6.9	701	704	702	677-727	751	726-776
	2	6.7	6.8	6.8	7.1	732	745	739	714-764	804	779-829
	3	6.9	6.9	6.9	7.0	**581**	**592**	**587**	**562-612**	**744**	**719-769**
Study Range		6.7-7.1				581-804					
High ORP Chlorine	1	6.9	7.1	7.0	6.9	794	802	798	773-823	840	815-865
	2	6.5	6.4	6.5	6.8	779	779	779	754-804	852	827-877
	3	6.7	6.7	6.7	6.8	748	739	743	718-768	845	820-870
Study Range		6.5-7.1				739-852					
Low ORP Chlorine Dioxide	1	3.3*	3.3*	3.3*	3.1*	741	743	742	717-767	757	732-782
	2	7.0	7.3	7.1	7.4	717	724	720	695-745	749	724-774
	3	6.6	6.6	6.6	6.7	692	689	691	666-716	727	702-752
Study Range		3.1*-7.4				691-757					
High OPR Chlorine Dioxide	1	3.0*	3.0*	3.0*	2.8*	790	794	792	767-817	816	791-841
	2	6.6	7.2	6.9	7.0	781	783	782	757-807	783	758-808
	3	5.8	5.8	5.8	5.7	733	732	733	708-758	769	747-794
Study Range		2.8*-7.2				732-816					

*Dump tank Acidified with phosphoric acid to lower pH ·Measurement not Taken [b]Apples entered the dump tank in this location

[c] Values are an average of ORP levels measured prior to apple treatment and after apple treatment

Table 2. **Commercial packing house ORP (mV) readings in a dump tank using portable pH/ORP meter and stationary pH/ORP Meter for four replications: water, low organic load chlorine in flume (low and high ORP) and high organic load.**

Dump Tank ORP (mv)								
Treatment	**Rep**	**Portable**					**Stationary Built-in**	
		Start of Dump Tank Average[a]	*1st Bend by probes Average*[a]	*Just before hyperwash incline Average*[a]	*Overall Average*[b]	Range[c]	*Average*[b]	Range[c]
Low Organic Load Target 930 mV	1	805.15	829.9	829.95	821.67	780-855	945.5	921-971
	2	818.65	918.8	835.45	857.63	794-944	935	910-960
	3	791.35	787.75	796	791.7	763-821	929.5	905-955
	4	794.4	803.2	802.05	799.88	769-828	930	905-955
Low Organic Load Target 930 mV	1	827.7	858	849.85	845.18	803-883	940	915-965
	2	848.95	862.3	860.5	857.25	824-887	933	908-958
	3	812.6	813.35	805.6	810.52	781-838	933.5	909-959
	4	819.05	827.25	832.75	826.35	794-858	929	904-954
High Organic Load Target 930 mV	1	839	829	832	833.33	807-864	879.5	873-923
	2	778	796.35	819.1	797.82	753-844	905.5	881-931
	3	792.1	800.2	804.95	799.08	767-830	913.5	889-939
	4	803.5	829.65	816.45	816.53	779-855	930	905-955

[a] Average of multiple measurements; data not shown
[b] Averages include all individual measurements used in previous calculations
[c] Ranges include all measurements used for calculations; the low range value uses the lowest individual reading minus instrument sensitivity, the high range value uses the highest individual reading plus instrument sensitivity

Table 3. **Commercial packing house ORP (mV) readings in a flume using portable pH/ORP meter and stationary pH/ORP Meter for four replications of treatments: water, low organic load chlorine in flume (low and high ORP) and high organic load normal production.**

Flume ORP (mv)										
Treatment	**Rep**	**Portable**					**Stationary**			
							Built-in		**Constant Read WSU**	
		Just after hyperwash[a]	*mid-flume*[a]	*End of flume*[a]	*Average*[b]	Range[c]	*Average*[b]	*Range*[c]	*Average*[b]	*Range*[c]
Low ORP Chlorine 750 mV	1	**656.7**	**651**	**640.5**	**649**	**599-702**	**752.5**	**713-792**	**688.5**	**548-829**
	2	**655.35**	**609.05**	**594.45**	**622.95**	**459-730**	**741**	**695-787**	**934.5**	**901-968**
	3	**667.2**	**666.2**	**649.2**	**660.87**	**596-709**	**755.5**	**722-789**	**902**	**873-881**
	4	**609.05**	**592.85**	**560.75**	**587.55**	**454-668**	**755.5**	**727-784**	**912**	**883-941**
High ORP Chlorine 850 mV	1	**818.55**	**823.4**	**830.25**	**824**	**785-863**	**241**	**195-287**	**240**	**215-265**
	2	814.6	815.6	808.4	812.87	768-849	855	829-881	902.5	877-928
	3	**721.85**	**796.2**	**797**	**771.68**	**637-827**	**848.5**	**819-878**	**814.5**	**774-855**
	4	786.5	782.5	775.25	781.42	743-819	851.5	826-877	879.5	850-909
High Organic Load 860 mV	1	780	787	789.5	785.5	755-815	862.5	833-892	834	809-859
	2	**835.4**	**747.28**	**861.55**	**814.74**	**612-893**	**885.5**	**857-914**	**813**	**785-841**
	3	820.15	819.7	821.55	820.47	784-856	872	845-899	870.5	829-912
	4	791.8	797.2	791.4	793.47	746-836	860.5	830-891	838.5	809-868

[a] Average of multiple measurements; data not shown
[b] Averages include all individual measurements used in previous calculations
[c] Ranges include all measurements used for average calculations; the low range value uses the lowest individual reading minus instrument sensitivity, and the high range value uses the highest individual reading plus instrument sensitivity

FINAL PROJECT REPORT

Project Title: Apple microbial risk factors

PI:	Richard Pleus	**Co-PI (2):**	Gretchen Bruce
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Cooperators: Intertox Decision Sciences, LLC

Other funding sources

Agency Name: The Center for Produce Safety
Amt. awarded: $66,807
Notes:

Total Project Funding: $133,614

Budget History:

Item	2012	2013	2014
Salaries	17,698.62	20,370.60	
Benefits	8,141.38	9,667.44	
Wages			
Benefits			
Subcontract		1,850.00	
Shipping			
Supplies	254.45		
Travel	899.51		425.00
Expert Panel		7,500.00	
Miscellaneous			
Total	$26,993.96	$39,388.04	$425

Footnotes: The 2013 funded amount of $39,388.04 included a 2012 budget carryover of $9,129. Of the expended funds, $1,850 was spent on subcontractor data collection and analysis work, $7,500 was invoiced for Expert Panel member payments and the remainder was spent on salary and benefits. 2014 funds requested were $425 for travel to present the research results at the Northwest Horticultural Council Meeting in March 2014 and at the Center for Produce Safety Symposium in June 2014.

OBJECTIVES

This project had five objectives. As of December 31, 2014, all five objectives have been completed. The final objective was completed in January 2014. A review of the objectives and a discussion of the major findings are provided below.

1. **Gather pathogen testing data and information about mitigation measures from apple growers.**

   In early 2013, an industry survey was conducted that focused on food safety practices growers use to protect against microbial risks. Sixty-eight companies completed surveys. In the survey, growers were asked to identify their water sources, irrigation types and evaporative cooling details. For food safety practices, growers were asked to describe their sanitation and maintenance procedures and worker training. Finally, growers were asked about microbial testing types and frequencies.

2. **Correlate pathogen levels in water used in fresh market apple production and packing operations at different points in the system to levels measured on apples before they leave the packinghouse.**

   In addition to the water and microbial test data collected from testing laboratories, IDS collected packing line data consisting of pH, ORP, chlorine and temperature readings for various points along individual packing lines. Efforts were made to correlate the available data with environmental and product tests results.

3. **Characterize potential exposure to pathogens from consumption of fresh market apples and describe potential human health effects associated with these exposure levels; combine the results to estimate the risk of becoming ill from eating contaminated apples.**

   The exposure assessment was completed in 2013. The exposure assessment was combined with the hazard characterization in an apple-specific quantitative microbial risk assessment (QMRA) model and FDA-iRISK modeling tool to provide risk estimates for illness from eating contaminated apples.

4. **Prepare a written risk assessment report about the findings of Objectives 1-3.**

   A written risk assessment was completed in December 2013.

5. **Submit the risk assessment model and report for review to an advisory panel consisting of the WTFRC, the Northwest Horticultural Council and other experts in the field of quantitative microbiological risk assessment.**

   In December 2013, the risk assessment model and report were submitted to representatives from the WTFRC and the Northwest Horticultural Council (NHC) for review. After completion of the WTFRC and the NHC review, the risk assessment was submitted to an expert panel with whom a call was held in January 2014.

SIGNIFICANT FINDINGS

- No foodborne illness outbreaks associated with whole fresh apples were identified in either the CDC's FOOD database or in further Internet research.
- Research studies relating pathogens to apples were identified for *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* (*L. monocytogenes*). However, *Salmonella* and *L. monocytogenes* contamination risk assessments were ruled out at this time due to a lack of data and research related to the behavior of these pathogens on apples.
- In both the Intertox-developed risk assessment model and the FDA-iRISK® model, the risk of illness from *E. coli* O157:H7 is negligible for a given orchard treated with evaporative cooling water at *E. coli* levels found in Washington.

RESULTS & DISCUSSION

The major finding from this study is that the risk of *E. coli* O157:H7-related illness from the consumption of Washington apples following a water-related contamination event in the orchard (associated with evaporative cooling) is negligible. This conclusion was drawn after modeling Washington apple production and packing processes using available literature study data along with data collected from packinghouses and orchards. For the QMRA, a model was developed using Microsoft Excel and Palisade @Risk software to estimate potential exposure levels to these pathogens and the risk of becoming ill from eating contaminated fresh market apples. The model is based on potential contamination and growth/reduction of pathogens occurring in the field, during cooling, storage and packing. The model estimates exposures for adults children, pregnant women and the elderly using national fresh apple consumption rates, and incorporates probabilistic methods to characterize the uncertainty and variability in model inputs as well as outputs (e.g., where possible, input parameters such as temperature, time, etc., are included). Risks are characterized as the probability illness along with the total estimated number of cases. In addition to using the Intertox model, the risk of illness was also assessed using the FDA-iRISK® modeling tool in the same manner as the FDA used this tool to assess the risk of illness from consuming contaminated leafy greens. As in the Intertox model, the FDA-iRISK® model characterized the probability of illness along with the total estimated number of cases. While results from both models are comparable, the Intertox model included parameters that had greater specificity for apples.

As part of the QMRA process, data limitations and needs for further research were identified. In several cases, assumptions regarding pathogen growth and decrease were drawn from studies that may not reflect conditions consistent with commercial packing, (e.g., sanitizer concentrations, available nutrients, characteristics of the apple variety). Studies estimating pathogen growth and decrease in fresh market apples are also extremely limited. Even with the data limitations, however, this study is of value. The QMRA provides a baseline estimate of risk for the industry and indicates where further research efforts are needed.

EXECUTIVE SUMMARY

A quantitative microbial risk assessment (QMRA) model was developed using data from industry and scientific research in order to estimate risk of illness from the consumption of fresh market apples. The model can be used by the industry to predict apple pathogen levels from the orchard through departure from the packinghouse, using various contamination scenarios. The model currently provides an initial risk estimate for consumption of apples that have been contaminated by pathogenic *Escherichia coli* (*E. coli*) in evaporative cooling water applied to apples in the orchard prior to harvest. The QMRA model estimates the potential for change in apple pathogen levels during various stages of primary production and packing using relevant orchard, storage, and packing facility parameters. Based on the results, preventive measures should be in place during production to minimize the potential for apple contamination.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-14-101A

Project Title: Assessment of overhead cooling practices for apple food safety

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Cooperators: This study involves partnerships with the WSU Wenatchee Tree Fruit Research and Extension Center and WSU Prosser Research and Extension Center for field studies, as well as industry partners and input from regulatory personnel.

Total Project Request: Year 1: $92,363 **Year 2: 97,887 Year 3:** $104,183

Other funding sources

Agency Name: Western Center for Food Safety
Amt. requested/awarded: $80, 768 (requested) / $80,768 (awarded)
Notes: The Western Center for Food Safety, an FDA Center of Excellence, provided funding for validation of field experimental methods and selection of appropriate surrogate organisms. Dr. Killinger attended meeting with scientists funded by the Western Center for Food Safety to discuss methods used in field experiments in order to better align methods between investigators nationally and discuss future strategies for research.

Agency Name: Washington Specialty Crop Block Grant
Amt. requested/awarded: $45,304 (awarded)
Notes: Funding from a Washington Specialty Crop Block Grant related to irrigation water treatment would provide funds for additional testing and allow for examination of irrigation water treatment in a second orchard location.

WTFRC Collaborative expenses:

Item	2014	2015	2016
Wages	6,400	15,000	16,000
Benefits	1,600	2,000	2,500
Total	8,000	**17,000**	18,500

Footnotes: Wages and benefits for assistance from WTFRC staff.

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

Item	2014	2015	2016
Salaries		41,615	48,961
Benefits		16,682	20,156
Wages	5,800	3,000	1,500
Benefits	563	66	33
Equipment	65,000	8,000	
Supplies	10,000	10,000	12,000
Travel	3,000	1,524	3,033
Plot Fees			
Miscellaneous			
Total	84,363	**80,887**	85,683

Footnotes: Footnotes:
[1] Technical support and undergraduate students in Pullman.
[2] Equipment for field and laboratory experiments.
[3] Fruit, chemicals, measurement devices, microbial supplies and analysis/management fees.
[4] Travel to central Washington for inoculation studies and fruit collection.

OBJECTIVES

1) Investigate foodborne pathogen and surrogate survival in laboratory studies and develop inoculation methods for field experiments

2) Examine non-pathogenic surrogate survival in field studies to understand potential risks associated with standard overhead cooling water application practices

SIGNIFICANT FINDINGS

- Preliminary data suggests that generic *E. coli* is reduced at rates greater than or equivalent to the proposed 0.5 log per day reduction proposed by FDA for mitigation of water that exceeds the proposed standards for geometric mean and statistical threshold value in a water quality profile.
    - For Gala and Golden Delicious apples that were not treated with overhead evaporative cooling water, a 99% reduction was observed within the first 8 hours after a simulated contamination event (in the absence of sunlight) followed by reductions averaging 0.8 log (less than 90%) over the next two days.
- Field inoculation methods and staff training programs were developed to generate a consistent method for application of generic *E. coli* surrogates in an orchard setting.
- Appropriate non-pathogenic, surrogate isolates with rifampicin (antibiotic) resistance for identification and isolation from background flora were identified and acquired. Rifampicin resistance to regionally acquired *Salmonella* spp. and *E. coli* O157:H7 was developed for growth curve analysis. Growth curve methods were optimized.

METHODS

Year 1. Objective 1. Strain Development and Acquisition. For field experiments, it was identified that non-pathogenic, surrogate isolates would need to be marked for identification and isolation from background flora. Most strains are marked through resistance to an antibiotic or a florescent protein. Surrogate strains resistant to rifampicin were obtained from collaborators (TVS 353, TVS 354, TVS 355, LJH 1238). These isolates have been utilized in other studies associated with microbial survival on produce and are likely to be acknowledged as acceptable by regulatory and academic reviewers.

There is a need to compare the survival of surrogate strains to pathogenic isolates from the region in laboratory studies, based on input from scientists associated with the Center for Produce Safety and the Western Center for Food Safety. Therefore, pathogen isolates from regional studies performed by the Killinger laboratory were selected for development of resistance to rifampicin. Isolates were streaked onto tryptic soy agar (TSA) supplemented with rifampicin at increasing concentrations from 10µl/ml until resistance to 120µl/ml was achieved. Rifampicin resistance was developed for three *E. coli* O157:H7 and four *Salmonella* isolates that were collected from irrigation water sources in central Washington by the Killinger laboratory. Additionally, rifampicin resistance was developed for three non-pathogenic, surrogate *E. coli* isolates used in previous studies by the Killinger laboratory and other scientists performing research on apples.

Growth Curve Method Development. To compare the bacterial isolates based on growth characteristics, methods needed to be standardized. A collaborator was identified with access to a SPETRAmax Plus 384 spectrophotometer. The first series of experiments focused on identifying the appropriate media for experiments (buffered peptone water versus tryptic soy broth). Buffered peptone water was selected, and the next series of experiments focused on identifying the appropriate concentration of bacterial inoculum and appropriate methods for inoculation to deliver consistent results. Appropriately diluted cultures were added to a sterile 96-well polystyrene microtiter plate and placed in the SPETRAmax Plus 384 spectrophotometer with incubation at 37ºC for 35h for healthy growth curve analysis. An optical density (OD_{600}) reading was taken every 15 minutes throughout incubation. The data were retrieved using SPETRAmax Plus 384 software provided by the manufacturer and analyzed using Microsoft Excel 2010.

Year 1. Objective 2. Field Examination of Non-pathogenic Surrogate Survival.

Field study locations in the Wenatchee and Prosser Research and Experiment Stations were identified; only the Wenatchee location had overhead cooling sprinklers. Therefore, all inoculation methods and experiments were conducted at the Wenatchee location in Year 1. The water sources for both orchards were evaluated for bacterial water quality through another study funded by the Washington Tree Fruit Research Commission (AP-12-108).

For method development, numerous variables needed to be considered. Strain preparation has been demonstrated to influence bacterial survival; therefore, surrogate isolates were prepared in a way to generate cells that would be hardy under field conditions. The individual strains were then combined to create a cocktail for transport to the field location. Additional preparation in the field was necessary to create sufficient inoculum amounts (approximately 9.6L per batch). Each inoculum batch was prepared in a backpack sprayer at the orchard within 30 minutes of inoculating apples. A sample was collected to confirm the concentration of the initial inoculum.

To ensure apples were inoculated as consistently as possible, four preliminary experiments were performed to optimize methods and ensure worker safety during inoculation. The inoculation process involves an individual navigating a ladder with the backpack sprayer of inoculum and spraying each individual apple on the tree as consistently as possible. Input from scientists associated with the Western Center for Food Safety indicated that performing the inoculation after sunset was likely to present the highest risk for bacterial attachment. To perform the experiments in the dark, a team of "spotters" were needed to illuminate the trees for the individual spraying the apples. The sprayers were equipped with a backpack sprayer, gloves, face mask and face shield, and the spotters were equipped with face masks, flashlights and gloves. This technique required a significant amount of teamwork and communication, which could only be achieved through adequate training.

Gala variety apples were picked at 0, 8, 24, 32 (1.3 days), 56 (2.3 days), and 168 (7.3 days) hours after inoculation. Golden Delicious variety apples were picked at 0, 8, 24, 32 (1.3 days), 56 (2.3 days), and 168 (7.3 days), 336 (14.3 days), and 504 (21.3 days) hours after inoculation. The apple harvest time points were selected to reflect industry harvesting practices (i.e. start of picking at dawn). Immediately after an entire tree was inoculated, apples were picked and placed into individual bags for enumeration of the initial inoculation level (time 0). The remaining apples were picked,

bagged, and transported at specific time points depending on the apple variety and availability. All apple samples were transported on ice to the laboratory for processing.

For all time points, the inoculated apples were examined by adding 10ml of 0.1% peptone water. The apples were rubbed for 1 minute, shaken for 30 seconds, and rubbed again for 1 minute to remove attached bacteria. Media for enumeration involved Chromagar ECC with rifampicin. Enrichment in tryptic soy broth (TSB) followed by plating on Chromagar ECC with rifampicin and filtering prior to plating were also used for samples expected to have lower microbial counts. Control apples of the same varieties were examined for total coliforms and generic *E. coli* as well as pathogenic *E. coli* and *Salmonella*.

Years 2-3. Objectives 1-2.

Laboratory and field experiments will be performed using methods developed in Year 1. Laboratory experiments will be require new arrangements for access to a SPETRAmax Plus 384 spectrophotometer. Growth curve analysis of regionally relevant rifampicin resistant pathogens for comparison to surrogates used in the field would strengthen the study. For field experiments in Wenatchee, an orchard with a replicated 4 block design will be assessed throughout the season to determine the breadth of study (number of varieties, number of fruit per treatment, etc.). Investigators will determine if overhead, evaporative cooling can be installed in the Prosser location for assessment of Fuji apples. An option exists to include irrigation water treatment through funding from a Washington Specialty Crop Block grant. Assessment of water sources will be performed at least one month prior to harvest and if funds permit, additional samples will be collected and parameters such as turbidity will be examined. Statistical analysis of year 1 data will be performed.

RESULTS AND DISCUSSION

Year 1, Objective 1. Development of Field Inoculation Methods.

Through the four preliminary experiments, it was determined that team members should be assigned to specific roles. Preliminary data was collected to evaluate and identify which team members were the most adept at delivering consistent microbial levels. Preliminary experiments also examined the potential for compositing samples; preliminary results and availability of fruit influenced the decision to sample individual apples.

Several considerations were discussed for timing the initial inoculation and sample collection time points; adjustments were required based on orchard conditions. The team concluded that the appropriate time for inoculation required a balance of several considerations including fruit maturity and projected harvest date, ambient temperature and other weather conditions. *Consensus from conversations with industry representatives indicated the minimum amount of time between the last overhead cooling event and harvest was 12 hours; some reviewers would view this minimum time as the highest risk for food safety.* The time points for collecting apples were selected to reflect industry harvesting practices rather than time after the inoculation event in most cases; therefore, most sampling events aligned with initial harvest time (approximately 6:00am). Gala apples were

harvested at 6 time points (9 originally planned). Golden Delicious harvested at 8 time points (12 originally planned).

Fruit counts in the orchard were lower than expected, so the experimental design of the study had to be adjusted. It was decided to designate high and low canopy treatments within each tree; a middle zone was originally planned. Fruit from individual trees were counted. In many cases, individual trees could not serve as an experimental unit, due to an inadequate number of apples; groups of trees were identified to serve as experimental units in the orchard blocks. For Gala apples, 18 apples were harvested per time point (approximately 9 each from the high and low canopy zones), and for Golden Delicious, 28 apples were harvested from per time point (approximately 14 from the high and low canopy zones).

Statistical analysis has not been performed. It is uncertain if data is sufficient at this time to draw conclusions. Therefore, all results should be considered preliminary and additional years of data collection are needed to verify preliminary results.

For Gala and Golden Delicious apples that did not receive overhead evaporative cooling (untreated), microbial reduction was fairly similar between varieties from time 0 up to 1 week after inoculation (Figure 1). Eight hours after the simulated contamination event (i.e. contaminated water application near sunset with picking at dawn), generic *E. coli* were reduced approximately 2.5 log cfu/apple (over 99%). For the next two days, generic *E. coli* were reduced over 0.5 log cfu/apple each day. It should be noted that microbial die-off tapered, with a 1.6 log reduction between days 2-7 (over 90% reduction). Apples that required pre-enrichment for detection of stressed cells are not reported for the preliminary analysis. Further statistical analysis will provide more accuracy, and pre-enriched samples will be included in this analysis. Control apples (20 per variety) were tested; only one Gala apple had detectable levels of generic *E. coli*, approximately 353 cfu/apple (2 log cfu/apple).

For Gala and Golden Delicious apples that received overhead, evaporative cooling (treated), results will need to be carefully compared to additional replications in years 2-3. A shortage of water for irrigation and overhead, evaporative cooling throughout the season may have impacted these results; comparison with additional years is needed. For the Gala apples treated with overhead evaporative cooling, reductions in generic *E. coli* appeared to be equivalent or slightly greater than the untreated Gala apples from 8 hours up to 1.3 days (Figure 1). This observation was more pronounced for Golden Delicious. Preliminary data suggests that generic *E. coli* was reduced at rates greater than or equivalent to the proposed 0.5 log per day reduction proposed by FDA for mitigation of water that exceeds the proposed standards for a water quality profile in the proposed produce FSMA standards (for up to approximately 14 days based on preliminary Golden Delicious data).

The averages for Gala apples collected from the low and high canopy zones are provided in Figure 2. Figure 3 provides the range in microbial values from each time point for untreated and treated Gala and Golden Delicious apples. It is important to note that the range recovered from apples at each time point varied dramatically. For untreated Golden Delicious apples, three weeks after a simulated contamination event, 14 apples had over 1,000 generic *E. coli* present on their surface (Figure 4).

Figure 1. Overall average of generic *E. coli* levels on Gala and Golden Delicious apples with (treated) and without (untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Values reported in $\log_{10}$ colony forming units (cfu)/apple (5=100,000 cfu/apple, 3=1,000cfu/apple).

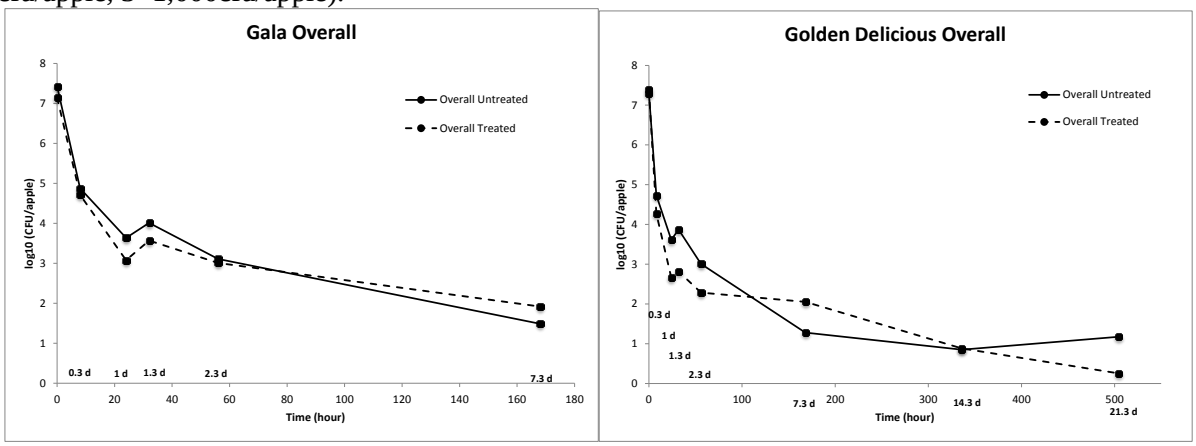


Figure 2. Overall average of generic *E. coli* levels on Gala apples with (treated) and without (untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Averages for apples collected in high and low canopy zones for each treatment also included. Values reported in $\log_{10}$ colony forming units (cfu)/ apple (5=100,000 cfu/apple, 3=1,000cfu/apple).

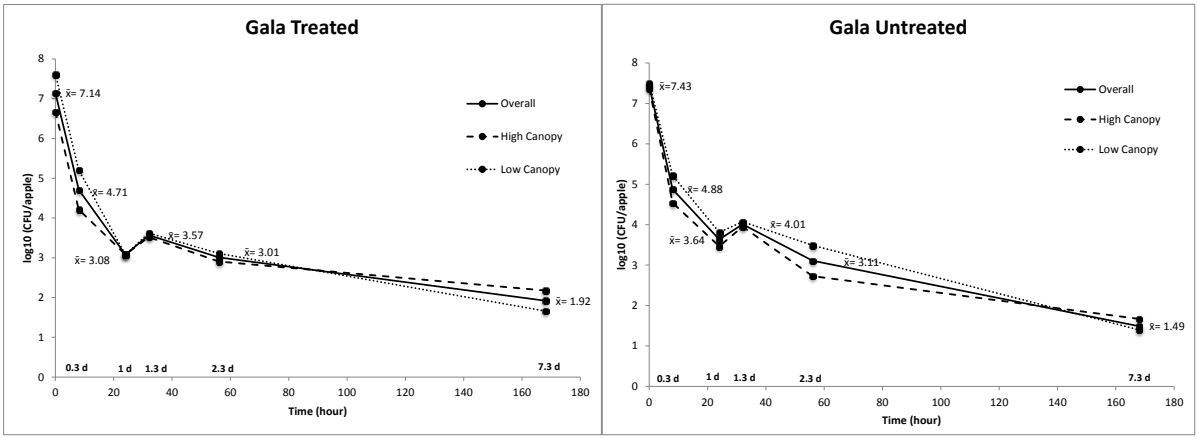


Figure 3. Overall average of generic *E. coli* levels on Gala apples with (treated) and without (untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Data points for individual apples also included. Values reported in $\log_{10}$ colony forming units (cfu)/apple (5=100,000 cfu/apple, 3=1,000cfu/apple).

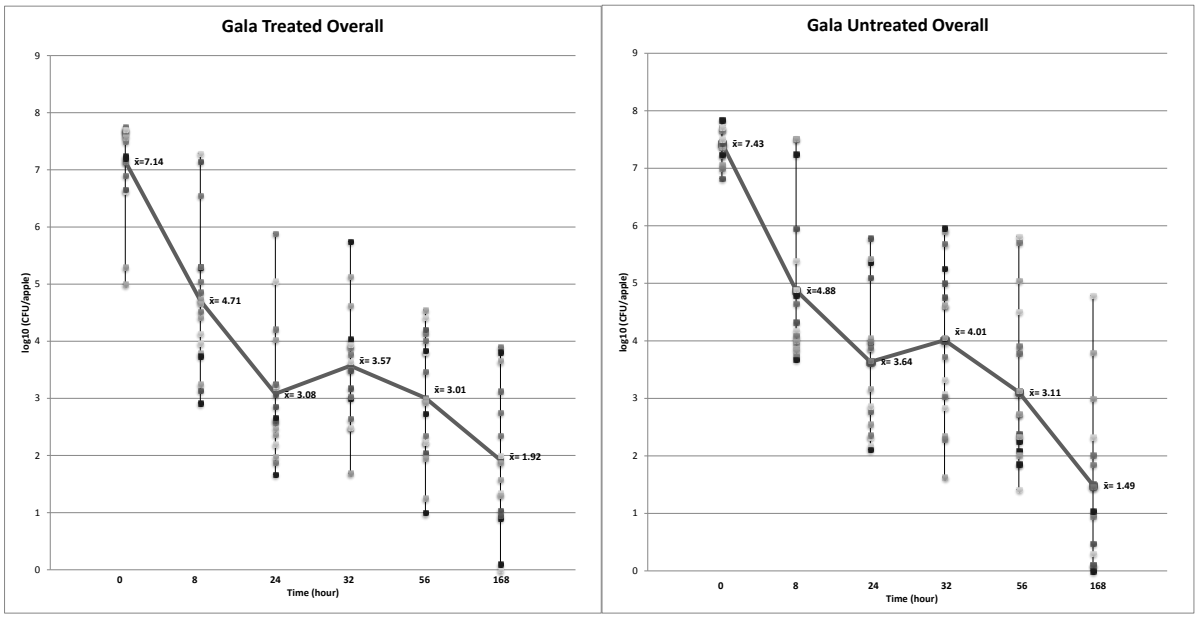
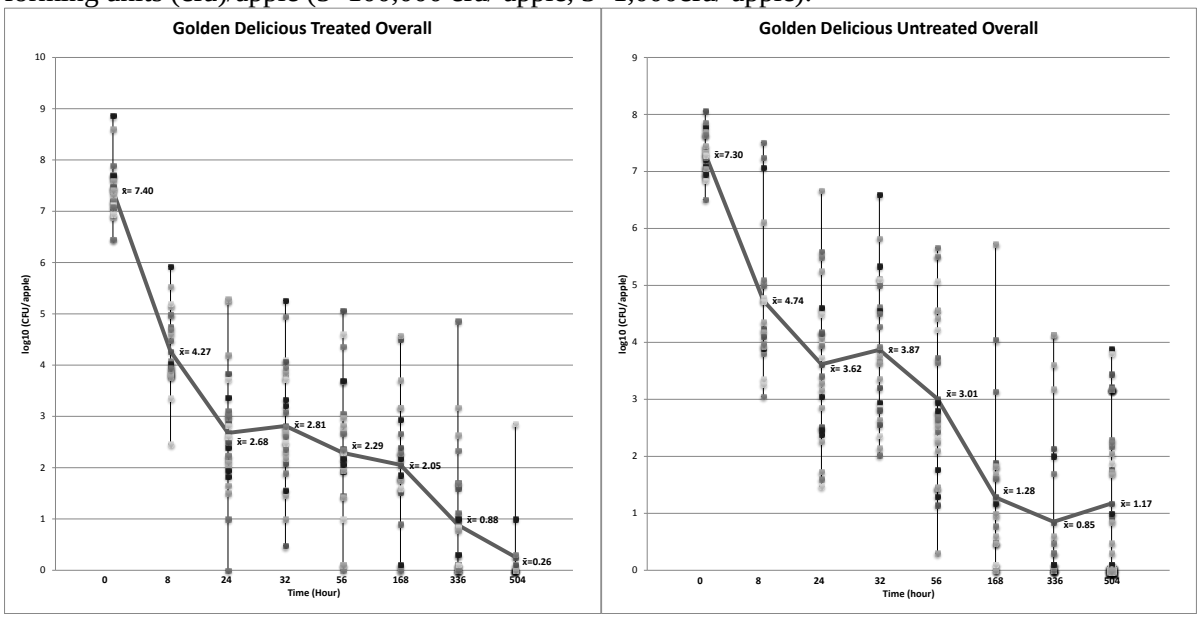


Figure 4. Overall average of generic *E. coli* levels on Golden Delicious apples with (treated) and without (untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Data points for individual apples also included. Values reported in $\log_{10}$ colony forming units (cfu)/apple (5=100,000 cfu/ apple, 3=1,000cfu/ apple).



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

Project Title: Analysis of packinghouse preventive controls for dump tank water

PI: Diane Wetherington **Co-PI (2):** Susan Leaman
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Cooperators: Joan Rosen, Ph.D., JC Rosen Resources

Total Project Request: **Year 1:** $47,000 **Year 2: $47,000**

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1 $94,000
Organization Name: Intertox Decision Sciences **Contract Administrator:** Eileen McCormack
Telephone: 206-257-3589 **Email address:** diane@idecisionsciences.com

Item	2014	2015	
Salaries	28,000	28,000	
Benefits	5,600	5,600	
Wages			
Benefits			
Equipment			
Supplies			
Travel	1,400	1,400	
Miscellaneous	2,000	2,000	
Contractors	10,000	10,000	
Total	47,000	**47,000**	

OBJECTIVES

1. **Collect industry data: As part of their previous work, IDS has collected individual company water, environmental and product test data from third-party laboratories for apples, pears and cherries. In addition IDS has worked with individual companies to obtain and analyze actual packing line monitoring data. In 2014 IDS proposes extending the data collection efforts to other packinghouses.**

   Washington tree fruit packing companies follow HACCP-type programs to safeguard food quality and provide safe tree fruit to consumers. In accordance with project objectives to improve HACCP systems for tree fruit packing facilities, an analysis of different control points and operations was proposed, with a focus on the first critical step, the dump tank. Data analysis correlating dump tank water parameters to microbial concentration was planned in order to understand the dynamic changes of dump tank water quality during tree fruit packing operations and its effect on microbial populations.

   To maintain dump tank water quality, WA tree fruit packing companies add antimicrobial chemicals or sanitizers to the water, and monitor the water by measuring the sanitizer level and taking oxidation reduction potential (ORP) readings. In addition, companies test their dump tank water on a routine basis (e.g., quarterly) for microbial load. Yet few companies analyze these data sets for correlation to assess whether the preventive controls being administered (i.e., sanitizer use) is achieving the desired result (i.e., control of dump tank water microbial load). IDS intent was to use existing data compiled in previous work to look for correlations between the ORP readings/sanitizer concentrations and the microbial load. HACCP and dump tank water testing data from previous years were voluntarily provided to IDS by individual companies. Data was then analyzed using a regression analysis in order to identify trends. However, the individual company microbial load data was inadequate to significantly correlate dump tank water monitoring parameters to microbial levels measured in the same system due to the lack of microbial testing and presence. Due to inadequate data, the methods to collect industry data have changed and are explained in the Methods section below.

   To date, IDS targeted 10 packing companies for participation in the project. Four companies are participating in the program; however, scheduling and line changes limited their availability in 2014. In early December, IDS met with two of these packing facilities (Facilities 1 and 2) to lay out a sampling plan, identify the packing line(s) areas where sampling will occur, and assess and chart the dump tank area to prepare for sampling. In addition, IDS conducted a literature search and identified similar studies validating dump tank water preventive controls for other fresh produce commodities. Based on the site visits and the literature review, IDS prepared a sampling plan that is under review by our collaborator, Dr. Joan Rosen. A questionnaire for project participants was developed to collect relevant information prior to sampling. The sampling schedule for 2015 is based on seasonal activities and commodities. Sampling is scheduled to begin at Facilities 1 and 2 in mid-January.

2. **Data Analysis: Correlate dump tank water monitoring parameters (e.g. temperatures, pH, ORP readings, sanitizer levels and exposure by fruit and variety) to microbial levels. To the extent possible, examine how various sanitizers perform given the fruit and environmental conditions. Examine critical control points, how they were determined, and how frequently they are exceeded.**

   Data analysis will begin in February 2015 and continue throughout the year following each data collection event at participating facilities.

3. **Review HACCP plans: Based on the data analysis, develop a dump tank decision tree for packinghouse use. Recommend appropriate parameters for dump tank water in tree fruit packinghouse HACCP plans.**

   Per our proposal, IDS has engaged Dr. Joan Rosen as a food safety expert to assist with this objective. Work on Objective 3 will commence in the last quarter of 2015 following collection and analysis of the data.

SIGNIFICANT FINDINGS

- Existing individual company data is inadequate to assess the effectiveness of dump tank water preventive controls.

METHODS

1. Collect industry data:

   The following parameters will be used to correlate dump tank water monitoring data to water microbial levels:

   - Conductivity – measures the ability of water to pass an electrical current; it is affected by the presence of inorganic dissolved solids such as cations and anions.
   - Oxidative Reduction Potential (ORP) – measures disinfectant activity
   - Turbidity – measures water clarity – the amount of light scattered by suspended particles; a proxy for organic pollutants which in turn reduce sanitizer efficacy
   - pH and temperature – affect bacterial growth and survival
   - Sanitizer level - Free chlorine or peracetic acid levels

   In lieu of using existing company data, IDS purchased equipment and will take direct measurements at companies that have agreed to participate in the project. Prior to purchasing equipment, IDS consulted with Dr. Trevor Suslow regarding equipment he used to conduct a similar project at three tomato packing facilities (Tomas-Callejas et al., 2012). IDS will also collect water samples for microbial analysis to be performed by a contracting third-party laboratory. Water sampling kits will be supplied by our contracting laboratory. Sampling points, volumes, and time intervals have been ascertained based on conversation with other researchers and published studies (Lopez-Velasco et al., 2012; Tomas-Callejas, 2012; Zhou et al., 2014). With the exception of microbial load (total coliform and generic *E. coli*), all samples will be processed at the time of sampling.

2. Data Analysis: Correlate dump tank water monitoring parameters outlined above to microbial levels measured in the same system. To the extent possible, examine how various sanitizers perform given the fruit and environmental conditions. Examine critical control points, how they were determined, and how frequently they are exceeded.

   Water quality is a critical factor when examining the risk of contamination in fresh-pack apples. In apple packing operations, dump tank water is a critical control point where microbial levels are controlled with use of sanitizers that are typically monitored by ORP measurements. If enough data is available, we propose analyzing how various sanitizers perform under varying conditions. We will examine critical control point exceedances and attempt to identify the root cause. We will use regression analysis to test for correlations between microbial levels measured in dump tank water and ORP readings and sanitizer levels, temperature and pH readings, to evaluate sanitizers and to examine critical control point exceedances as the data permits.

3. Review HACCP/HARPC plans: In the Preventive Controls Rule, the FDA proposed a new requirement, HARPC plans, for most food facilities including fresh produce facilities where water comes in contact with produce that is being packed. Similar to HACCP plans, HARPC requires preventive controls to be implemented and monitored to address any hazards identified in the system. Based on the correlation between wash water system parameters and microbial levels, appropriate parameters for dump tank water will be recommended for sanitizing agents commonly used in tree fruit packing facilities. These parameters would be made available as guidance for use by the industry in their operation-specific HACCP/HARPC plans. Also for use in operation-specific HACCP/HARPC plans, IDS will prepare a decision tree based on the research findings.

RESULTS & DISCUSSION

Using data provided to us by participating companies, it was not possible to validate the preventive controls that these companies used in their dump tank systems. Analysis of existing individual company data revealed that the amount of microbial data was inadequate to correlate it to ORP monitoring data.

Searches of the scientific literature identified recent studies conducted to validate preventive controls for dump tank water for the tomato packing industry. These types of studies are critical as the tree fruit industry prepares to meet the preventive control validation requirements of HARPC following finalization of the proposed Preventive Controls Rule.

REFERENCES

Lopez-Velasco G, Tomas-Callejas A, Sbodio A, Artes-Hernandez F, Suslow TV. 2012. Chlorine dioxide dose, water quality and temperature affect the oxidative status of tomato processing water and its ability to inactivate *Salmonella*. *Food Control*, 26:28-35.

Tomás-Callejas A, López-Velasco G, Valadez AM, Sbodio A, Artés-Hernández F, Danyluk MD, Suslow TV. 2012. Evaluation of current operating standards for chlorine dioxide in disinfection of dump tank and flume for fresh tomatoes. Journal of Food Protection, 75(2):304-13.

Zhou B, Luo Y, Turner ER, Wang Q, Schneider KR. 2014. Evaluation of current industry practices for maintaining tomato dump tank water quality during packinghouse operations. *Journal of Food Processing and Preservation*, 38(6):2201-2208.

CONTINUING PROJECT REPORT **YEAR:** 2014
WTFRC Project Number: N/A

Project Title: Programs to increase packouts of apples

PI: Ines Hanrahan
Organization: Washington Tree Fruit Research Commission
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S 18th St. Suite 100
City/State/Zip: Union Gap, WA, 98903

Cooperators:
WTFRC internal program: Manoella Mendoza, Tory Schmidt, Udel Mendoza, Felipe Castillo
Scientists: James Mattheis, Dave Rudell, Jaqueline Gordon
Product suppliers: Valent Biosciences, Extenday
Others: Grower collaborators, WTFRC seasonal crew and interns, Glade Brosi (Stemilt)

Other funding sources
All supplies and chemicals were donated by industry suppliers.

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

Item	2013	2014	2015
Salaries	9,000	7,120	10,000
Wages	8,336	12,351	10,000
Equipment + supplies	0		
RCA rental	1,800	1,800	1,800
Travel	0		
Total gross costs	19,136		
Reimbursements	(8,000)		
Total net costs	**11,136**	**21,271**	**21,800**

Footnotes:
Entire budget is based on fiscal year August 1st– July 31st the following year, i.e. 2014 reflects costs from Aug.1, 2013 until July 31st, 2014.
Salaries: incl. benefits, proportional time spent on outlined projects for Hanrahan, Castillo, Schmidt
Wages: incl. benefits, covers time slip expenses, benefit rate at 42%
Supplies: experimental fruit, storage boxes and trays donated
RCA rental: numbers based on fiscal year (@ approx. $6,300/room/year)
Reimbursements: monetary contributions by chemical suppliers

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

OBJECTIVES

1. Determine harvest maturity and storage behavior of red strains of Honeycrisp.
2. Investigate practical use parameters for the DA meter in Honeycrisp orchards. Determine if the I_{AD} index correlates to common maturity parameters. (NEW)*
3. Document Honeycrisp fruit quality in local store displays. (NEW)
4. Determine an easy and cost effective way to measure titratable acidity. (NEW)
5. Expand collaborative efforts with other research programs working on fruit quality management.

*seasonal adjustment of objectives based on industry feedback and Hanrahan program capacity.

SIGNIFICANT FINDINGS

Objective 1: Two red Honeycrisp strains retained quality well and did not exhibit chilling disorders or bitterpit regardless of preharvest ethylene pathway inhibitor use after long term storage. Final eating quality varied depending on storage time, strain, and preharvest ethylene pathway manipulation.

Objective 2: The DA meter offers a fast, non-destructive way to monitor maturity progression of Honeycrisp. I_{AD} values are poorly correlated to any other common maturity parameter in Honeycrisp.

Objective 3: Honeycrisp were available until June in local stores. Compared to previous years, eating quality of fruit has improved, but off flavor and bland tasting fruit are found in half the stores visited between February and June.

Objective 4: Titratable acidity can be determined reliably and cost effectively with an off the shelf test.

Objective 5: Ongoing collaborative efforts across disciplines, institutions, and regions leverage funding and increase relevance and impact of research in the areas of fruit quality and food safety.

METHODS

Red strains of Honeycrisp: Fruit representing 2 commercial strains, was picked by a commercial crew and obtained from a collaborator at the warehouse on the day of harvest. Lot 1 did not receive a preharvest treatment. Lot 2 was divided in batches of fruit treated with: a) untreated control, b) Retain, c) Harvista. All fruit was stored for 1 week at 50F, and then cooled down to 36F in a) three months RA, or b) six months CA with 1% carbon dioxide and 2% oxygen. Standard maturity and defect analysis was performed at harvest, and after 7 days at room temperature following cold storage.

DA meter in Honeycrisp: To optimize DA meter performance when used routinely in WA orchards we tested: number of measurements needed/fruit, positioning on the fruit, time of day, shading of sample fruit, and retrofitting of visor. Correlations to common destructive maturity indices were made on 200 fruit each from 2 orchards in 2014, utilizing an average DA value of 2 readings (front, back)/fruit.

Supermarket survey: Eight Yakima supermarkets were visited monthly from February until June 2014. Visual and sensory quality of fruit was determined.

Titratable acidity test kits: A tube test (www.resultsnowtests.biz) and a semi automated titrator (Hana Instruments, HI 84432) were compared to an automated titrator (Methrom).

RESULTS & DISCUSSION

My program has continued to focus on Honeycrisp fruit quality in 2014.

Red strains of Honeycrisp: *Lot 1* had 100% red color, yellow background color, no bitterpit, splits or other grade reducing defects at harvest (Table 1). Box size ranged from 91-97 (i.e. smaller than standard Honeycrisp selections) and common maturity parameters fell within parameters considered optimum for long term storage in Washington. Fruit held up well in three month RA or six month CA storage and subsequent week at room temperature (Table 1). Flavor was excellent after three months RA (100% ripe apple flavor), but fruit became bland (40% of fruit) and off flavor developed after six months in CA storage (35% of fruit) (data not shown). *Lot 2* fruit at harvest was initially firmer when treated with Retain (1st pick), but Harvista treated fruit retained firmness on the tree through the 2nd commercial pick. All fruit stored well in RA and CA with minimum disorder development (data not shown). Off flavor developed in control and Retain treated fruit, regardless of storage time, while Harvista treated fruit exhibited no off flavor (not shown). These results align with earlier data, were 1-MCP treated fruit had an altered/improved overall flavor profile. However, off flavors are not completely eliminated by blocking ethylene signal perception, since other orchards (see Mattheis report) frequently received Harvista and/or 1- MCP treatment also, without exhibiting results that extreme.

Table 1: Lot 1 fruit quality and disorder prevalence at harvest and during storage in 2013-14.

TREATMENT	HARVEST	3 MONTHS RA	6 MONTHS CA
Sugars (% brix)	13.1 ns	13.1	13.4
Acids (%)	0.63 a	0.47 b	0.44 c
Firmness (lbs)	14.6 ns	14.3	14.6
Diameter (in)	3.11 ns	3.03	3.08
Box 40 lb (size)	91	97	93
WC (%)[x]	1 ns	0	0
IB (%)[y]	0 ns	0	0
Splits (%)	0 ns	8	*
Color (1-4)	4 ns	3	4
BG Color (1-4)[z]	3 ab	2 b	3 a
Starch (1-6)	4.9	*	*

[x] water core; [y] internal browning; [z] background color

DA meter in Honeycrisp: The DA meter has been utilized in the internal WTFRC program for two years on Honeycrisp alone. Fruit maturation on the tree can be monitored easily and non-destructively (Mattheis cont. report, 2014). For consistent results measurements should be taken at the same time of the day (data not shown). Direct sunlight will interfere with the ability of the meter to take readings, hence blocking light or retrofitting a visor is advised (Figure 1). It is sufficient to take two measurements/fruit (towards the center), one in the front and one in the back, but care should be taken to avoid slipping on the backside, especially when using the DA meter without retrofitted visor.

Figure 1: Use of DA meter A) when blocking light; B) when retrofitted with visor

Correlating actual DA meter values to other commonly assessed fruit maturity parameters was tested using fruit from two orchards in 2014. Orchard 1 was picked Sept. 10 (single pick of 100 fruit from range of canopy positions). DA values recorded ranged from 0.09 to 1.55. A large spread in background and red color was visually observed, and verified with laboratory readings. DA values correlated in the field to background color, but regression analysis showed only a weak actual correlation (Figure 2). Fruit on sun side looked very similar in 0.31-1.2 range. Fruit in commercially harvestable range (based on visual clues) had DA-values between 0.5-0.7. A linear regression analysis was performed for all maturity indices. Background color and starch exhibited the strongest relationship with the I_{AD} index (r^2 = 0.43 and 0.46). Firmness and fruit weight presented the weakest correlation (r^2 = 0.08 and 0.04, not shown). Fruit in the second orchard had a very narrow range in DA readings, with 70% of the fruit falling within I_{AD} of 0.61-0.9 and no readings below 0.3 (not shown). Since the grower had already executed a 1st pick, we recommended to go ahead with a single remaining pick. Regression analysis revealed red color to be the strongest correlated to the I_{AD} index (r^2 = 0.26), while all other values had weak correlations below r^2 of 0.1.
In summary, the results of this study indicate no strong correlation between commonly used maturity indicators for Honeycrisp apples and DA meter values.

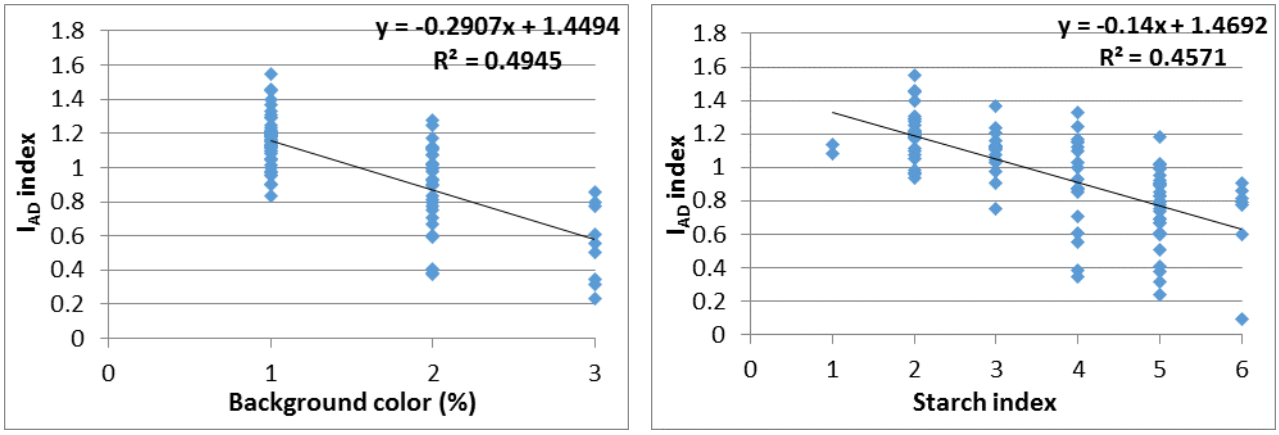

Figure 2: Example of linear regression of background color and starch degradation with I_{AD} index of Honeycrisp at harvest (n=100).

Supermarket survey: We visited eight Yakima area supermarkets six times from February 20 until June 11, 2014. Seven locations offered Honeycrisp until March 18, and one location (Fred Meyer) carried domestic fruit until May 28. Imported fruit was available May 28 and June 11 in one location (Figure 3). Every time (except June 11) we found lots with good eating quality and several batches that had a pleasant appearance but tasted bland (Figure 3). However, we also found lots with off flavor. Compared to previous years, the percentage of fruit with minimum acceptable flavor in March (criteria: no off flavor, bland is acceptable) has increased significantly (30 to 71% from 2012 to

2014), but efforts to further minimize off flavor development in lots designated for long term storage should continue to ensure repeat sales in future years.

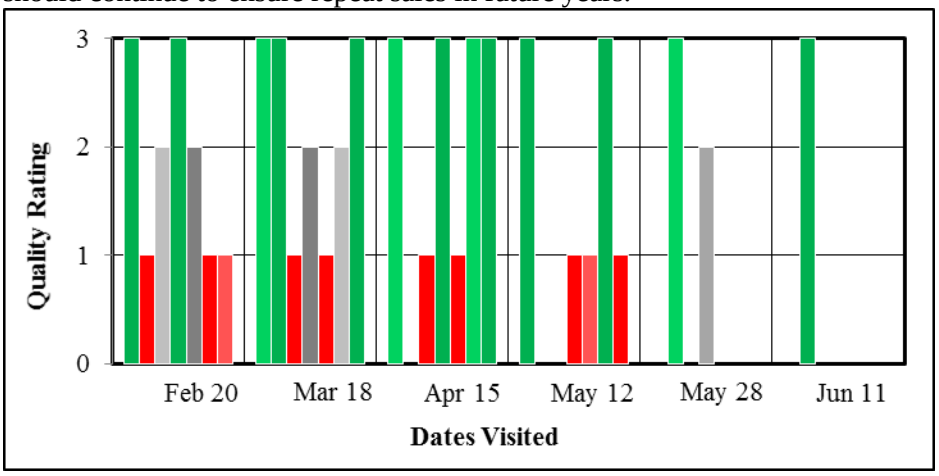

Figure 3: Quality rating (1 = off flavor, 2 = bland, 3 = apple) for Honeycrisp purchased in Yakima area supermarkets between February 20 and June 11, 2014.

Titratable acidity test kits: To ensure good storability and eating quality of Honeycrisp after CA storage it is advisable to select lots with higher titratable acidity (TA) levels at harvest. Malic acid is the prevalent acid in apples. It is used as metabolic substrate during storage and is typically highest in fully mature fruit. Traditionally, TA could only be determined with specialized titration equipment. This method is time consuming and expensive, thus not very practical for commercial packing and storage facilities. We decided to compare three possible TA determination methods in 2014: A tube test (TT) (www.resultsnowtests.biz) and a semi automated titrator (HI) (Hana Instruments, HI 84432) were compared to an automated titrator (Methrom) used at the WTFRC quality lab in Wenatchee (Figure 4). Both tests (TT and HI) are less accurate than the Methrom (not shown). HI has a wide range of readings. Especially problematic is the fluctuation of readings, i.e. values are not consistently higher or lower than the Methrom, but rather fluctuate above or below the Methrom readings. The TT consistently reads above Methrom. For example, if you want to be 70% confident that the TT segregates out the samples at TA 0.5 or above, your test should read 0.65 (between yellow and orange). We consider the performance of the TT sufficient to segregate lots based on the generally observed acid range. The TT method is easy to learn, reproduce and does not require specialized equipment. The reading is based on a color scale with easily distinguishable colors. Operator error can be kept to a minimum, if basic instructions are followed carefully. Further, TT is the cheapest method at $2-3/sample (incl. costs for test and labor).

Figure 4: Titration methods used in WTFRC lab in 2014: A) tube test; B) semi-automated titrator; C) automated titrator

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 and food safety (Table 2).

Table 2. 2014 WTFRC collaborations on pre- and post- harvest fruit quality management projects.

COLLABORATOR(S)	PROJECT	COMMENTS/HANRAHAN ROLE
Rudell	Disorder toolbox	Cooperator on SCRI project
NSure*	Honeycrisp disorder ID	WA field + storage testing
Killinger	Packingline microb. safety	Field support (see AP-09-906)
Killinger	Overhead cooling	Field support (see AP-12-108)
Extenday*	Drape nets	Field and storage evaluation
Brunner, MSU	SSCDS	Cooperator on SCRI project
Evans/Auvil	WSU Breeding: P3	Storage evaluation (see Auvil/Evans final)
Mattheis/Rudell	Extend HC storage life	CO-PI
Killinger	Microbial safety of bins	Collaborator on CPS project
NHC/WSU	China apple export	Collaborator harvest + storage

*project costs completely covered by companies

CONTINUING PROJECT REPORT **Year: 1 of 1 (Extension)**
WTFRC Project Number: AP-14-102

Project Title: Improving food safety of fresh apples by hot air impingement drying

PI: Girish M. Ganjyal
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Co-PI: Karen Killinger
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Cooperators: Laura Grunenfelder – Northwest Horticultural Council, Van Doren Sales, Inc., Stemilt Growers LLC., Pace International LLC., US Syntec, Hansen Fruit Company, Washington Fruit & Produce Company and many others packing houses.

Total Project Request: Year 1: $56,743
Note: We have submitted a new proposal with funding request for additional three years as the results from the work proposed in this one year project proved to be viable for the industry.

Other funding sources: Part of the PI's new faculty start-up funds were used to support this project for covering part of the equipment, supplies and salaries.

Budget 1
Organization Name: WSU **Contract Administrator:** Ben Weller/Carrie Johnston
Telephone: 509-335-0052/355-4564 **Email address:** wellerb@wsu.edu carriej@wsu.edu

Item	2014-15
Salaries	$32,295
Benefits	$4,945
Wages	$4,291
Benefits	$416
Equipment	$5,000
Supplies	$6,796
Travel	$3,000
Miscellaneous	$0
Plot Fees	$0
Total	$56,743

Footnotes:
The majority of the funding requested was to cover graduate student wages and part time technician wages. Funds are also requested for wages to support an undergraduate student to help with the experiments. Some funds were requested to cover part of the cost for impingement dryer, supplies for using analytical equipment and other laboratory supplies to accomplish the various planned experiments. Travel funds were requested to cover travel costs related to the project work, such as trips to the packing facilities in Wenatchee and Yakima.

RECAP OF ORIGINAL OBJECTIVES

The objective of this proposal was to conduct a feasibility study on the potential of hot air impingement technology for drying waxed apples. Specifically we wanted to investigate if the high temperature drying will maintain the quality of the wax coating and does not negatively impact the fruit quality.

The more specific objectives of the proposal were:

1) Develop a thorough understanding of the current drying process and properties of the waxes commonly used in the apple packing process.
    - Specifically, to understand the upper limits of the wax drying in terms of temperature.

2) Determine the impingement drying characteristics of wax coated apples and the impact on quality of the apples.
    - Identify the effects of the temperature and drying time on the wax and apple quality.
    - Design and build a pilot scale hot air impingement dryer and test it to determine if this technology has the potential to dry the waxed apples at higher temperature without negatively affecting the quality.

SIGNIFICANT FINDINGS

In the year 2014, significant progress was made on the preliminary evaluation of this technology with the one year funding provided by the WTRFC.

<u>The following were the major tasks completed during the first year of work:</u>

- Rheological properties of two kinds of waxes (Carnauba and Shellac) were studied.
- An assessment of the current drying conditions in different packing houses was conducted by more than 12 plant visits.
- Drying characteristics of apples coated with both Carnauba and Shellac waxes with convection air drying method were thoroughly studied at a drying air temperature range of 100 to 200^{o}F and drying times of 1 to 3 minutes, as determined by assessment of current packinghouse conditions.
- Quality assessments were conducted for apples dried with the (conventional) convection drying method over a three week period.
- An impingement dryer was purchased after thoroughly researching the options available in the market.
- The new impingement dryer purchased was modified to:
    - Increase the clearance for drying bigger apples
    - Conveyor belt was modified to suit the apple drying process
- Preliminary plant trials were conducted in a packing facility to determine the potential effectiveness of the impingement drying of waxed apples.

<u>The following were the major findings from the first year of work:</u>

- Both types of wax (shellac and carnauba) behave differently under higher heat conditions.
- Viscosity of both waxes reduces significantly with increase in temperature.
- Both Carnauba and Shellac waxes can be effectively dried at higher temperatures (up to 200^{o}F), in a regular convection air dryer.
- The Shellac wax tends to have flaking issues around 100^{o}F. However, with an increase in temperature beyond 150^{o}F, it provides a high level of gloss on apples.

- Carnauba wax provides good performance in the temperature range of 100 to 200ºF.
- In general, at higher temperature, shorter drying time was better for maintaining gloss.
- The overall quality of the apples (as determined by measurement of total solids, moisture loss and pH) were comparable with the control apples (without waxing, but similar drying treatment) over the 3 week storage period.
- In-plant impingement drying tests provided very encouraging results on the drying of waxed apples.
- We observed that the drying temperatures could be increased to 300ºF for both the Carnauba and Shellac waxes without negatively affecting the glossiness on the dried apples.
- With higher temperatures of 250ºF and 300ºF, shorter drying times (less than 1 min) was found to be more beneficial.
- Longer drying times at higher temperatures had negative effects on the wax quality.

Overall, we had very encouraging results from the first year of the project. Thus a new project is proposed to conduct thorough studies evaluating this technology on a broad scale in packing house settings, along with its effects on the fruit quality and microbial loads. Finally, we are also proposing to thoroughly assess the economic feasibility and scale-up options.

METHODS

All the materials (apples and waxes) were obtained from the various co-operators.

For the first objective, initially visits were made to different packing facilities and data collected. The drying temperature and times were recorded. Along with this the temperatures of the apples surface and core were also recorded. These measurements were taken with standard temperature recorders and thermocouples.

Waxes were tested using a rheometer to understand the effects of their viscosity behavior as an effect of temperature. Viscosity and melting characteristics will help us to understand the spread-ability of the waxes. This will be useful to understand the impacts on the dryer designs. These drying characteristics will also help determine the best drying conditions for the impingement drying system.

Initial drying studies were conducted using a convection oven (Model 414004-568, VWR International, LLC, Bridgeport, NJ). The details of the experiments are as described below in the results section. The already cleaned apples were obtained from the packing house. Wax was applied by hand and dried at different conditions. The quality of the apples (glossiness before and after drying, pH, ºBrix, weight loss, as well as assessments of wax quality, such as dripping, flaking, and cracking) were monitored over a period of 3 months). Standard procedures were followed.

For the Impingement frying studies, a new oven was purchased (PS628E, WOW^2, Middleby Marshall, Elgin, IL). The oven was disassembled to understand the air flow mechanisms. Air flow ducts were redesigned and the oven was modified to have about 6 inch clearance for drying apples of all sizes. The oven was further modified by removing the original metal mesh conveyor belt with a conveyor belt similar to that in the packing house dryers. Plant trials were conducted to understand the performance of the newly modified dryer. Apples were waxed using a wax brush system and dried in the modified dryer at different settings. The glossiness of the apples were measured using a Glossmeter. The waxed and dried apples were stored over a period of 4 weeks to study the glossiness changes.

RESULTS & DISCUSSION

i) Current drying conditions in packing houses:

We visited various apple packing houses and assessed the drying conditions (more than 15). Thanks to all the packing houses that provided tours of their facilities and helped us with drying temperature readings and information regarding drying conditions. We found that the drying conditions range very broadly, with the air temperatures ranging from 80°F to 150°F. The drying times were narrow, ranging from 2 ½ minutes to 3 minutes.

ii) Convection air drying of waxed apples:

A study was conducted to understand the drying characteristics of the Carnauba and Shellac waxes using a convection dryer in the laboratory.

Cleaned and un-waxed Fuji apples (one pallet) were hand-collected from an apple packing line. The apples were transported to Pullman for testing with the convection drying process. Two kinds of waxes (Carnauba and Shellac) were obtained from Pace International LLC and US Syntec (each) for testing. Drying studies were conducted in a convection oven (Model 414004-568, VWR International, LLC, Bridgeport, NJ). Drying temperature of 100 to 200°F were studied along with drying times of 1, 2 and 3 minutes. Apples were hand waxed following the procedures described to us by our co-operators. After hand waxing, the apples were dried at various conditions of temperature and time combinations. After drying, the Apples were tested for Gloss, °Brix and pH. Further, the apples were stored for three weeks in a walk-in cooler in the pilot plant facilities in Pullman. Apples were pulled out every week and the quality parameters were tested.

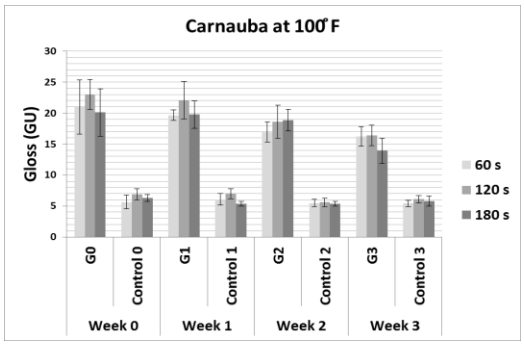

Fig 1. Gloss data for Fuji Apples coated with Carnauba.

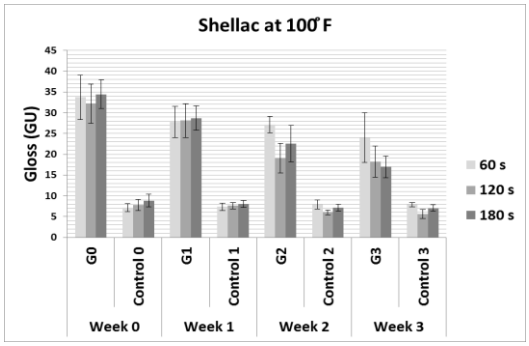

Fig 2. Gloss data for Fuji Apples coated with Shellac.

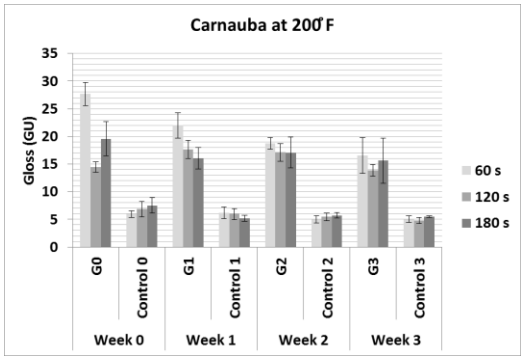

Fig 3. Gloss data for Fuji Apples coated with Carnauba.

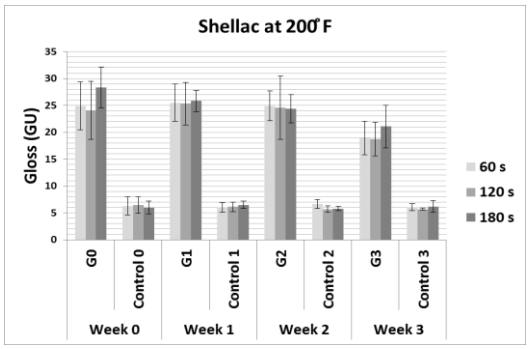

Fig 4. Gloss data for Fuji Apples coated with Shellac.

Figures 1 through 4 show the gloss results of the apples coated with either Carnauba or Shellac wax and dried at different drying conditions. From these results we saw that there were no statistically significant differences in the Apple glossiness at different temperatures and times of drying. However, we generally observed that the Shellac wax has more glossiness at higher temperatures than the Carnauba wax. "G" in the figures above represent the wax treated samples.

Shellac was found to be more sensitive to drying temperatures at 100°F. At this temperature, the wax coated well, but during storage started to deteriorate. The apples pulled out of the storage at the end of the first week showed lot of flaking of the coating (Fig 5). This phenomenon was observed only for the Shellac wax, at lower temperatures (100°F) and longer drying time of 2 minutes or greater. This phenomenon was not present at higher drying temperatures for this wax. This result was encouraging for the use of higher temperature drying with this wax. Carnauba wax did not show any issues in these drying experiments.

Fig 5. Shellac wax showing flaking at 150°F.

Figures 6 through 9, show the data of the °Brix of the apples dried at 100 and 200°F for 1, 2 and 3 minutes. The figures show the data for both Carnauba and Shellac coated apples. We did not see any significant differences in these apples, suggesting that the higher temperature drying did not have any negative impacts for this quality parameter, although additional studies are warranted.

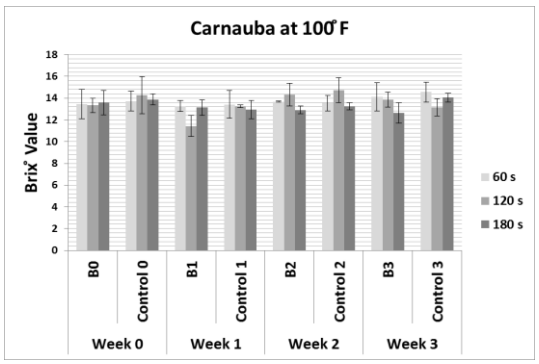

Fig 6. °Brix data for Fuji Apples coated with Carnauba.

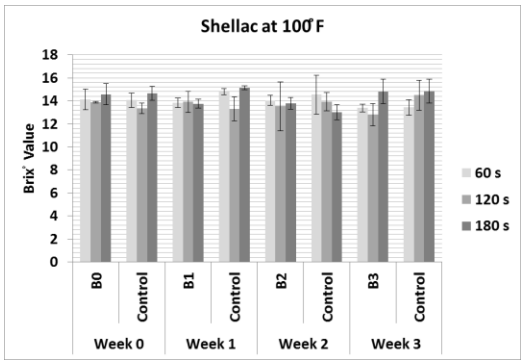

Fig 7. °Brix data for Fuji Apples coated with Shellac.

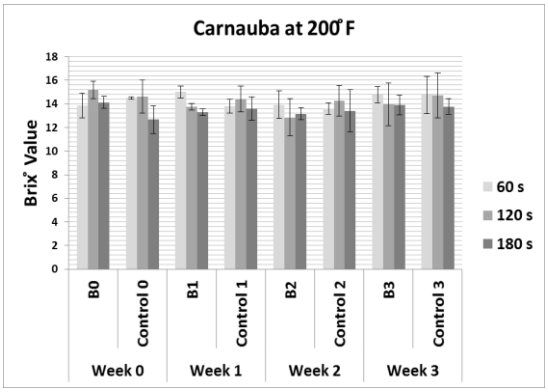

Fig 8. °Brix data for Fuji Apples coated with Carnauba.

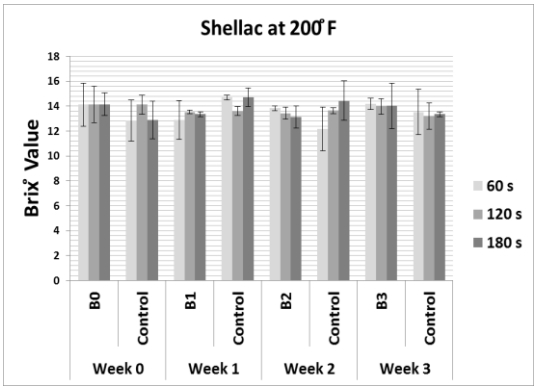

Fig 9. °Brix data for Fuji Apples coated with Shellac.

The results for the pH of the apples were also consistent among all the treatments. We did not see any visual deterioration of the apples for all the treatments.

Overall, from this experimental work we learned that higher drying temperatures (up to 200°F) can be used without negatively impacting the quality of the wax and the apples in general. We also did trials with two other varieties (red delicious and gala) of the apples, although not as broad of study as completed for Fuji. We saw similar results for other varieties of the apples, although additional, in-depth studies are needed.

iii) Impingement air drying of waxed apples:

During the last year, we simultaneously started working on the impingement drying process. Initially, we had thought to buy a small unit that can fit in the laboratory. After conducting a lot of research on different off-the-shelf impingement drying units, we purchased the Hot Air Impingement Oven (PS628E, WOW^2, Middleby Marshall, Elgin, IL). This unit was more flexible and had the range of air velocities and temperatures needed.

This unit had a wire mesh conveyor with a clearance of only 3 inches. If the conveyor was modified to resemble current apple dryers, the clearance would be even more limited.

This forced us to take close look at the dryer design. The dryer air flow paths were modified, and the clearance increased to 6 inches. Further, we replaced the conveyor belt with the roller belt conveyor, similar to what is currently used in the packing lines. Please see Fig. 10, for the modified impingement dryer.

Fig 10. Modified impingement dryer in use in packing house

Following the completion of the modifications, we took the dryer to a packing house. One of our co-operators was very helpful and welcoming to us, so we could test the dryer in their facility.

During the first trials, we used the dryer to test the upper limits of the drying temperatures for drying of the apple waxes, testing both the Carnauba and Shellac waxes with drying air temperatures ranging from 150 to 300°F. ***Interestingly, the higher drying temperatures were found to be more favourable for efficient drying of the waxes in terms of the wax finish and glossiness.***

For Carnauba wax, the drying temperature of 250°F was found to be optimum from this preliminary testing. The appearance of the wax was still good up to 300°F, beyond which the wax became too thin and started to flow away from the apple surface. We completed a experimental design to study the gloss of the apples coated with Carnauba wax. Fig. 11 shows the gloss data of the Carnauba coated apples at different impingement drying conditions. We can see the glossiness of the apples immediately after drying and after a week of storage time.

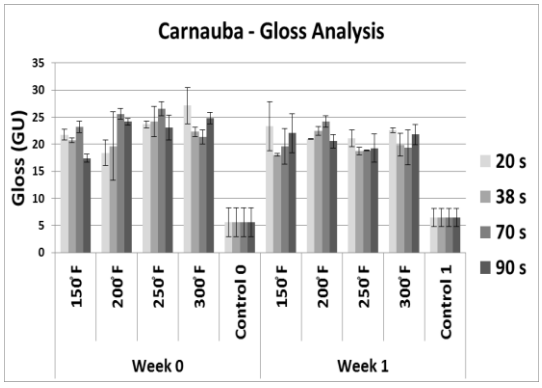

Fig 11. Glossiness data of Apples coated with Carnauba and dried by hot air impingement dryer.

As indicated in Fig. 11, the glossiness of the apples did not change significantly after one week of storage at 40°F. We will continue to monitor these apples for a total of 5 weeks.

For Shellac wax, the impingement drying temperature of 300°F proved to be more effective. At this high temperature of drying, only 20 seconds of drying time was required. In general we observed that the Shellac wax performed better at higher temperatures. However, the Shellac wax did not perform well above 300°F, similar to Carnauba wax.

iv) Internal temperature of the apples during impingement drying process:

During the impingement drying tests, the surface and internal core temperatures of the apples were recorded. We found that with the complete range of the impingement drying tests conducted, the core temperature did not significantly change compared to the untreated control apple. Please see Fig. 12, showing the data of the surface and core temperatures of apples at various drying conditions.

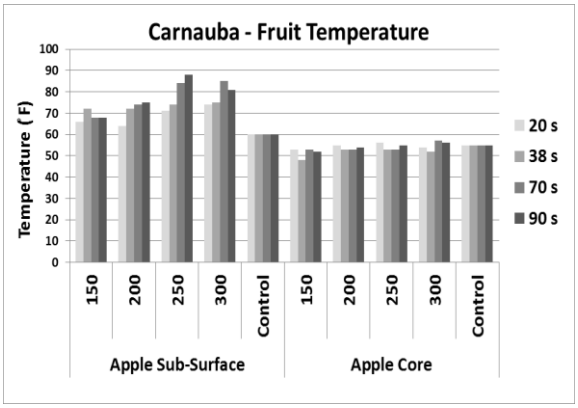

Fig 12. Temperature data of apples coated with Carnauba and dried by hot air impingement dryer.

These results are encouraging not only because of apple quality, but also for the positive impact it can have on the energy requirements for downstream cooling of the apples.

v) Next steps:

As a part of this one year project, we plan to conduct additional in-plant trials with the modified impingement dryer and observe the quality of the fruit over a period of 4 to 5 weeks in storage, according to current industry standards. With this additional data, we will submit the final report for this one year project.

CONTINUING REPORT **YEAR:** 1 of 3
WTFRC Project Number: AP14-103A

Protect title: WA 38 rootstocks and training systems

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Total Project Request: **Year 1:** $98,903 **Year 2: $74,523** **Year 3:** $69,093

Other funding sources: none

WTFRC Collaborative expenses

Item	2014	2015	2016
Wages[1]	6,000	7,000	9,000
Travel[2]	1,500	1,800	1,800
Total	7,500	**8,800**	10,800

Footnotes:
[1] Pruning, floral evaluation, harvest and fruit evaluations (second and third years).
[2]Travel to the orchards (Roza and Sunrise) from Wenatchee.

Budget 1
Organization Name: WSU **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509-335-4564/509-663-8181 **Email:** carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2014	2015	2016
Salaries[1]	35,632	39,601	33,249
Benefits[2]	6,057	7,112	4,863
Wages[3]	4,080	4,243	4,412
Benefit[4]	395	411	428
Equipment[5]	25,000	0	0
Travel [6]	7,591	4,849	5,823
Supplies[7]	4,688	1,688	1,587
Miscellaneous[8]	2,760	2,819	2,931
Plot Fees[9]	4,000	4,000	4,000
Goods and Services[10]	1,200	1,000	1,000
Total	91,403	**65,723**	58,293

Footnotes:
[1] Salary for Ag. Research Assistant (Musacchi) and Research Associate (Gallardo).
[2]Benefits costs include increase of 4% per year.
[3]Student employee for 1.4 wks: 40/wk at $10/hr (Musacchi) and Non-Student Temporary (Whiting).
[4] Benefits at 9.7%.
[5] Ethylene reader and dry matter reader.
[6] Travel to Prosser and Sunrise Orchard (Musacchi) and Travel to Wenatchee and Yakima to facilitate focus group meetings (Gallardo).
[7] Supply costs to complete structure, pollinator trees, mineral analysis, trellis.
[8] Labor for installing trellis, planting trees and pruning,
[9] Standard annual plot fee, Sunrise Orchard and Roza Station.
[10]Fee for the venue of the focus group meetings and cost of refreshments to be served during the meetings ($50/meeting x 4 meetings = $200).

OBJECTIVES

1. Identify growth and productivity characteristics of WA 38 on M9-Nic29 and Geneva 41 trained to conventional vertical (spindle) and angled (V) systems.
2. Identify growth and productivity characteristics of WA 38 on M9-Nic29 and Geneva 41 trained to a bi-axis (fruiting wall) with and without mechanization.
3. Conduct an economic analysis of WA 38 production in the three training system scenarios.

SIGNIFICANT FINDINGS

Objective 1: Identify growth and productivity characteristics on spindle and V systems.

Vegetative parameters

- Sunrise:
  - The highest trunk cross-sectional area (TCSA) was reported for trees trained to spindle.
  - G41 rootstock had significantly higher TCSA than Nic29.
  - The "bending" pruning technique had higher TCSA than the "click" technique.
  - The average number of rootsuckers per tree was higher in spindle and Nic29.
- Roza:
  - By November 2014 the highest TCSA was for trees trained to spindle.
  - Nic29 had higher TCSA than G41; this difference was statistically significant for spindle but not for V-system.
  - The pruning techniques didn't show any difference in TCSA at the end of the season.
  - The average number of rootsuckers per tree was higher in Nic29 than in G41, but only within the V-system.

Pruning and bending

- At Roza, the V-system management took significantly longer than spindle system. At Sunrise, tree growth was affected by the lack of water and there were no significant differences.
- Trees on G41 rootstock required more pruning time than Nic29 because G41 is more vigorous.
- The "click" pruning technique took significantly more time during both winter and green pruning for both Sunrise and Roza orchards, but this extra time was generally offset by the reduced training time.

Yield and quality parameters (Roza only)

- Trees on the V-system had more fruit per tree and higher yield per tree and per acre than those on spindle.
- Trees on G41 rootstock had more fruit per tree and higher yield per tree and per acre than those on Nic29.
- There was no difference in fruit size or yield between the two pruning techniques.

Light interception measurements

- For Sunrise and Roza, the V-system had significantly higher light interception than spindle system at mid-morning and midday.
- Light interception at Roza was higher than in Sunrise throughout the entire growing season.
- The increase between the first and last measurements of the season was greater at Roza than at Sunrise for both training systems, indicating better canopy growth in Roza.

Objective 2: *Identify growth and productivity characteristics on bi-axis.*

- Trees have been trained to have two strong leaders in order to produce a flat canopy or fruiting wall which will be easier to be mechanized in the future management of the orchard.
- No data has been collected yet.

- Bi-axis tree grow very well in Roza. Trees already fill the 80% of space available for the fruit wall.
- Different situation has been observed in Sunrise where the sandy soil and the lack of water during the first part of the season compromise the trees growth.

Objective 3: Conduct an economic analysis of WA 38 production.

- Results from this trial are comparable to commercial pruning time and costs.
- Commercial pruning time (Gala 1,089 trees per acre, M9 rootstock and Red Delicious 900 trees per acre, M106 rootstock) ranges from 0.006 to 0.008 hours per tree, and the cost ranges from $0.08-$0.09 per person per tree
- The pruning time for the Roza and Sunrise WA 38 plots ranges from 0.006 to 0.0106 hours per tree and the cost ranges from $0.072-$0.128 per person per tree.

METHODS

Two thousand trees of the new WSU scion variety WA 38 (Cosmic Crisp) propagated on M9-Nic29 and G41 rootstocks were planted in June 2013 in two locations at the WSU Sunrise (Wenatchee) and WSU Roza (Prosser) orchard to compare vegetative and productive performance. In both sites, two main training systems are compared: spindle (3 ft x 10 ft) and V-system (1.5 ft x 10 ft). Another trial with WA 38 bi-axis trees (1 year younger) on the same rootstocks has been set up to assess the possibility of mechanized thinning, pruning and harvest.

Both orchards are irrigated with sprinklers and drippers; a weather station including soil moisture probes was also installed. At the beginning of summer 2014, water deficit issues were evident at Sunrise orchard and a timer for the irrigation was set in order to have a specific daily schedule. This modification significantly improved the water situation in the orchard and soil moisture levels have been more constant since the timer installation.

Soil samples were collected from the field at two different depths: 0-30 cm and 30-60 cm. Texture analyses on both sites revealed that WSU Sunrise orchard tends to be sandier than the WSU Roza site, which shows a higher percentage in clay and silt.

Specific fertigation plans were decided according to the needs of each site that differ in fertility. In Sunrise, we applied urea to the ground and through foliar application for six weeks starting from May. In Roza, weekly applications of MAP, potassium nitrate and calcium nitrate were dispensed. Two Promalin applications were done to promote side branching. Pest management was mainly focused against powdery mildew and rosy apple aphid through the season.

In 2014, trees were managed according to the training system in order to achieve the correct structure/shape; first by completing the trellis structure and wires and then by pruning and bending (string and tape) both in winter and summer. Two different pruning treatments were used within each training system and rootstock combination. The “bending” method involved minimal pruning, retaining long branches, removing competitive vertical shoots, and concentrating mainly on the bending of the lowest-middle branches. The “click” pruning technique focused mainly on removing crowded branches, choosing the flattest one and trying to develop more buds close to the stem to avoid the “blind wood” issue that characterizes the “type IV habitus” apple varieties.

The Roza orchard was harvested on October 1, 2014. Fruit size and yield were measured for all treatments and quality analyses were performed on fruit from the “bending” pruning treatment. There was no harvest at Sunrise because we removed the flowers early in the season to reduce tree stress and invest in tree growth.

RESULTS AND DISCUSSION

Objective 1: Identify growth and productivity characteristics on spindle and V systems.

Vegetative parameters

At Sunrise, spindle had the highest TCSA and G41 rootstock had significantly higher TCSA than Nic29 (Fig. 1). The average number of rootsuckers per tree was higher in spindle and Nic29. The "bending" pruning technique resulted in higher TCSA than the "click" technique (data not shown).

By November the highest TCSA at Roza was for trees trained to spindle (Fig. 1). Nic29 had higher TCSA than G41; this difference was statistically significant for spindle but not for V-system. The average number of rootsuckers per tree was higher in Nic29 than in G41, but only within the V-system. The pruning techniques didn't show any difference in TCSA at the end of the season (data not shown).

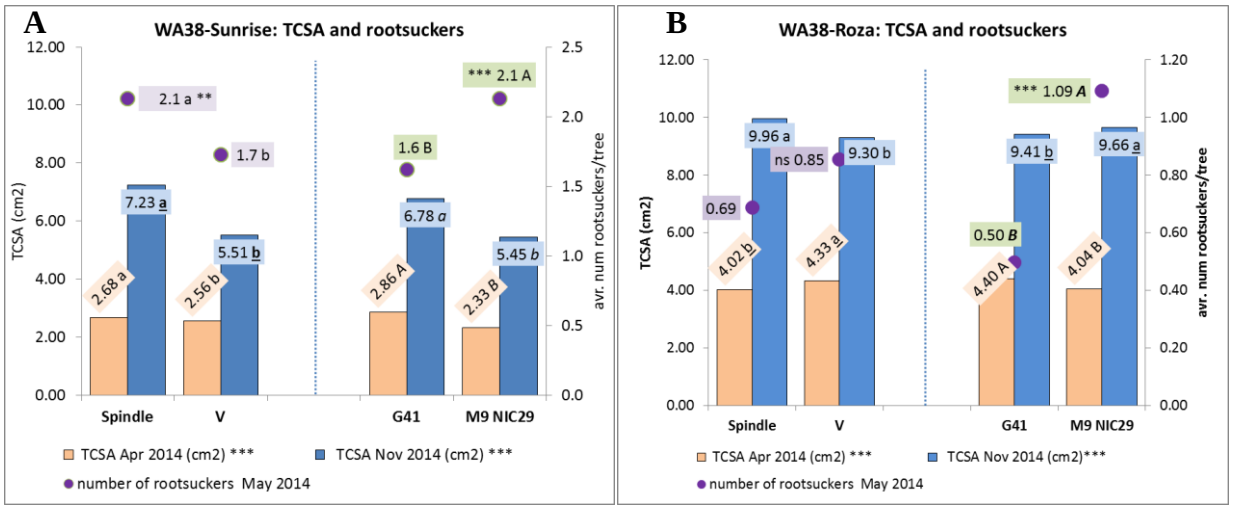

Figure 1. TCSA (cm^2) and average number of rootsuckers in Sunrise (A) and in Roza (B). Comparison between training systems, and rootstocks, year 2014.

Pruning and bending

The V-system management took significantly longer (more than 23 hr/acre) than spindle at the Roza site (Table 1). Growth of trees at Sunrise was affected by the lack of water so less time was spent on managing training overall, and differences in total time/acre spent on each system were not significantly different. More pruning wood was removed during winter pruning from trees trained at spindle because they needed more cut adjustments at the beginning to build up the correct structure/shape.

Trees on G41 rootstock required more pruning time than Nic29 because the G41 rootstock is more vigorous; significantly more wood was removed from trees grafted on G41 rootstock during winter pruning at both sites. Trees on G41 required 32-33 hours more in management than trees on Nic29 for both sites (Table 1).

The "click" pruning technique took significantly more time during both winter and green pruning for both Sunrise and Roza orchards, but the extra time for "click" pruning was generally offset by the reduced amount of time that training "click"-pruned orchards required. Significantly more time was spent for "bending" technique for both first and second bending at both orchards, with the exception of the first bending at Roza. This is due to the minimal pruning applied with this technique retaining longer branches that need to be tied. The "bending" and "click" pruning techniques are shown in Figure 2.

Generally speaking Roza's pruning and bending management in the first year took around 60 hr/acre more than in Sunrise; this reflects the differences in vigor and canopy developments of the two sites.

Yield and quality parameters

The Roza orchard was harvested on October 1, 2014. Fruit size and yield were measured for all treatments and quality analyses were performed on fruit from the "bending" pruning treatment (Table 2). There was no harvest at Sunrise because we removed the flowers early in the season to reduce tree stress and invest in tree growth.

The quality analyses were carried out only on fruit from "bending" treatment in Roza. They were divided in two pullouts: T0 was done 2 weeks after storage and T1 will be analyzed in March 2015 (6 months of storage). Fruit from the spindle system had a lower I_{AD} index, meaning they were riper, sweeter (Brix) and had a more vivid background color (yellowish) than those harvested from V-system. There were no significant differences in internal ethylene concentration, overcolor (blush), red intensity, firmness or starch levels (data not shown).

Table 1. Training and pruning labor hours at Sunrise and Roza orchards, 2014. Labor time is presented as hours per person per acre.

Training	Rootstock	Pruning treatment	Winter pruning		First training (bending, stringing or taping)		Green pruning		Second training (bending, stringing or taping)		Total time (hr per person per acre)	
Sunrise			**16-Apr-14**		**30-Apr-14**		**27-May-14**		**27-May-14**			
V-system			15 hr 23 min	a	47 hr 2 min	b	16 hr 10 min	a	20 hr 49 min	a	99 hr 22 min	
Spindle			10 hr 26 min	b	61 hr 31 min	a	10 hr 53 min	b	14 hr 31 min	b	97 hr 18 min	
Sig[1,2]			***		**		***		***		*ns*	
	G41		14 hr 38 min	a	62 hr 46 min	a	16 hr 36 min	a	20 hr 44 min	a	114 hr 43 min	a
	Nic29		11 hr 10 min	b	45 hr 48 min	b	10 hr 26 min	b	14 hr 35 min	b	81 hr 58 min	b
	Sig[1,2]		***		**		***		**		***	
		Bending	9 hr 56 min	b	75 hr 20 min	a	8 hr 55 min	b	24 hr 56 min	a	119 hr 53 min	a
		Click	16 hr 51 min	a	26 hr 13 min	b	19 hr 40 min	a	7 hr 58 min	b	70 hr 40 min	b
		Sig[1,2]	***		***		***		**		***	
Roza			**17-Apr-14**		**1-May-14**		**30-May-14**		**11 to 24-Jun-14**			
V-system			18 hr 8 min	a	37 hr 46 min	b	36 hr 52 min	a	77 hr 11 min	a	169 hr 58 min	a
Spindle			10 hr 43 min	b	74 hr 26 min	a	22 hr 12 min	b	39 hr 14 min	b	146 hr 35 min	b
Sig[1,2]			***		***		***		***		***	
	G41		14 hr 47 min		55 hr 6 min		33 hr 7 min	a	71 hr 10 min	a	174 hr 12 min	a
	Nic29		14 hr 4 min		57 hr 5 min		25 hr 57 min	b	45 hr 15 min	b	142 hr 22 min	b
	Sig[1,2]		*ns*		*ns*		**		***		***	
		Bending	10 hr 53 min	b	58 hr 30 min		22 hr 16 min	b	66 hr 48 min	a	158 hr 28 min	
		Click	17 hr 58 min	a	53 hr 41 min		36 hr 48 min	a	49 hr 37 min	b	158 hr 59 min	
		Sig[1,2]	***		*ns*		***		**		*ns*	

[1] $p<0.05$, *; $p<0.01$, **; $p<0.001$, ***; *ns*, not significant for Type III sums of squares model significance

[2] Student-Newman-Keuls *post hoc* test to assign letter groups to means where model was significant

[3] Arithmetic means are presented; *post ho*c tests were done with LSMEANS option and the Bonferonni adjustment for multiple comparisons

Figure 2. WA 38 trained on spindle with "bending" technique (A) and V system with "click" technique (B) in Roza (May 2014).

Table 2. WA 38 fruit yield at the Roza research station in Prosser, WA, Oct. 1, 2014[1].

Training system	Rootstock	Pruning	Yield (kg/tree)		Avg. fruit weight (g)		No. fruit per tree		Yield (MT/acre)		Yield (ton/acre)	
V-system			1.1	a	327.1		3.24	a	3.17	a	3.49	a
Spindle			0.7	b	324.2		2.29	b	1.11	b	1.22	b
Significance[2]			*		*ns*		*		***		***	
	G41		1.1	a	323.7		3.40	a	2.58	a	2.85	a
	Nic29		0.7	b	327.8		2.13	b	1.69	b	1.87	b
	Significance[2]		*		*ns*		*		*		*	
		Bending	1.0		330.1		2.93		2.22		2.44	
		Click	0.8		321.1		2.60		2.06		2.27	
		Significance[2]	*ns*		*ns*		*ns*		*ns*		*ns*	

[1] No yield data from Sunrise orchard; blossoms were removed to reduce tree stress induced by insufficient irrigation from the Wanapum Dam draw-down. Only fruit from the "bending" pruning treatment were collected for fruit quality analysis.
[2] *ns*, not significant; *, p <0.05; **, p<0.01; ***, p <0.001 according to SNK *post hoc* test.

Light interception measurements

Light interception measurement was conducted during the growing season after bloom period at both sites. Photosynthetically active radiation (PAR) data was collected at mid-morning and midday on a clear day, every 2 weeks throughout the growing season. The mid-morning light interception was expected to mainly reflect the canopy vertical growth, and the midday light interception would mainly reflect the canopy horizontal expansion.

On both experimental sites, V-system had significantly higher light interception than spindle system at mid-morning (data not shown) and midday (Figure 3, for all comparisons, $p < 0.05$), with seasonally-averaged difference 4% and 11% respectively in Roza, and 6% and 4% respectively in Sunrise. Light interception at Roza was higher than in Sunrise throughout the entire growing season. The increase between the first and last measurements (89-day interval at Roza and 85-day interval at Sunrise) was greater in Roza than in Sunrise for both training systems, reflected by the steeper trend lines in Figure 3(a) and (c) than Figure 3(b) and (d). This phenomenon indicates the canopy growth at Roza was better than at Sunrise. One explanation would be the irrigation shortage at Sunrise this year, which substantially limited the growth of trees.

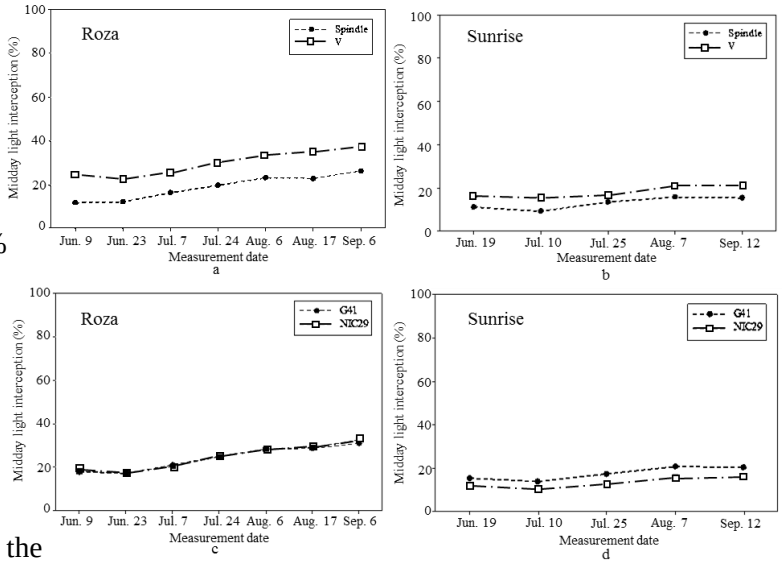

Figure 3. Seasonal midday light interception of different training systems and rootstocks at Roza and Sunrise experimental sites.

As shown in Figure 3(a) and (b), the difference in midday light interception between the two training systems was nearly constant throughout the growing season. This is likely because of the inherent difference on the horizontal exposure area between the two training systems. Regardless of the tree growth status, V-system has larger midday projection area on the ground than spindle, resulting in higher midday light interception. In addition, plots with V-system had higher tree density than plots with spindle, which contributed to the higher light interception of V-system plots.

At Sunrise, trees with G41 rootstock had significantly higher light interception than those with Nic29 rootstock (Figure 3(d), $p < 0.05$, seasonal-averaged difference as 6% and 4% at mid-morning and midday, respectively); indicating G41 was more vigorous than Nic29. At Roza, no such significant difference was observed. This is likely due to the good field conditions at Roza, which weakened the effect of rootstock on tree growth. As mentioned above, Sunrise experimental site had irrigation issue during the growing season. Under such negative conditions, G41 showed its advantage compared to Nic29.

Objective 2: Identify growth and productivity characteristics on bi-axis.

In 2013, a head back cut 2 ft from the ground was performed in order to build a 2-leader system by selecting two new branches to become the axes of this trellis. The bi-axis trees planted along the row were trained in 2014 to have two strong leaders in order to produce a flat canopy or fruiting wall which will be easier to be mechanized in the future management of the orchard.

Pruning was done to remove branches heading internally the two main leaders, eliminating competitors in the apex and building relatively short lateral limbs. Some notching was done in some areas in the tree to stimulate bud break. Trees have been fertilized with the same program as like the whole orchard. No data have been collected yet.

Objective 3: Conduct an economic analysis of WA 38 production.

We used an ANOVA model to estimate effects of different treatments (location, time of the year, trellis system, rootstock, pruning technique) on the pruning time, expressed as hours per person per tree, and the pruning cost, expressed as dollars per person per tree. To estimate the cost we considered that pruning labor is paid on average $12.00 per hour in Washington State commercial orchards. We used a Tukey test to investigate if there were statistically significant differences across treatments.

Results show that in general all five treatment combinations (Table 3) pose statistically significant effects on the pruning time. Overall, pruning per tree was faster and less costly in Sunrise, in April, with the V-angled trellis system, using the M9-NIC29 rootstock and bending as the pruning technique.

Results from this trial are comparable to commercial pruning time and costs. Commercial growers indicated that pruning one acre of trees in the second year takes on average 7 hours. Considering the density of trees (Gala 1,089 trees per acre, M9 rootstock and Red Delicious 900 trees per acre, M106 rootstock), commercial pruning time ranges from 0.006 to 0.008 hours per tree, and the cost ranges $0.08-$0.09 per person per tree. The pruning time for the plots under study ranges from 0.006 to 0.0106 hours per tree and the cost ranges $0.072-$0.128 per person per tree.

Table 3. Tukey test results – Comparison across treatments

	Pruning time (hr/tree)	Pruning cost ($/tree)
Roza	0.0101 a[1]	$0.121 a
Sunrise	0.0062 b	$0.074 b
April	0.0063 a	$0.076 a
May	0.0099 b	$0.119 b
Spindle	0.0090 a	$0.109 a
V	0.0072 b	$0.087 b
G41	0.0091 a	$0.109 a
Nic29	0.0072 b	$0.086 b
Clicking	0.0106 a	$0.128 a
Bending	0.0060 b	$0.072 b

[1] Different letters indicate numbers are statistically significant different at 5% level.

CONTINUING PROJECT REPORT **YEAR**: 2014

Project Title: Apple rootstock and scion evaluation

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WTFRC Staff cooperators: Felipe Castillo, Tory Schmidt, Jim McFerson,

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

Cooperators: Dave Allan, Mark Wilcox, Dale Goldy, Dave Taber, Jim Divis, Scott McDougall

Total Project Request: **Year 1:** 24,200 **Year 2:** 39,420 **Year 3:** 31,500

WTFRC expenses:

Item	(2014)	(2015)	(2016)
Salaries[2,]	11,500	11,500	11,500
Benefits[2,]	3,700	3,700	3,700
Crew Wages[2]	5,000	14,400	10,000
Crew Benefits[2]	1,000	4,320	3,300
Stemilt RCA room			
Shipping			
Supplies			
Travel[1]	3,000	5,500	3,000
Miscellaneous			
Total	24,200	39,420	31,500

Footnotes:
[1]Fuel and maintenance.
[2]Apple rootstock trials.

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

OBJECTIVES:

1. Evaluate commercially available disease resistant apple rootstocks compared to industry standards, in commercial settings in Washington State.
2. Integrate the processes of evaluation and industry implementation of new rootstocks and scions into modern production systems.
3. Evaluate horticultural traits, fruit quality and storage traits for P3 scions in WSU apple breeding program.

Scion evaluation activities and findings:

- 100 trees were grafted at Quincy Phase 3 (P3) site in 2014 to WSU 68 onto discontinued P3 genotypes.
- The Prosser P3 site will have some advancing genotypes grafted onto stumps of discontinued genotypes in 2015.
- 7 bins of fruit was picked in from the 2012-13 WA 38 grafts in Quincy. Color was red, not mahogany, no bitter pit or lenticel issues were apparent. About 2 bins were picked from the Roza WA 38 trial also with no bitter pit. Roza trees are very vigorous and had no cooling.
- Hanrahan, Evans and Ross conducted WA 38 and Honeycrisp consumer preferences in Spokane in March 2014 with WA 38 outscoring Honeycrisp.
- WSU 36 has been discontinued, trees being removed due to concern with viruses.
- WSU 64 and WSU 65 from P2 will planted in 2015 in Quincy. This shift in tactics is aimed to screen for internal browning potential prior to distributing to other P3 sites. Internal Browning after 4 to 6 months storage has been the most common fatal flaw found as with WA 5.
- BPAC advised no additional storage samples be evaluated on WA 2.
- Held a field days in September in Prosser and October in Quincy to present WA 38. Two post harvest report meetings in August, one of them affiliated with Yakima Pom Club.
- Completed three sequential harvests of WA 38 in 2014 from replicated plots in Quincy and Prosser**.** Figure 1 indicates at harvest maturity measures. The texture of WA 38 has similar and unique 'crisp' characteristics not related to firmness measurements.

Figure 1: WA 38 harvest maturity from sequential harvests in Prosser and Quincy.

2014	Prosser			Quincy		
Harvest date	**Oct 1**	**Oct 8**	**Oct 14**	**Oct 2**	**Oct 8**	**Oct 14**
Firmness (lbs)	21.7	22.0	17.0	20.6	20.6	15.4
SSC (° brix)	13.9	14.1	14.4	12.0	12.1	12.2
TA (% malic acid)	0.52	0.58	0.50	0.50	0.51	0.44
Starch (1-8)	2.2	3.1	4.0	4.3	3.7	6.0

- WSU 46 was third leaf in 2014. Fruit quality under bird netting in Prosser was excellent. WSU 46 needs stop drop. No disorders were displayed in 2014. Fruit with more Red and less green background have a more balanced profile at harvest than apples with less red and more green background. After two months of storage the difference was not detectable. About 20%

of the fruit was picked prior to CA condition Gala. If the less red, less starch conversion maturity line is used, first pick of WSU 46 may be a week prior to peak Gala harvest.

Rootstock findings and activities:

- G.41, G.890, G.935, G.30 G.210, G.214, are all replant disorder tolerant and fire blight resistant. G.969 has been released and in eastern trials, fall in similar performance with G.935 and G.214 and is rated as replant tolerant in New York.
    - Several of the eastern trials are planted at much wider in-row tree spacing. This will significantly increase the estimated canopy volume due to horticultural practice, not necessarily rootstock genetics.
    - G.210 in Wapato with Gala scion planted at 3 feet in row spacing is very similar to G.935 and G.214.
- Vigor of non-bearing Geneva rootstock is typically higher than M.9 clones. Yield on Geneva rootstocks is higher than industry standards.

- Yield of the Geneva replant tolerant genotypes continue to increase compared to static yields of control /commercial standards in replant sites.
- Precocity of the more vigorous Geneva genotypes (G.30, G.210 and G.890) remain high even as the production canopy matures. The crop density of these genotypes are significantly better than the Malling stocks they replace (M.26, M.7 and M.106) and compare favorably with Mark, Bud 9 and M.9 clones.
- Yield efficiency as calculated by Kg of fruit per trunk cross sectional area cm^2 (TCSA) declines as canopy volume stops increasing, crop volume stabilizes and trunk /limb diameters increase.
- Fumigation in small blocks or rocky soils has become difficult. Replant tolerant, especially the more vigorous and precocious rootstocks may provide a solution to these replant sites. Also, vigorous scions such as WSU 38 may also assist mitigating replant disorders' negative effect on canopy development.

New rootstock trials 2015:

- A Red Delicious and Gala rootstock trial of 8 Geneva rootstocks (G.11, G.41, G.214, G.935, G.210, G.969, G.890, G.30) plus 3 standards (B.9, M.9 337, M.9 Nic29) is scheduled to be planted in unfumigated ground at the WTFRC orchard in Wapato in 2015. New to Washington State is G.969. Cornell rates G.969 as replant tolerant and woolly aphid resistant with a canopy volume similar to G.935/G.210. Red Delicious was chosen as a very sensitive to replant disorder variety, and also the most sensitive to rootstock effect on fruit shape/length.
- Three trials with the same rootstocks but different scions will be planted by growers in 2015

2003 to 2014 summary:

- There is not a 'best' rootstock. Horticultural practices play a big role in the vigor and size of the canopy, including pruning, nutrition, water management and tree density of the planting. There are seven replant tolerant rootstocks (G.41, G.214, G.935, G.210, G.969, G.890, G.30), each have been better (healthier canopy and more yield) than M.9 or Mark or other commercial rootstocks that have been in the trials. The most recent releases (G.890, G.210 and G.969) need more evaluation to compare to M.9 standards.
- G.11 has out performed all M.9 clones in fumigated and unfumigated plots. However, G.11 has not consistently filled its allotted space in unfumigated plots when compared to G.41, G.214 and the other 'replant tolerant' genotypes.

Replant Tolerance:

- Four fumigated/unfumigated rootstock trials show the genetic potential of disease and insect resistance in Washington State. Six genotypes(G.41, G.214, G.210,G.935, G.890, G.30), commercially released from the Cornell-Geneva apple rootstock program demonstrate significant improvement in health, growth rate, canopy development and yields when compared to industry standard controls. Four of the replant tolerant rootstocks are woolly apple aphid resistant (G.41, G.214, G.210, G.890).
- Yields of all of the replant tolerant rootstocks and G.11 are equal to or higher than M.9 or Mark.

Fire Blight resistance:

- All commercially available Geneva rootstocks have fire blight resistance. A Cornell University trial using finished tree of Gala sprayed at full bloom with fire blight bacteria did have tree loss with G.11. Other Geneva genotypes such as G.41 had 100% survival. In comparison, B.9 liners will not survive direct inoculation with fire blight, but will survive when only the scion is inoculated.

Liner Production:

- Additional rootstock producers have been licensed to propagate Geneva rootstocks. Tissue culture labs are getting licenses to sell direct to growers and finished tree nurseries.

- Tissue Culture of G.41 and the establishment of stooling beds have substantially increased the production of G.41. Licensed tissue culture operations may now sell Geneva rootstocks to all nurseries and growers.
- G.11 has the most liner production. It grows the smallest canopy due to its high precocity of the commercial Geneva rootstocks, and is not as robust mitigating replant as G.41 or G.210. It is rated by Cornell as being woolly aphid resistant.
- Increasing production of G.890, G.969 and G.210 are being encouraged.
- G.935 is not woolly aphid resistant. Two similar rootstocks that are woolly aphid resistant are G.214 and G.210.

Finished Tree Production:

- Some bud unions have been broken. There is not one rootstock + scion combination that is consistently a problem in all nurseries. Different nurseries have different combinations with problems. Honeycrisp is a consistent scion with weak bud unions, Pajam 2 being a noticeable problem.
- Extra large trees (3/4" to 1" caliper) have more breakage medium size trees (1/2" to 5/8").
- Some combinations of rootstock and scion have synergistic response. G.41 and Fuji is vigorous non-bearing combination.

What's next:

- Nurseries are searching for efficient propagation systems for Geneva rootstocks. There are several differing tactics being deployed. Tree buyers will benefit by checking the Cornell University website to get contact information of licensed nurseries to visit nurseries and observing production practices and results. The range of products (liners, sleeping eyes, bench grafts, one year nursery trees, two year bench graft, two year budded trees, etc.) has never been greater.

- As more sites, scions and Geneva rootstocks are planted, the better and best combinations will be identified. Soil types and scion traits will provide selection preference for rootstocks. It is unlikely that ‘one’ will be the best in all sites or with all scions.

CONTINUING PROJECT REPORT **YEAR:** 1 OF 3
WTFRC Project Number: AP-14-105A

Project Title: After RosBREED: Developing and deploying new apple DNA tests

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Cooperators: Paul Sandefur (PhD student, WSU Pullman), Sook Jung and Sushan Ru (WSU Pullman), Fred Bliss (Davis, California)

Total Project Request: **Year 1**: $89,000 **Year 2**: **$89,000** **Year 3**: $91,000

Other funding sources

Agency Name: WTFRC
Amount *requested*: $771,688 (2015–2017)
Notes: "Apple scion breeding" PI: Evans. Co-PI: Peace.

Agency Name: WTFRC Apple Review
Amount *requested*: $107,000 (2015-2017)
Notes: "Combining fire blight resistance and horticultural quality in Washington apples" PI: Norelli. Co-PI: Evans.

Agency Name: WTFRC
Amount awarded: $862,261 (2012–2014)
Notes: "Apple scion breeding program" PI: Evans. Co-PI: Peace.

Agency Name: WTFRC/OSCC
Amount awarded: $125,000 (2014–2016)
Notes: "After RosBREED: Developing and deploying new sweet cherry DNA tests" PI: Peace.

Agency Name: WTFRC
Amount awarded: $53,254 (2014–2015)
Notes: "Adding apple map, marker and trait data to the Genome Database for Rosaceae" PI: Main. Co-PIs: Evans, Peace, and Jung.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $10.0 M (Sep 2014 – Aug 2019)
Notes: "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars." PI: Iezzoni. Co-PIs include Peace, Oraguzie, and Main.

Agency Name: USDA-NIFA Specialty Crop Research Initiative

Amount awarded: $7.2 M (Sep 2009 – Aug 2014)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae." PI: Iezzoni. Co-PIs include Peace, Main, and Evans.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $2.7M (Sep 2014 – Aug 2019)
Notes: "Genome Database for Rosaceae: Empowering specialty crop research through big-data driven discovery and application in breeding". PI: Main. Co-PIs include Jung, Evans, and Peace.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $2.0 M (Sep 2009 – Aug 2014)
Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture." PI: Main. Co-PIs include Peace and Evans.

Agency Name: USDA-NIFA NRSP
Amt. requested: $1.99 M (Oct 2014- Sept 2019)
Notes: "Database resources for crop genomics, genetics and breeding research". PI: Main.

Budget 1
Organization Name: Washington State University **Contract Administrator:** Carrie Johnston
Telephone: (509) 335 4564 **Email address:** carriej@wsu.edu

Item	2014	2015	2016
Salaries[a]	31,008	**32,249**	33,540
Benefits	16,127	**16,965**	17,850
Supplies[b]	9,865	**9,786**	9,610
Travel – within-state	2,000	**2,000**	2,000
Travel – international[c]		**3,000**	3,000
Miscellaneous – workshop[d]	5,000		
Total	64,000	**64,000**	66,000

[a] Half-time support of Paul Sandefur, PhD student and RosBREED two-time "breeding trainee"; 0.40 FTE Terry Rowland, genetic screening technician of the Washington Tree Fruit Genotyping Lab (WSU, Pullman); 0.10 FTE Taein Lee,
[b] DNA extraction and PCR supplies, minor equipment maintenance, and computing supplies as necessary
[c] USA to Australia or Europe return trip for Dr. Peace, 2015 and 2016 only
[d] Travel support for one-day workshop in Wenatchee associated with RGC7 conference, 2014 only

Budget 2
Org. Name: University of Queensland **Contract Admin.:** Dr. Craig Hardner
Telephone: +61 7 3342 4095 **Email address:** craig.hardner@uq.edu.au

Item	2014	2015	2016
Salaries[a]	20,000	**20,000**	20,000
Travel[b]	5,000	**5,000**	5,000
Total	25,000	**25,000**	25,000

[a] Consultancy fees for Dr. Hardner, 5 weeks. Note that 3 additional weeks of Dr. Hardner's time will be provided by the Queensland Alliance for Agriculture and Food Innovation
[b] Queensland, Australia to Washington, USA return trip for Dr. Hardner

OBJECTIVES

Overall goal
Improve prospects for apple breeding efficiency, accuracy, creativity, and pace by developing and strategically deploying predictive DNA tests targeting valuable traits

Specific objectives

1. DNA test development:
    a. Develop new DNA tests, first for current genomics discoveries (acidity, sweetness, firmness), and continue with future discoveries (maturity time, size, texture, storage disorders)
    b. Establish a streamlined statistical approach to predict performance from DNA test outcomes

2. DNA test deployment strategies:
    a. Deploy new DNA tests strategically by devising and trialing strategies for the WABP aligned with existing tests and breeding operations; host an international workshop on this topic
    b. Establish a streamlined statistical approach for DNA test deployment under complex scenarios

SIGNIFICANT FINDINGS

- **Acidity** DNA test used in 2014 parent selection, updated for use in 2015 seedling selection, and used on new parent material to help guide 2015 crossing decisions
- New **fructose content** DNA test ready for 2015 seedling selection and used on new parent material to help guide 2015 crossing decisions
- **Texture** DNA test updated to efficiently target multiple genomic regions; ready for 2015 seedling selection
- DNA test development for **powdery mildew, fire blight,** and **flesh color** initiated
- Workshop "DNA Test Deployment Strategies for Rosaceae Crop Breeding" hosted and well attended by international researchers and affiliated scientists, keeping us on the cutting edge

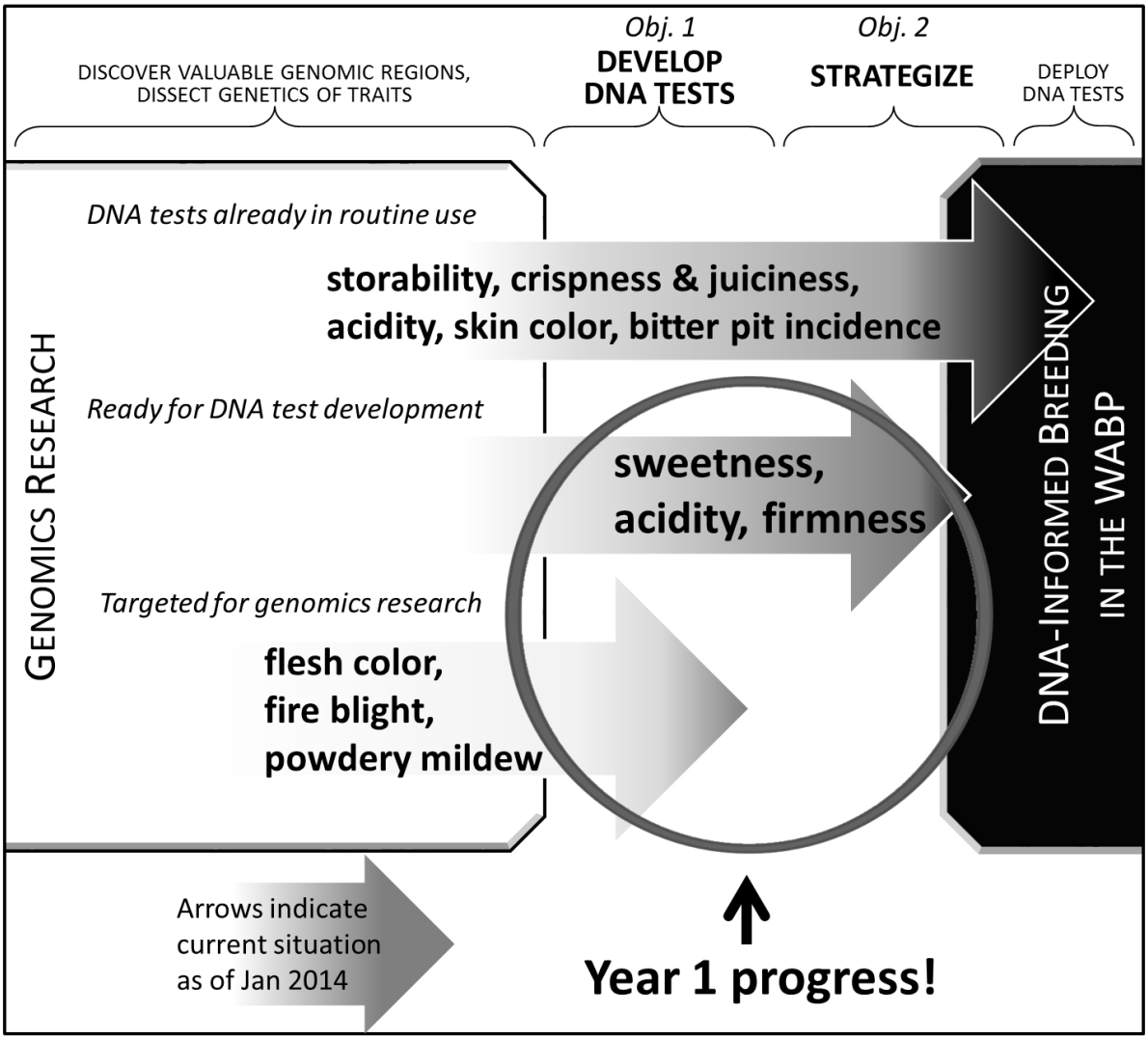

Figure 1: Progress made in Year 1 to continue to improve the efficiency, accuracy, creativity, and pace of the Washington Apple Breeding Program (WABP) via DNA-informed breeding.

METHODS

Activity 1: DNA test development

DNA test development relies on discoveries by RosBREED and project collaborators of genomic regions associated with fruit acidity, sweetness (e.g., fructose content), texture (crispness, firmness, and juiciness), other aspects of fruit quality from harvest through to extended storage, and other breeding-relevant traits such as productivity and pest and disease resistance. The trait-predictive DNA tests developed will reveal the genetic factors underlying superior genetic potential of new parents, seedlings, and elite selections.

DNA test development process

A) *Predictive genotype pattern identification*: Phenotypic data for each trait and genotypic data generated during the RosBREED project are combined, from which trait-predictive genotype patterns are identified.
B) *Initial marker design*: Using the Genome Database for Rosaceae's apple GBrowse tool (http://www.rosaceae.org/gb/gbrowse/malus_x_domestica_v1.0-primary), genomic sequences from the targeted regions are retrieved. At least five sites in each region are targeted with candidate markers, with assay primers designed using the software tools of BatchPrimer3 v1.0 (http://probes.pw.usda.gov/batchprimer3) and Primer3Plus (http://primer3plus.com). All primer pairs designed are custom ordered through Integrated DNA Technologies (http://www.idtdna.com/site).
C) *Candidate marker trials*: Each candidate marker is trialed using a low-throughput genotyping platform (polyacrylamide gel electrophoresis) on a small set of related individuals that represent the range of trait-predictive genotype patterns. Marker trialing includes PCR amplification and visualization of the candidate marker products followed by examination of marker outcomes to choose those that match the original genotype patterns. If necessary, multiple markers are combined to obtain pattern matching. Trialing of the most promising markers is conducted across a larger set of breeding-relevant individuals (n~50). If results are confirmed, the candidate marker(s) are elevated to the status of a trait-predictive DNA test for the breeding program. Trait-predictive genotypes can now be obtained for parent individuals.
D) *High-throughput conversion*: The low-throughput DNA test is converted to a high-throughput platform (ABI 3730 DNA Analyzer) by ordering primer pairs with appropriate fluorescent chemistries. If required, primers are re-designed to increase or decrease product size to facilitate combination of existing DNA tests into efficient, multi-trait DNA tests.
E) *Trait prediction refinement*: Because only DNA tests with products that match the original trait-predictive genotype patterns are chosen for high-throughput conversion, no additional confirmation of trait effects associated with DNA test outcomes is required for derived breeding material. As larger datasets become available (e.g., larger family sizes and additional years of phenotypic evaluation), DNA tests are screened on additional germplasm. Statistical analyses are then used to refine the accuracy of a DNA test's trait level predictions. Expanding beyond simple statistics, we will develop new statistical methods to streamline prediction of the average effect on trait performance of the various genotypes arising from DNA tests. The RosBREED phenotypic data on unselected seedling families will be used to ensure that the effect of a DNA test genotype is predictable in WABP germplasm. The opportunity to combine data collected as part of WABP P2 trials with the RosBREED data will be explored. Further data will be collected if necessary.

Activity 2: DNA test deployment strategies

We will devise and evaluate alternative deployment strategies that consider how the trait performance level that each DNA test helps achieve fits within the general breeding scheme and how it fits with other available DNA tests. Where possible, evaluation of alternative deployment strategies will use existing datasets to test hypothetical outcomes of applying DNA tests. Such existing large datasets, with both genotypic and performance data, include the RosBREED dataset of almost a thousand apple individuals and datasets from the routine DNA testing of seedlings since 2010 for which performance data is beginning to accrue.

A one-day "DNA Test Deployment Strategies for Rosaceae Crop Breeding" workshop will be hosted at WSU-TFREC in Wenatchee immediately prior to the 7th Rosaceae Genomics Conference in June 2014. The workshop will discuss experiences, successes, constraints, and opportunities to deploying DNA information for parent selection, seedling selection, and elite candidate selection. Workshop outcomes will be used to guide subsequent deployment of DNA tests for WSU's tree fruit breeding programs and guide the development of our streamlined statistical approach (below).

Statistical tools involving software programming will be developed and used that provide genetic potential predictions from DNA test results, combine additional sources of information such as performance and pedigree to identify elite candidates, and identify the most efficient strategy for deployment of these tests. In addition, because we envisage that a large number of individuals with multiple sources of DNA information will be available for selection decisions, a robust, objective, and rapid method is required to identify the elite candidates. Tradeoffs across multiple traits will be required because it is unlikely that individuals that are elite for all genomic regions will be available. The WABP's breeding database will be expanded to describe the predicted genetic potential from DNA tests. Methods for robust, objective selection that combine results from multiple DNA tests will be developed. To begin, two genomic regions (the *Ma* locus and one other) will be studied in detail. We will use socio-economic outcomes from RosBREED and choice modeling studies of decisions in the WABP to develop weights that reflect the quantitative preference of the breeder to alternative DNA test genotype combinations. These weights will be applied to historical data collected in the WABP to evaluate the efficiency of this weighted selection index approach. Based on the results of this evaluation, the method will be refined and extended to all available DNA tests.

RESULTS AND DISCUSSION

Activity 1: DNA test development

Significant progress was made in 2014 in developing and/or refining DNA tests for the valuable traits of **fruit acidity**, **fructose content**, and **texture**. In addition to storability, crispness, juiciness, bitter pit, and skin color, DNA test results for fruit acidity was used to guide 2014 cross decisions. The new and refined DNA tests will be applied for seedling selection beginning in early 2015. Progress was also made in developing DNA tests for **fruit flesh color** and **powdery mildew** and **fire blight susceptibility**, with the candidate marker design completed and trials initiated.

Acidity

A new marker, "LG8A-SSR", was developed for the second of two genomic regions associated with the bulk of observed differences among WABP individuals for fruit acidity. The LG8A-SSR when combined with the previously used "Ma-indel" DNA test allows for maximum prediction of apple acidity. This new combined DNA test, "Ma×A Acidity", is now ready for deployment in 2015 seedling selection. Ma×A Acidity clearly differentiates breeding-relevant acidity levels (Figure 2).

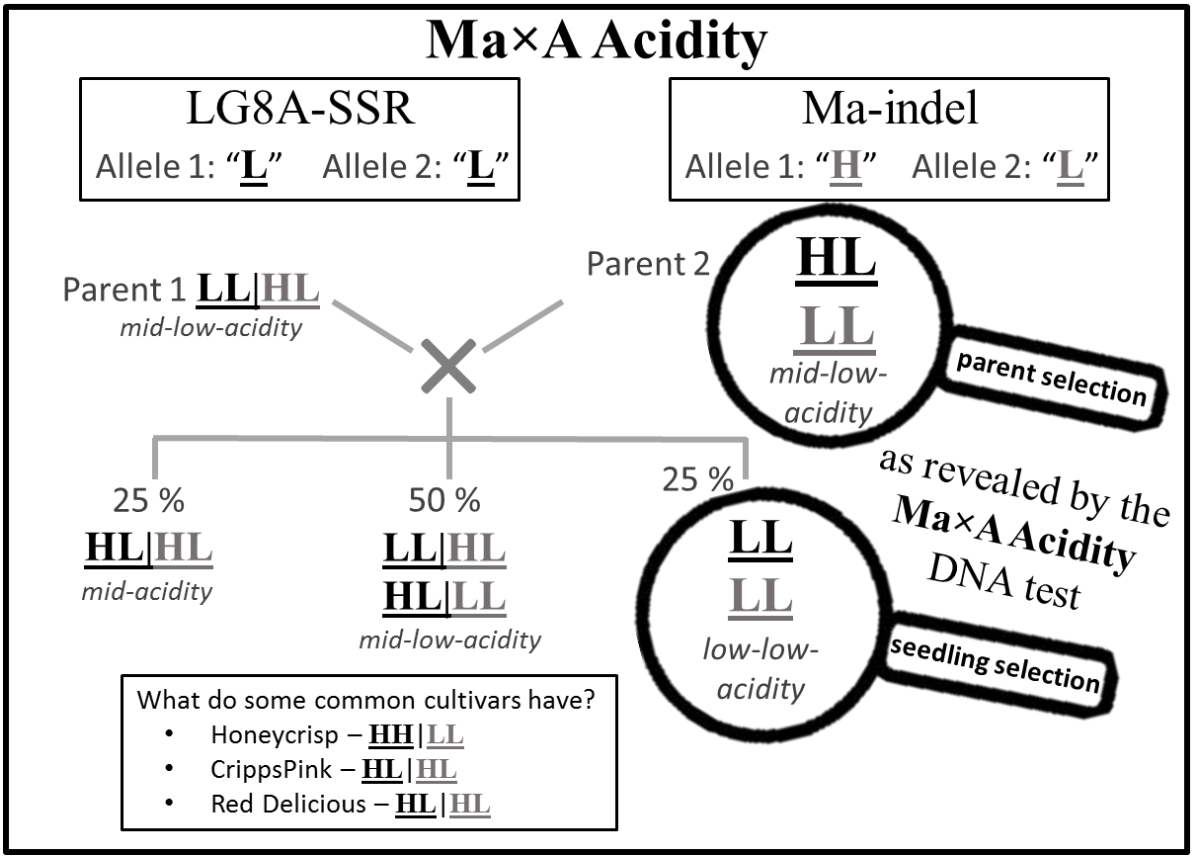

Figure 2: The Ma×A Acidity DNA test reveals contrasting genetic factors (alleles) that predict fruit acidity level ("H" = high; "L" = low), enabling selection of parental cross combinations that are likely to produce a high proportion of seedlings with enhanced acidity levels and for selecting seedlings with desired genetic potential. In the example cross shown, the magnified low-low acidity seedlings would likely be culled as they would not have the genetic potential to produce fruit with enough acidity for a desired sugar-to-acid balance.

Fructose content

A new DNA test, "LG1Fru-SSR", for prediction of fruit fructose content was developed. This new DNA test differentiates very high, medium, and low fructose content individuals (Figure 3) and will be deployed for 2015 seedling selection. The various combinations possible among these three alleles can be used to predict fructose content of any individual. When LG1Fru-SSR is used in combination with the Ma×A Acidity DNA test described above, parents and seedlings with the genetic potential to produce fruit with a superior sugar-to-acid balance can be differentiated from those lacking the flavor consumers demand.

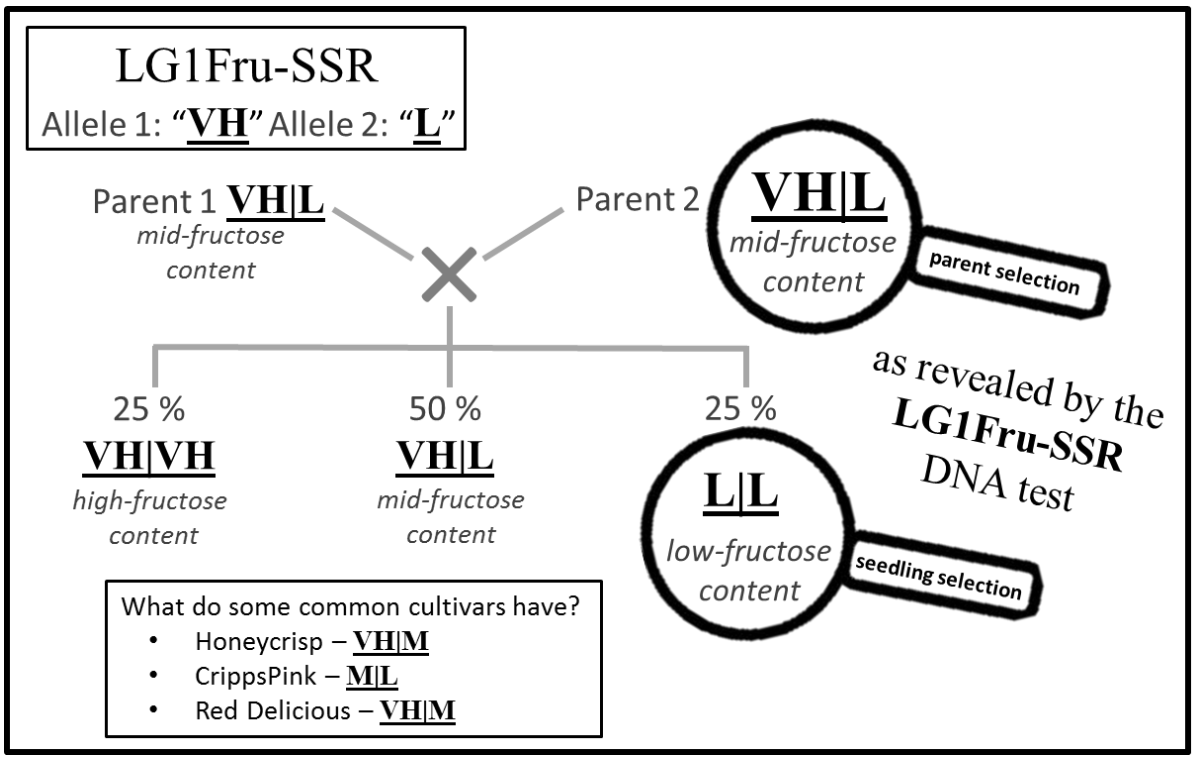

Figure 3: The LG1Fru-SSR DNA test reveals contrasting genetic factors (alleles) that predict fruit fructose content, enabling selection of parental cross combinations that are likely to produce a high proportion of seedlings with enhanced fructose content and for selecting seedlings with desired genetic potential.

Disease resistance and flesh color

Candidate DNA tests were designed for flesh color, specifically pink and red flesh, and resistance to powdery mildew and fire blight. Screening has been initiated for these tests and will continue in early 2015. At least one of these tests should be ready for deployment in late 2015.

Activity 2: DNA test development strategies

Interim spreadsheet-based solutions are being implemented for breeder use of DNA information from numerous DNA tests used on 2015 seedlings, prior to application of more detailed strategies to be devised in Year 2. A one-day "DNA Test Deployment Strategies for Rosaceae Crop Breeding" workshop was hosted at WSU-TFREC in Wenatchee on 23 June 2014. The event was well attended, with more than 40 participants from at least 15 countries. Experiences, successes, constraints, and opportunities to deploying DNA information for parent selection, seedling selection, and elite candidate selection were discussed, with many valuable contributions from participants. A report summarizing the alternative strategies is being written as an invited scientific article for the journal Frontiers. The workshop outcomes are keeping our fruit breeding programs on the cutting edge. Some of these outcomes include new connections formed with researchers working toward the same goals who can help with deployment logistics and new understanding of the limitations faced by programs outside of WSU. Specific strategies discussed will helping guide subsequent deployment of DNA tests for WSU's tree fruit breeding programs and will guide the development of our streamlined statistical approach in Year 2.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-14-107

Project Title: Adding apple map, marker and trait data to GDR

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Co-PI(3):	Kate Evans	**Co-PI (4):**	Sook Jung
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City/State/Zip:	Wenatchee/WA/98801	**City/State/Zip:**	Pullman/WA/99164

Cooperators: Jim Luby (University of Minnesota), Susan Brown (Cornell University), Gennaro Fazio (USDA-ARS), Yanmin Zhu (USDA-ARS), Francois Laurens (INRA, France, PI of FruitBreedomics project)

Total Project Request: **Year 1:** 26,183 **Year 2: 27,071**

Other funding sources

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $2.7M (Sep 2014 – Aug 2019)
Notes: "Genome Database for Rosaceae: Empowering specialty crop research through big-data driven discovery and application in breeding". PI: Main. Co-PIs include Jung, Evans, Oraguzie, Wasko DeVetter, and Peace.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $2.0 M (Sep 2009 – Aug 2014)
Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture." PI: Main. Co-PIs include Peace, Evans and Jung.

Agency Name: USDA-NIFA NRSP
Amt. requested: $1.99 M (Oct 2014- Sept 2019)
Notes: "Database resources for crop genomics, genetics and breeding research". PI: Main. Writing team includes Jung, Peace and McFerson.

Agency Name: NSF DIBBS
Amt. *requested*: $1.48 M (Jan 2015–Dec 2017)
Notes: "Tripal Gateway, a platform for next-generation data analysis and sharing". PI: Ficklin. Co-PIs include Main and Jung.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $10.0 M (Sep 2014 – Aug 2019)
Notes: "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars." PI: Iezzoni. Co-PIs include Peace, Oraguzie, Evans and Main.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $7.2 M (Sep 2009 – Aug 2014)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae." PI: Iezzoni. Co-PIs include Peace, Main, and Evans.

Agency Name: WTFRC
Amount *requested*: $771,688 (2015–2017)
Notes: "Apple scion breeding" PI: Evans. Co-PI: Peace.

Agency Name: WTFRC Apple Review
Amount *requested*: $107,000 (2015–2017)
Notes: "Combining fire blight resistance and horticultural quality in Washington apples" PI: Norelli. Co-PI: Evans.

Agency Name: WTFRC
Amount awarded: $862,261 (2012–2014)
Notes: "Apple scion breeding program" PI: Evans. Co-PI: Peace.

Agency Name: WTFRC/OSCC
Amount awarded: $125,000 (2014–2016)
Notes: "After RosBREED: Developing and deploying new sweet cherry DNA tests" PI: Peace.

Agency Name: WTFRC
Amount awarded: $862,261 (2012–2014)
Notes: "Apple scion breeding program" PI: Evans. Co-PI: Peace. The Washington Apple Breeding Program will be a primary beneficiary of this proposal as will the USDA-ARS rootstock breeding program.

Agency Name: WTFRC
Amount requested: $269,000 (2014–2016)
Notes: "After RosBREED: Developing and deploying new apple DNA tests" PI: Peace Co-PIs: Evans, Hardner, Main.

Budget 1
Organization Name: Washington State University **Contract Administrator:** Carrie Johnston
Telephone: (509) 335 4564 **Email address:** carriej@wsu.edu

Item	2014	2015
Salaries[a]	16,900	17,576
Benefits	5,283	5,495
Wages		
Benefits		
Equipment		
Supplies[b]	3,000	3,000
Travel[c]	1,000	1,000
Miscellaneous		
Plot Fees		
Total	26,183	**27,071**

[a] 0.25 FTE Dr. Sook Jung (Senior GDR data curator), 4% salary increase in year 2.

[b] Computational hardware housing cost (10%), disk backup storage supplies, minor equipment maintenance

[c] Twice annual in-person meetings for feedback and training and quarterly teleconferences (gotomeeting).

OBJECTIVES

Overall goal: To ensure the public apple map, trait and marker databases remain current in GDR and are fully integrated with the private WA Apple Breeding program to help enable efficient apple breeding.

Specific objectives:

1. Collect and curate new publicly available map, marker, trait loci and genotypic and phenotypic data for apple
2. Upload and integrate apple data within the Genome Database for Rosaceae
3. Integrate the curated apple map, marker and trait data with the WABP
4. Ensure optimal utilization of the WA Apple Breeding ToolBox and GDR through hands-on training for the WABP team and allied researchers.

SIGNIFICANT FINDINGS

1. Data from 24 publications including 27 genetic maps, 2,375 molecular markers, 573 QTL, 36 germplasm have been curated, uploaded and integrated with other GDR data. The curation effort includes the association of QTLs with Trait Ontology. These data will be used to help introgress genes/loci for traits of interest in the breeding programs.
2. Data on 55 *Malus* taxa, such as aliases, chromosome number, genome size, ploidy, growth habit, propagation method, usage, origin, hybrid parents, resistance to biotic and abiotic stress and references, have been curated and uploaded to GDR. These data will also be used to identify genes/loci associated traits of interest and introgress in new cultivars.
3. Collaborated with the FruitBreedomics bioinformatics team to standardize map, marker, genotype and performance data storage to facilitate future transfer of this data into GDR. This will mean that breeders will have access to all the apple phenotype and genotype data generated by the large European project, an equivalent to the US RosBREED project for use in the WA Apple Breeding Program.
4. Phenotypic data for the RosBREED project from NY and WA apple breeders have been uploaded and integrated with other GDR data, providing up to date phenotype data for the WA Apple Breeding Program. This allows performance data to be compared across locations and years through the Breeders Toolbox, helping efficient breeding.

METHODS

1. **Collect and curate new map, marker, trait loci and genotypic and phenotypic data for apple**: Using our standardized Microsoft Excel data templates we will collect and curate publicly available apple map, marker, trait loci and genotypic and phenotypic data from various sources including: (a) the RosBREED (www.rosbreed.org) project (b) the EU version of RosBREED - FruitBreedomics (www.fruitbreedomics.com) project, (c) other collaborators and (d) extracted from peer-reviewed publications. For these data to be fully integrated with other existing data and be useful to breeders and other users, additional curation effort is necessary. For example, multiple names and aliases are often used for markers, primers, germplasm, mapping population and trait descriptors. Details such as marker source organism, sequences, marker types, mapped positions of marker and trait loci and germplasm details, such as pedigree and description, can be also missing in publications and user-submitted data. We will work to standardize the names and obtain the details required to integrate data so it is useful to researchers. We will also associate trait loci with Trait Ontology (TO) so that users can browse standardized TO to find all the associated trait loci.

We are actively adding more tree fruit ontology terms to TO, originally developed to describe traits in grass species, to expand to other plant species.

2. **Upload and integrate data within the Genome Database for Rosaceae**: We will upload and integrate the apple map, marker and trait data to the GDR using our bulk data loader so that the GDR apple data mining and browsing tools remain current with up-to date data. We currently use Perl scripts to upload data in the Excel file. We will use the newly available Tripal bulk data loader. Curators need to create bulk loader templates only once using the Tripal online page and use it repeatedly to upload data in the same format. This will make adding more data types, if needed, easier than modifying Perl scripts.

3. **Integrate the curated apple map, marker and trait data with the WABP**: We will link the WABP data with the available public data and tools. The data mining tools include breeding data search tools by dataset, germplasm names, trait values, alleles and parentage. Breeders can download genotypic and phenotypic data of germplasm that meet the various categories and thresholds that users specified. In addition to the efficient management and retrieval of genotypic and phenotypic data, the integration of the breeding data with GDR will allow breeders to directly use the up-to-date genome information in DNA assisted breeding. Currently available breeding decision-support modules in GDR include *Trait Locus Warehouse*, *Marker Converter*, *Technology Portfolio* and *Cross Assist*. The *Trait Locus Warehouse* allows breeders to search for available QTLs for their trait of interest by various categories. When they find QTLs they can view them in a graphic viewer, GBrowse (Stein et al., 2002). *Marker Converter* helps breeders to view markers and re-sequencing data around the QTL to find sequence alleles that can be utilized in developing better markers for a trait locus. When breeders develop primers using the downloaded sequences, they can go to the *Technology Portfolio* to find companies who can perform the genotyping. All of these tools will be extremely useful when all the data underlying the tools, such as markers, trait loci and sequences of various germplasm and reference species, are up-to-date. We will make every effort to update the underlying data. *Cross Assist* is a breeding decision-support tool designed to predict the efficient parent combinations that can produce a target number of seedlings with specific traits thresholds specified by users. We will integrate the breeding values and DNA-based functional genotype data from the available parent pool of the WABP into *Cross Assist* to enhance Dr. Evans' cross-planning efforts.

4. **Ensure optimal utilization of the WA Apple Breeding ToolBox and GDR through hands-on training to the WABP team and other allied scientists:** We will conduct hands-on in-person training on data template completion and use of the toolbox and hold quarterly conference calls to ensure toolbox is kept current with data and functionality.

RESULTS AND DISCUSSION

Publicly available trait and marker data that is relevant to the WABP has been added to GDR and more performance and genotypic data specific to the breeding program will be added over the next couple of months. These additions will continue to provide an up-to-date breeding information management system for the WABP to facilitate routine marker-assisted breeding and more efficient development of new cultivars for WA apple growers. Ties with the European FruitBreedomics project are strong, with data-sharing strategies in place. Significant funding has been obtained from federal sources to continue to develop resources (GDR) and tools (markers for traits of economic importance) that will help make apple tree fruit breeding in WA even more efficient, accurate, creative, and rapid and keep WA apple growers competitive on a global scale.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-13-104

Project Title: Glyphosate fate in inland pacific northwest apple orchards

PI:	Ian C. Burke	**Co-PI (2)**:	Mark Mazzola
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Cooperators: Tim Smith

Total Project Request: **Year 1:** $34,386 **Year 2:** $40,536 **Year 3: $20,000**

Other funding sources: None

Budget 1
Organization Name: CSS **Contract Administrator:**
Telephone: 509.335.2562 **Email address:**

Item	2013	2014	2015
Salaries	25,587	28,1496	
Benefits	2,200	2,387	
Wages			
Benefits			
Equipment			
Supplies	6,599	10,000	20,000
Travel			
Plot Fees			
Miscellaneous			
Total	$34,386	$40,536	**$20,000**

Footnotes:

OBJECTIVES:

Objective 1: Recap of Objective 1: Experiment 1.1 and 1.2 will determine the fate of the glyphosate after application without a significant recent glyphosate use history in apple production systems, including fate of glyphosate absorbed through the bark.

Experiment 1.1: Fate of glyphosate in an orchard without a recent glyphosate use history.
Assessment first and second year data from field experiment 1 (Sunrise) is completed. Residue analysis from Sunrise is not completed. Assessment of first year data from field experiment 2 (Quincy NE) and field experiment 3 (Quincy SW) is completed. Goals and activities for the next year include the final assessment of tree injury at Sunrise in the spring of 2015 as well as the continuation of Quincy NE and Quincy SW.

	Field Experiment	Results Timeline	Result Type
Experiment 1.1	Sunrise (Located in block 3c of the Sunrise Orchard)	First year data by December, 2013 and second year data by December 2014.	Results of field experiments and residue data.
	[1]Quincy NE (located off Martin Rd, Quincy, WA)	First year data by December, 2014 and second year data by December 2015	Results of field experiments and [2]residue data.
	[1]Quincy SW (located off S Rd. NW, Quincy, WA	First year data by December, 2014 and second year data by December 2015	Results of field experiments and [2]residue data.

[1]The addition of Quincy NE and Quincy SW is a deviation from the original methods of Objective 1.
[2]Residue analysis of Quincy NE and Quincy SW is dependent on the residue analysis of Sunrise.

Experiment 1.2: Absorption and translocation of basal-applied and soil-applied glyphosate.
The greenhouse experiment for the absorption and translocation experiment (ATE) 1 is completed, providing glyphosate absorption data. The laboratory processing, to obtain translocation data, is not finished. Young apple trees have been ordered for ATE 2 and are expected to be available for pickup in January 2015. Goals and activities for the next year include completing the laboratory processing of ATE 1 as well as the completion of the greenhouse and laboratory processing associated with ATE 2.

	Tree Fruit	Results Timeline	Result Type
Experiment 1.2	Young Apple Trees – Gala/M9 ATE 1	Greenhouse experiment completed by June 2014 and laboratory processing completed by March 2015.	Detailed knowledge of the absorption and translocation of glyphosate in young apple trees.
	[1]Young Apple Trees – Gala/M9 ATE 2	Greenhouse experiment completed by June 2015 and laboratory processing done by December 2015.	Detailed knowledge of the absorption and translocation of glyphosate in young apple trees.
	[2]Young Apple and Cherry Trees	Experiment completed by December 2015.	Detailed knowledge of the absorption and translocation of glyphosate in young apple and cherry trees by soil application.

[1]The addition of ATE 2 is a deviation from the original objective 1 schedule and will replace the ATE in cherry.
[2]Postponing glyphosate absorption and translocation by soil application in apple and cherry is a deviation from original objective 1 schedule and will be dependent on the results from the duplicate apple absorption and translocation experiments.

Objective 2: Recap of Objective 2: Identify optimum conditions for microbial degradation to mitigate soil adsorption (and potential persistence) of glyphosate in inland Pacific Northwest orchards, and characterize shifts in bacterial and fungal communities in the soil.

Experiment 2.1: Genetic analysis of microbial communities.
Knowledge of the fungal community composition within the nontreated control and the plots treated with glyphosate at 1920 g ae/ha has been obtained. Further analysis of the fungal community composition in the remaining plots treated with glyphosate at 840 g ae/ha, as well as the bacterial community composition analysis, remains to be completed.

	Results Timeline	Result Type
Experiment 2.1	First year data by December 2013	Knowledge of the effect of glyphosate on overall microbial dynamics
	Second year data by April 2015	Knowledge of the effect of glyphosate on qualitative changes in community structure

SIGNIFICANT FINDINGS:

- Following the applications of glyphosate at Sunrise, Quincy NE, and Quincy SW, no visual injury has been observed.
- No differences in tree growth are present at Sunrise, Quincy NE, or Quincy SW, but a trend of decreasing tree growth with increasing glyphosate is present at Quincy SW.
- Glyphosate absorption by the bark treatments in ATE 1 was surprisingly higher than glyphosate absorption by the leaf treatment.
- Relative to the 2013 growing season samples, there appears to be a modest partitioning of both microbial communities among the two soil treatments at Sunrise.

METHODS:

Experiment 1.1: Fate of glyphosate in an orchard without a recent glyphosate use history.
Sunrise was established on April 24, 2013 in block 3C at the WSU Sunrise Orchard. Trunk diameter measurements as well as notes on trunk, graft, and overall bark condition were recorded for each tree. Sunrise was established with a randomized complete block design with a split-plot treatment arrangement and four replications. Main plots were 2.1 m wide by 24 trees, or ~24 m, in length and consisted of three treatments; 1) no postemergence glyphosate and maintained weed free by hand weeding or with a paraquat application at 140 g/ha, 2) glyphosate at 840 g ae/ha, and 3) glyphosate applied at 1920 g ae/ha. Split-plots were 2.1 m wide by 12 trees, or ~12 m, in length and were either 1) no vegetation facilitated by hand weeding or a directed application of paraquat or 2) a uniform stand of volunteer weeds. The trunk diameter measurements were converted to cross-section measurements of area. Quincy NE and Quincy SW were established on May 13, 2014 in two separate fuji blocks planted in 2013. Initial trunk measurements were recorded for each tree and the two field experiments were established with a randomized complete block design with four replications. Plots are 2.1 m wide by 24 trees in length. Each study includes three treatments; 1) no post emergence glyphosate and maintained weed free with applications of paraquat at 140 g/ha and hand weeding, 2) glyphosate at 840 g ae/ha, and 3) glyphosate applied at 1920 g ae/ha.

Prior to each glyphosate application at Sunrise, the no vegetation split-plots were hand weeded and the low hanging branches along with any suckers were trimmed. Glyphosate applications were applied to the whole plot and directed at the base of the tree. Glyphosate was applied on May 16, 2013, July 11, 2013, May 22, 2014, and July 31, 2014. To supplement the soil residue analysis as well as eliminate any concerns of glyphosate drift into the canopy during application, spray targets were placed systematically throughout the tree canopy and on the ground to document were the spray droplets were landing. Prior to each glyphosate application at Quincy NE and Quincy SW, the low hanging branches as well as any suckers were trimmed and the plots were hand weeded. Glyphosate

applications were applied to the whole plot and directed at the base of the tree. Glyphosate was applied on May 22, 2014 and July 31, 2014.

To quantify non-adsorbed and adsorbed glyphosate residue, soil samples were collected after each glyphosate application using a zero-contamination system (core diameter of 5 cm) set for 10 cm depth. Following each application at each field experiment site, two soil samples were systematically collected from within the plots at 0, 1, 8, and 15 days after application. After sampling was completed, samples were stored at -20 °C (-4 °F). In collaboration with Mark Mazzola and objective 2, the soil samples were removed from the freezer and split in half. One half of the soil was delivered to Mark Mazzola and the remaining half of the soil sample was returned to the -20 °C (-4 °F) storage until further analysis for free and adsorbed glyphosate and AMPA residues.

At Sunrise, tissue samples were collected 22 days after each application. Tissue samples were stored at -20 °C (-4 °F) until further analysis.

The harvest of Sunrise took place on August 28th 2013 (2013 report), but a whole plot harvest did not occur in 2014. A subsample of 20-40 apples, sized between 80 and 88, was saved from each split-plot for quality analysis and juice analysis in 2013 and for only juice analysis in 2014. No harvest or subsamples were collected from Quincy NE and Quincy SW.

Experiment 1.2: Absorption and translocation of basal-applied and soil-applied glyphosate.
Brookfield gala on M9 rootstock (no larger than 3/8'') were purchased from Willow Drive Nursery, Inc. and planted in tall tree pots in the greenhouse. Trees were allowed to grow until leaves were mature. Trees were arranged by height to utilize a randomized complete block design. Treatments included an application of 60 kBq of radiolabeled glyphosate, mixed in water and non-ionic surfactant, to either 1) a leaf, 2) bark above graft, or 3) bark below graft. After treatment, plants were allowed to grow in a greenhouse and destructively harvested at 1, 7, 14 and 28 days after treatment. Each harvest consisted of 4 replicates of each treatment. Each plant was divided into sections 30 cm in length, starting from the graft, and the soil and roots were allowed to dry and collected as well. The treated areas were rinsed with a mixture of water, methanol, and nonionic surfactant to obtain glyphosate not absorbed. Parts will be dried at 40 C, weighed, and larger samples ground and subsampled. The sub-samples will be oxidized and the evolved ^{14}C-CO_2 will be captured and quantified. Translocation of glyphosate will be determined from the recovered radioactivity in the oxidized samples.

Experiment 2.1: Genetic analysis of microbial communities.
A composite apple root sample with adhering rhizosphere soil will be collected from two trees in each treatment plot from a depth of 5-15 cm. DNA will be extracted from duplicate sub-samples (5 g) for each plot using the MoBio PowerMax Soil DNA extraction kits and resulting DNA will be pooled. Initial examination of microbial communities will utilize a genetic approach to identify quantitative shifts in populations. Bacteria will be quantified from the duplicate soil extracts by real-time quantitative PCR (qPCR) by targeting the 16S gene with the primer set 338F and 518R, and fungi using the primer set NSI1 and 5.8S.. Quantification will be achieved using the StepOne Plus Real Time PCR thermocycler. All reactions will be performed using three technical replicates. The standard curves for PCR quantification will be generated by diluting DNA plasmid containing cloned amplification product. The plasmid used for the bacterial 16S standard curve will be constructed with the 16S gene from *Methylobacterium* sp. amplified from soil using the primers 8F (50-AGA GTT TGA TCC TGG CTC AG-30) and 1406R (50-ACG GGC GGT GTG TRC-30). The fungal standard curve will be prepared from the ITS region of *Mortierella alpina* amplified from soil using the qPCR primers in which the complete and correct plasmid insert was previously verified by DNA sequencing. Qualitative changes in microbial community structure will initially be examined using a coarse genetic approach by employing terminal restriction fragment length polymorphism (T-RFLP)

analysis. This method will be used as a cost savings approach and will serve to identify the most appropriate community to target for examination by pyrosequencing. T-RFLP analysis of bacterial and fungal communities will be conducted using the methods previously described and commonly employed by the collaborators (Weerakoon et al., 2012). These data will be utilized to determine what, if any, microbial populations should be targeted for analysis by pyrosequencing. In the conduct of prosequencing analysis. The bacterial 16S gene will be targeted for amplification using the forward primer consisting of the 25-bp 454 A Adapter and an 8-bp barcode followed by the modified universal bacterial primer16S-27F (5´-AGRGTTTGATCMTGGCTCAG-3´). The reverse primer will consist of the 25-bp 454 B Adapter and the modified universal bacterial primer 16S-519R (5′-GTNTTACNGCGGCKGCTG-3′). Fungal DNA will be amplified using the 25-bp 454 A Adapter and an 8-bp barcode followed by the forward primer ITS1F and the reverse primer consisting of the 25-bp 454 B Adapter the universal primer ITS4. Duplicate reactions will be conducted for each primer pair/sample combination and the resulting amplicon population will be purified using a QIAquick Gel Extraction Kit. Purified amplicons will be quantified and pooled within samples for pyrosequencing. Pyrosequencing will be conducted using a 454 sequencer at Washington State University.

RESULTS AND DISCUSSION:

Experiment 1.1: Fate of glyphosate in an orchard without a recent glyphosate use history.

No injury was present following an application of glyphosate at Sunrise, Quincy NE, or Quincy SW and no injury was observed as Sunrise trees began to break dormancy in the spring of 2014.

After obtaining tree growth data from Sunrise, Quincy NE, and Quincy SW and further investigation of the yield and fruit quality data (2013 report), it is likely that any yield or fruit quality differences reported in 2013 were not a result of glyphosate treatment or the presence of vegetation, but were rather a result of variable fruit thinning practices. Measuring tree growth will more accurately provide the data necessary to determine if treatment effects are present within the study. The tree growth (Table 1) at Sunrise, Quincy NE, and Quincy SW was not affected by the application of glyphosate and the presence of vegetation had no effect on tree growth at Sunrise.

Table 1. Glyphosate treatment effects on tree growth from Experiment 1.1.

	Sunrise		
	Year 1 tree growth (mm^2 $tree^{-1}$)	**Year 2 tree growth (mm^2 $tree^{-1}$)**	**Total tree growth (mm^2 $tree^{-1}$)**
Treatment	**Mean**	**Mean**	**Mean**
Nontreated	5.6 ± 1.1	3.3 ± 0.7	8.8 ± 1.2
Glyphosate 840 g ae/ha	10.1 ± 0.8	4.2 ± 1.1	14.3 ± 1.4
Glyphosate 1920 g ae/ha	6.2 ± 1.3	8.6 ± 2.4	14.8 ± 2.7
Split-plot	**Mean**	**Mean**	**Mean**
Vegetation	6.7 ± 1.1	6.4 ± 1.3	13.0 ± 1.7
No Vegetation	8.0 ± 0.9	4.4 ± 1.5	12.4 ± 1.7

	Quincy NE	**Quincy SW**
	Year 1 tree growth (mm^2 $tree^{-1}$)	**Year 1 tree growth (mm^2 $tree^{-1}$)**
Treatment	**Mean**	**Mean**
Nontreated	41.7 ± 4.6	100.8 ± 5.8
Glyphosate 840 g ae/ha	49.6 ± 3.9	97.2 ± 7.6
Glyphosate 1920 g ae/ha	43.7 ± 5.8	83.9 ± 8.7

Although the glyphosate treatments at Sunrise, Quincy NE, and Quincy SW did not have a significant effect on tree growth, it is interesting to see the trend present at Quincy SW. As the rate of glyphosate increases, the tree growth in year 1 decreases. The trees at Quincy NE and Quincy SW were planted in 2013 and the trees at Quincy SW are smaller caliper trees than the trees at Quincy NE. Therefore, in the smaller and less mature trees, absorption and translocation of glyphosate may be occurring and resulting in reduced tree growth. Applications to Quincy NE and Quincy SW in the second year and evaluation of tree health in the spring will provide more definitive results pertaining to the presence and possible mobility of glyphosate in the vascular system of the tree.

Experiment 1.2: Absorption and translocation of basal-applied and soil-applied glyphosate.
In ATE 1, the increased absorption of glyphosate by the bark of young gala/M9 trees compared to the leaf was unexpected (Figure 1). The absorption of glyphosate by the bark does not mean that translocation to other parts of the tree is occurring. Processing of the remaining parts of the trees will provide information regarding the translocation of absorbed glyphosate to other areas of the tree. The completion of ATE 2 will be a valuable addition to objective 1.2, as the absorption results of ATE 1 suggest high levels of glyphosate absorption by the bark of both the rootstock bark and gala bark.

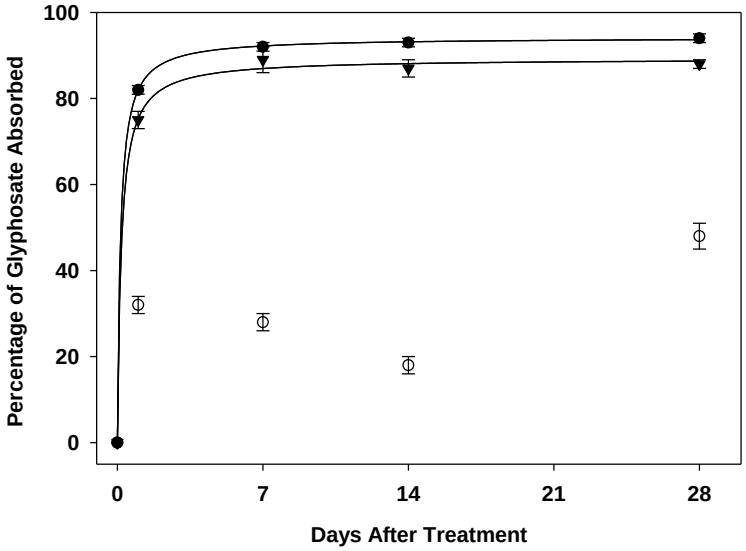

Figure 1. Percent of radiolabeled glyphosate absorbed by each treatment. The filled circles represent the absorption by the above graft bark, the inverted triangles represent the absorption by the below graft bark, and the open circles represent the absorption by the treated leaf.

Experiment 2.1 Genetic analysis of microbial communities.
In year one, there were clearly no treatment effects of glyphosate on fungal or bacterial community composition between the nontreated plots and the plots treated with glyphosate at 1920 g ae/ha (2013 report). In the current year, principal coordinate analysis was conducted of bacterial and fungal community derived T-RFLP data for samples collected after the first glyphosate application at 1920 g ae/ha at Sunrise in May 2014. Statistically, there were no significant differences in composition of either the bacterial or fungal community between the nontreated and glyphosate treated plots. However, relative to the 2013 growing season samples, there appeared to be only a modest partitioning of both microbial communities among the two soil treatments. Further analysis of the fungal and bacterial community composition in the Sunrise plots for samples collected in August of 2014 is ongoing at this time, and could help determine the need for processing samples treated with glyphosate at 840 g ae/ha as well as looking at multiple year effects.

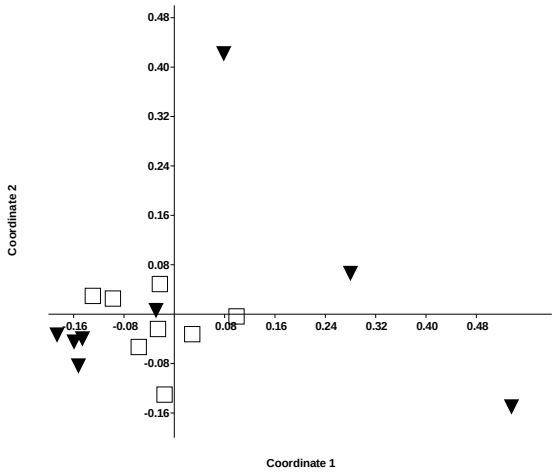

Figure 2. The effect of glyphosate on bacterial community composition based upon principal coordinate analysis of T-RFLP data. Open squares represent nontreated control and inverted triangles represent data points from plots treated with glyphosate at 1920 g ae/ha.

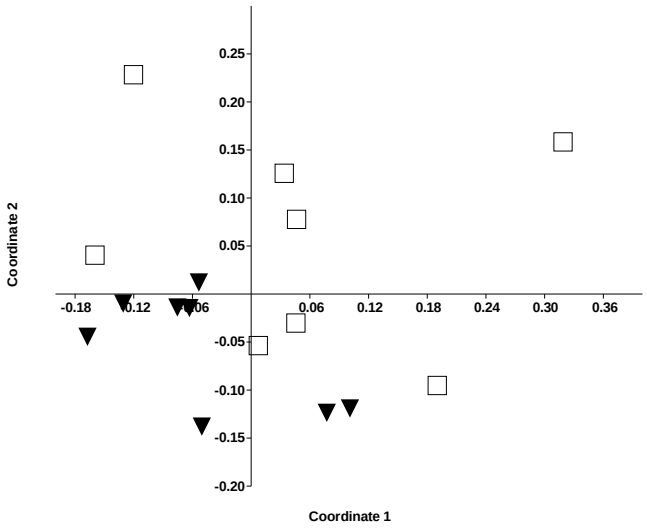

Figure 3. The effect of glyphosate on fungal community composition based upon principal coordinate analysis of T-RFLP data. Open squares represent nontreated control and inverted triangles represent data points from plots treated with glyphosate at 1920 g ae/ha.

CONTINUING PROJECT REPORT **YEAR**: 2014-15
WTFRC Project Number: AP13-103

Project Title: Identification of procedures to extend 'Honeycrisp' storage life

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Cooperator: Tory Schmidt, WTFRC

Total Project Request: **Year 1:** $72,714 **Year 2:** $68,714 **Year 3: $68,714**

Other funding sources: none

WTFRC Collaborative expenses:

Item	2013	2014	2015
Wages	$8,000	$8,000	$8,000
RCA Room Rental	$630	$630	$630
Miscellaneous	$4,000[1]		
Total	$12,630	$8,630	**$8,630**

Footnotes: [1]Funds for acquisition of a differential absorbance (DA) meter for maturity assessment

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

Item	2013	2014	2015
Salaries	$39,586	$39,586	$39,586
Benefits	$19,498	$19,498	$19,498
Wages			
Benefits			
Equipment			
Supplies	$1,000	$1,000	$1,000
Travel			
Miscellaneous			
Plot Fees			
Total	$60,084	$60,084	**$60,084**

Footnotes: Salary, benefits for GS-6 technician

OBJECTIVES

1. Characterize differences in orchards that produce fruit with a history of disorder resistance or susceptibility.
2. Determine utility of ethylene green life, fruit density, titratable acidity, chlorophyll fluorescence and chlorophyll absorbance as additional indicators of storability.
3. Identify alternatives to the 7 day 50 ^{o}F pre-conditioning protocol.
4. Identify factors contributing to CO_2 injury occurring during the initial 30 days after harvest.
5. Identify CA protocols that maximize quality retention and minimize disorders.

SIGNIFICANT FINDINGS

- Later picks of fruit from middle and low positions in v-trellis canopies displayed higher sensitivity to chilling injury in 2013.
- Fruit position within the canopy influenced at harvest fruit quality to a greater extent than netting in 2014
- Eating quality of fruit from orchards harvested with sub-optimal quality for longer storage was compromised after 6 month CA storage in 2013.
- Raynox application did not alter at harvest and storage performance of fruit in 2013, but fruit may exhibit improved taste after storage.
- Netting of orchards led to changes in fruit quality at harvest and after storage in 2014 (analysis on-going).
- Rapid CA reduced bitter pit incidence in 2013.
- Fruit chlorophyll fluorescence during cooling did not correlate with soft scald development.
- Fruit weight at harvest was related to soft scald (low weight) and bitter pit (high weight).
- High CO_2 during SmartFresh treatment did not cause CO_2 injury.
- Treatment with SmartFresh and/or storage in CA (2 % O_2, 1% CO_2) impacted fruit volatile production during storage compared to untreated fruit stored in air.

METHODS

1. *Preharvest factors*:

1.1. Fruit position in tree: 2013 set up: in a mature v-trellis block 12 trees (6 east, 6 west- facing) were divided into 3 sections: upper (> 7ft.), medium (4.5-6.5ft.), low (<4ft.). In each section 6 fruit were marked and evaluated twice weekly until harvest for: red color, fruit size, sunburn, DA meter. Four trees were harvested at each of 3 harvest dates. Fruit was placed in 33F cold storage and DA meter and disorder readings performed weekly for 3 months. 2014 set up: the same v-trellis block was used, and the canopy divided into sections on trees facing west with similar crop load in either a netted or un-netted section (4 reps each); fruit was picked from one tree each at three weekly harvest dates and placed in 33F cold storage. Bi-weekly DA meter and disorder readings were performed for 12 weeks after which fruit was destroyed performing maturity analysis. In addition, at harvest 10 fruit for each tree/pick date/location within tree were picked and DA meter values and common maturity determined.

1.2. Altered light and temperature: In 2013, a pilot study was performed using single drape nets approx. 30ft. long, supplied by Extenday, in 3 orchards. Netted fruit were compared to fruit receiving evaporative cooling (EC) vs fruit receiving Raynox + EC. Temperature sensors were placed under the nets and within the EC section. Fruit conforming to commercial maturity requirements were harvested sequentially, conditioned 7 days at 50F and then stored in CA for 6 months. In 2014, 2 mature orchards (with medium crop load), both located in Gleed, were covered until harvest June 27 and 30 with drape nets in 4 sections each. In-season measurements included: light intensity (as PAR), daily temperature (with Hobo sensors), sunburn development (reading before net installation and prior to 1st pick), and amount of bird pecks. Fruit was harvested in 3 sequential weekly picks starting Sept. 1. Care was taken to obtain mixed samples of commercial WAEXF quality and procedures

included: ≥55% red color, even sampling from east and west as well as top, middle, bottom of all trees within the section. Fruit maturity was determined at harvest and after storage. After storage, evaluations included external disorder evaluation of 50 fruit/trt/rep, flavor of 20 frt/trt/rep (apple, bland, off flavor). Storage treatments were: 3 months RA, 3 months RA + 1-MCP, 6 months CA, 6 months CA + 1-MCP.

2. *Postharvest factors:*

Maturity assessment: Fruit from multiple lots will be harvested over a several week period covering pre- through post- commercial maturity. Fruit from each orchard/harvest will be conditioned 7 days at 50 °F and then stored in air or CA for up to 8 months. SmartFresh application is performed during preconditioning. At harvest, starch index, firmness, soluble solids content, titratable acidity, weight, red and background color, internal ethylene content will be assessed as will chlorophyll fluorescence, density, and dry matter content.

2.1 Bitter Pit and Rapid CA Rapid CA during preconditioning of two orchard lots was evaluated for impacts on bitter pit development. All fruit were held at 50 °F for 7 days, then at 37 °F. Fruit were treated or not with SmartFresh, stored in air, or stored in CA (2% O_2, 1% CO_2) after 2 days of preconditioning (rapid CA) or after completion of preconditioning plus 2 days at 37 °F.

2.2 Short-term Rapid CA and Disorders

Fruit from the same two orchards as in 2.1 were held in rapid CA at 37 °F for 1 to 8 weeks then was transferred to air at 37 °F.

2.3 Fruit Chlorophyll Fluorescence during Cooling

Chlorophyll fluorescence of fruit from 11 orchard lots at harvest was assessed during cooling to 33 °F. Fruit were stored in air at 33 °F and disorders assessed periodically.

2.4. Utility of Fluorescence, Specific Gravity, and Dry Matter as Honeycrisp Maturity Indices

Fruit from 11 orchard lots were analyzed at harvest, fruit specific gravity was calculated from fruit weights determined in air and under water, and % dry matter was calculated from weights prior to and after drying for 2 days at 160 °F. Additional fruit from each lot were conditioned at 50 °F for 7 days, then stored in air at 37 °F.

2.5 Honeycrisp CO_2 Exposure during SmartFresh Treatment

Fruit were exposed to 0 or 1 ppm SmartFresh at 50 °F with 0, 2, or 4% CO_2 added to treatment chambers. Chambers were opened after 24 hours and fruit held an additional 6 days at 50 °F. Fruit were then stored at 37 °F for 4 months.

2.6 Honeycrisp Volatile Production During Storage

Fruit were treated or not with SmartFresh the day of receipt, conditioned for 7 days, cooled to 37 °F over 2 days, then held in air or CA (2% O_2, 1% CO_2). Volatiles were collected while the fruit was in cold storage.

RESULTS & DISCUSSION

1. Orchard factors

1.1. Fruit position in tree:

(2013 results, 2014 experiments in progress)

2013: - soft scald development was influenced by harvest sequence and location of fruit within the tree; disorder sensitivity increased with advance in harvest date for fruit grown in the middle and lower parts of the canopy while fruit grown in top parts of the canopy exhibited soft scald beginning with the second pick, but at much lower overall levels (Figure 1).

2014: - DA-value range was similar to 2013 (1.19 – 0.65 from 1st to 3rd pick)

-maturity general: fruit grown in the top section of the canopy was generally firmer, sweeter and more acidic (data not shown),

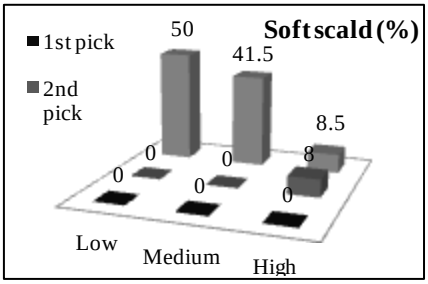


Figure 1: Development of soft scald in Honeycrisp apples stored for 12 weeks at 33F. Fruit was harvested in 3 picks from three canopy positions in 2013.

while starch degradation rates, background color change, fruit size, and DA meter values appeared to be independent regardless of position of fruit within the canopy
-1st pick: the lone maturity parameter affected by netting was higher colored fruit in the upper netted section as compared to the lowest untreated section
-2nd pick: netted fruit had lower sugar concentration and higher DA meter values
-3rd pick: netted fruit had lower sugar levels

1.2 Altered light and temperature:
2013 (storage): - fruit treated with a sunburn protectant (Raynox) + EC did mature similarily to fruit treated with EC alone (example of orchard 1 in Table 1) or EC + Net (pilot study); fruit from orchard 1 was considered of optimum quality for long term storage, while the other two locations had compromised fruit quality at harvest (data not shown)
- maturity after storage was not altered by preharvest sunburn protectant application or netting (data not shown)
-disorder pressure after storage was low in all orchards, and severity was not influenced by in-field or storage treatments (orchard 1: Table 2) including netting (not shown); results on grease development are inconclusive based on preharvest sunburn protectant use (Table 2)
- eating quality of fruit from orchards harvested with sub-optimal quality was compromised (not shown); Raynox + EC treated fruit tasted better after 6 months CA storage than fruit treated with EC alone (Figure 2), especially when picked early (1st and 2nd pick)

	At harvest					
	Raynox			UTC		
Picking sequence	1st	2nd	3rd	1st	2nd	3rd
SSC (ºbrix)	0.54 ns	0.49 ns	0.48 ns	0.53	0.49	0.45
TA (%)	13.1 ns	13.7 ns	13.2 ns	12.7	13.6	13.4
Firmness (lbs)	15.2 ns	14.1 ns	13.2 ns	14.8	13.9	13.4
Starch (1-6)	5.2 ns	4.7 ns	5.3 ns	5.3	4.6	5.2
Background color (1-4)	2	2	2	2	2	2
DA-meter (0-5)	0.86	0.69	0.69	0.98	0.75	0.70

Table 1: At harvest quality of Honeycrisp from orchard 1.

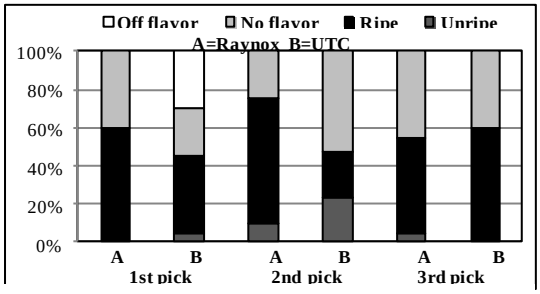


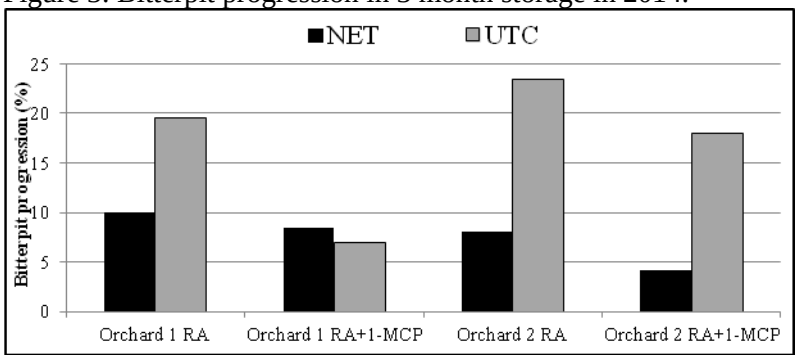
Figure 2: Eating Quality of Honeycrisp after 6 month CA storage.

	3 months RA						6 months CA					
	Raynox			UTC			Raynox			UTC		
Picking sequence	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Soggy breakdown (%)	0 ns	0 ns	0 ns	0	0	0	0 ns	1 ns	9 ns	1	0	8
Soft scald (%)	0	0	0 ns	0	0	0	0 ns	2 ns	4 b	0	1	12 a
No Grease (%)	na	na	na	na	na	na	98 a	77 b	74 ns	55 b	87 a	82

Table 2: 2013 chilling disorder and greasiness developemnt of Honeycrisp from orchard 1

2014: - in-season light intensity (as PAR), sunburn severity at harvest, amount of bird pecks, and maximum temperature were reduced in both locations (data not shown)
-At harvest red color development was delayed in one orchard, un-netted fruit was sweeter, firmer, and less mature (data not shown)
-preliminary results (1st pick only) indicate after 3 month storage, bitterpit incidence increased in both orchards faster in un-netted sections (from zero to ± 20%) (Figure 3), while chilling injury levels were low (soft scald, soggy breakdown); eating quality of fruit was compromised by presence of considerable amounts of off flavor (5-38%) in all fruit, best tasting fruit (regardless of orchard location) had received 1-MCP treatment (data not shown)

Figure 3: Bitterpit progression in 3 month storage in 2014.



2.1 Bitter Pit and Rapid CA In 2013, lot one fruit developed extensive bitter pit during storage except for fruit treated with SmartFresh then stored in rapid CA (Table 3). Some rapid CA controls and SmartFresh fruit developed large, rough, brown peel lesions (leather blotch) during storage but the % fruit affected was lowest for rapid CA fruit. No soft scald developed in fruit from either lot. All fruit with leather blotch also had bitter pit. Lot two (data not presented) had a low incidence of bitter pit and treatment effects were not significant. No symptoms attributable to rapid CA (external or internal browning, off-odors) were observed in fruit from either lot.

Lot 1	% bitter pit	% leather blotch	% total disorder
CA	69ab	0b	69ab
Rapid CA	44bc	9b	44b
air	87a	0b	87a
SF CA	51ab	43a	51b
SF Rapid CA	12c	3b	12c
SF air	56ab	38a	56ab

Table 3. Incidence of bitter pit and leather blotch on Honeycrisp apples, 2013 harvest, through 7 months. CA: 2% O_2, 1% CO_2. SF: SmartFresh.

To date in 2014, lot 1 (same as in 2013) has developed less bitter pit compared to 2013 but rapid CA is not significantly different compared with other treatments for lot 1 and lot 2 incidence is low (Table 4). Lot 2 has some soft scald.

	% bitter pit		% soft scald	
	lot 1	lot 2	lot 1	lot 2
CA	35a	10a	0	7bc
Rapid CA	17ab	1b	1	17ab
air	35a	4ab	0	1c
SF CA	14b	10a	1	8bc
SF Rapid CA	22ab	1b	0	14ab
SF air	29ab	1b	0	8bc

Table 4. Incidence of bitter pit and soft scald on Honeycrisp apples, 2014 harvest, through 2 months. CA: 2% O_2, 1% CO_2. SF: SmartFresh.

2.2 Short-term Rapid CA and Disorders
Through 2 months, no effects on bitter pit of a shortened CA storage period compared to fruit held continuously in CA were evident (results not shown).

2.3 Fruit Chlorophyll Fluorescence during Cooling

A range of fluorescence (Fα) values at harvest was observed with the highest initial value in the same lot as in 2013. No relationship between fluorescence at harvest and soft scald or bitter pit after 2 months was observed (Figure 4). Typically lots with one disorder had a low or no incidence of the other disorder.

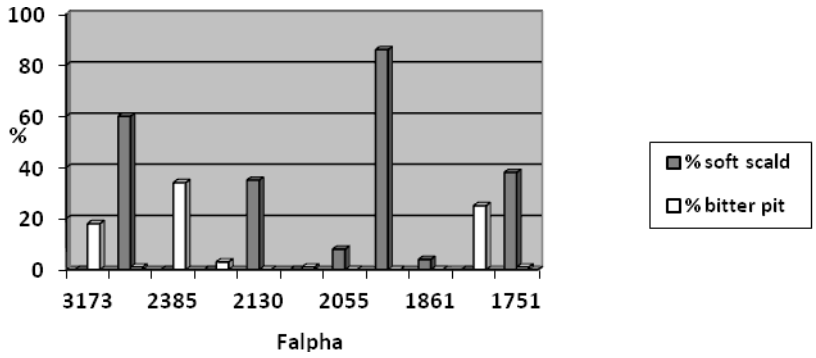


Figure 4. Honeycrisp disorders and fruit chlorophyll fluorescence during cooling at harvest.

2.4. Utility of Fluorescence, Specific Gravity, and Dry Matter as Honeycrisp Maturity Indices

Fluorescence (Fα), density, and dry matter varied with orchard and were not correlated with other maturity indices (Table 5).

Lot	1	2	3	4	5	6	7	8	9	10	11
Fα	3173	2413	2385	2105	2130	1912	1861	1751	2279	1831	2055
weight g	273	243	276	281	246	273	220	190	250	281	247
under water g	336	301	348	334	257	337	265	238	305	334	306
specific gravity	0.81	0.81	0.79	0.84	0.96	0.81	0.83	0.80	0.82	0.84	0.81
dry matter %	--	--	13.9	14.2	13.8	20.6	20.9	17.5	19.4	23.2	19.2
SSC %	14.4	13.5	12.9	12.5	12.8	13.6	13.5	10.7	11.8	15.0	11.4
TA %	0.530	0.523	0.492	0.556	0.489	0.438	0.373	0.451	0.363	0.553	0.411
starch 1-6	4.6	5.0	5.2	3.3	5.4	5.7	6	5.9	6	5.5	5.8
IEC ppm	12.5	4.9	18.3	3.4	21.6	21.2	65.2	0.55	33.2	28.0	17.7
color 1-5	3.3	3.6	3.1	3.6	2.7	3.7	3.1	2.2	3.4	3.9	2.9
lbs	13.5	14.1	12.6	13.8	12.5	13.9	13.8	12.3	11.9	14.1	12.3

Table 5. Honeycrisp maturity indices at harvest. Fα: fluorescence; SSC: soluble solids content; TA: titratable acidity; IEC: internal ethylene content; color: peel ground color rating, 1=green, 5=yellow.

After two months in storage, soft scald risk was highest in low weight fruit, while bitter pit risk was highest in high weight fruit (Figure 5).

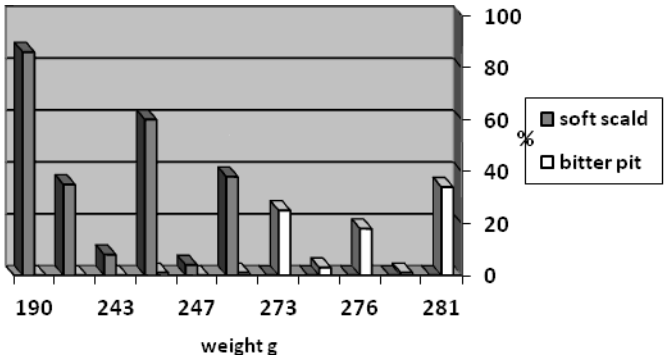


Figure 5. Honeycrisp disorders and fruit average weight by lot at harvest.

2.5 Honeycrisp CO_2 Exposure during SmartFresh Treatment

Similar to 2013, incidence of cortex browning after 4 months in air storage was low and not related to CO_2 or SmartFresh (Table 6). No impacts on fruit quality were observed attributable to high CO_2 during the 24 hour treatment period.

Orchard	% CO_2	% cortex browning	
		Control	SmartFresh
A	0	0	0
	2	0	0
	4	0	0
B	0	11	2
	2	6	2
	4	2	2

Table 6. Honeycrisp CO_2 injury after storage.

2.6 Honeycrisp Volatile Production During Storage

The storage chamber volatile profile is predominantly ethyl esters, compounds that are made from ethanol, during the first month in storage for all treatment combinations except CA no SmartFresh which had lower amounts of these compounds. Between 1 and 2 months and thereafter for air no SmartFresh, a large group of non-ethyl esters predominates. An increase in some of these compounds was detected in CA no SmartFresh chambers starting at 7 months. The patterns for SmartFresh in air and SmartFresh in CA are very similar for all volatile compounds throughout the 8 month storage period, with amounts for most volatiles lower compared with air or CA no SmartFresh. Incidence of disorders (soft scald, soggy breakdown, cavities, internal browning) was very low and no consistent treatment relationships were present.

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

Project Title: Commercial testing of early scald risk assessment tools

PI: David Rudell
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Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Drs. Jinwook Lee, Bruce Whitaker, and Christopher Watkins

Total Project Request: **Year 1:** $54,881 **Year 2:** $56,275 **Year 3: $57,675**

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. 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).

Agency Name: AgroFresh, Inc.
Amt. awarded: $90,000
Notes: Continued development of systems for implementation of biomarker-based tools developed from the above SCRI project as well as finding additional biomarkers.

Budget
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510)559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2013	2014	2015
Salaries	$38,417	$39,302	$40,372
Benefits	$16,464	$16,972	$17,302
Wages			
Benefits			
Equipment			
Supplies[1]			
Travel			
Total	$54,881	$56,275	**$57,675**

Objectives:

1. Determine if risk assessment tools accurately represent scald risk in multiple commercial lots of Granny Smith apples.
2. Test scald risk assessment tools using Delicious apples.
3. Validate additional biomarkers for CA storage.
4. Extend search for biomarkers for at-harvest superficial scald risk assessment tools.

Goals and activities for the next year: Perform a harvest maturity experiment using Delicious to test risk assessment. Repeat Granny Smith and Delicious storage monitoring trials in commercial storages. Test gene expression-based risk assessment tools. Compile scald risk assessment biomarker (SRAB) monitoring data to establish threshold values for absolute SRAB values.

SIGNIFICANT FINDINGS:

1. Delaying CA imposition results in enhanced ethylene and SRAB levels.
2. SRAB levels increase with higher O_2 levels in CA storage.
3. Scald risk increases with SRAB levels for Delicious in test chambers.
4. SRAB levels can assess Granny Smith scald risk as early as 1 month in storage.
5. SRAB monitoring can be used to monitor how multiple factors associated with room loading, impacts of other fruit in the same room, and room atmosphere/integrity affect scald risk.

Methods:

Equipment and Cooperative Summary: Tissue sampling, processing and analysis of biomarkers using analytical instrumentation (gas and liquid chromatography-mass spectrometry, spectrophotometry) will be performed at ARS-TFRL, Wenatchee. Storage experiments will be performed both at ARS-Wenatchee and in commercial storages. Parallel trials were performed in-house by AgroFresh with our consultation. Additional chemical identification will be performed in cooperation with Dr. Bruce Whitaker (BARC, USDA-ARS, Beltsville, MD).

Procedures:

Year 1

Validating SRAB tools for Delicious apples

Scarlet Spur Delicious apples were harvested 3 weeks prior to commercial harvest. Fruit maturity and quality were evaluated at harvest. Apples were stored at 33 ^oF in CA at 0.5% or 2% O_2 (all 1% CO_2), and SRAB levels monitored monthly. One of the 2% O_2 chambers was pulled down to 0.5% O_2 once SRAB levels indicated risk was elevated. Scald was evaluated in all treatments at 0 and 7 days (at 68 ^oF) at 3, 6, and 9 months storage.

Lot to lot scald assessment in commercial Granny Smith storages

Multiple lots (10 lots in 3 storage facilities) of Granny Smith were commercially harvested and stored in organic CA rooms. Fruit were moved to a common CA storage set at 0.5% O_2/ 0.5% CO_2 once commercial rooms were opened. Monthly estimation of scald risk was performed using biomarker-based scald risk assessment tools and scald was evaluated monthly through 10 months. Another study funded and performed by AgroFresh evaluated biomarkers on fruit from 4 orchards. SRAB levels and scald were monitored monthly using our protocols through 4 months.

Impacts of rapid CA and ethylene scrubbing on scald development and risk assessment

A single lot of Granny Smith with historically high risk for scald development was stored in 4 research CA rooms (30 bins/room). Room atmospheres of 0.5% O_2, 0.5% CO_2 were imposed either immediately upon loading or after 1 week with or without ethylene scrubbing. Scald development and SRAB levels were evaluated monthly as well as room ethylene and other volatile compounds including volatile SRABs.

Impact of rapid CA imposition of non-superficial scald peel injury development
Granny Smith apples harvested 1 month prior to commercial harvest were stored in 0.5% O_2, 0.5% CO_2 in USDA, ARS experimental CA chambers. Storage atmosphere was imposed while fruit were still warm or after fruit had reached 33 ^oF. Peel injury and scald development was monitored from 3 through 9 months plus 7 days at room temperature, after 10 months apples were held 14 days at room temperature.

Year 2

Lot to lot scald assessment in commercial Granny Smith and Delicious storages
Fruit (8 lots Delicious, 11 lots Granny Smith) were sorted at the receiving station into doorway boxes to be sampled from multiple 2000 (Delicious) or 800-1000 (Granny Smith) bin commercial rooms Room atmospheres were set between 0.5 and 1% O_2 by our commercial cooperator according to their protocol. Peel samples are being collected at 0, 1, 2 weeks and 3, 4, 6, and 9 months with scald evaluated at 0, 7, and 14 days after removal from storage. SRAB levels are estimated immediately following removal using the spectrophotometric assay. Apples will be transferred at 3 months from each storage and moved to 0.5 % O_2 to assure equal time in equal storage environments or air to simulate long-term holding as commercial rooms are emptied and delivered to the supply chain.

Impacts of rapid CA imposition and ethylene scrubbing on scald development and risk assessment

Fruit from three Granny Smith orchards are being stored in 2 RCA rooms (30 bins/room). Room atmospheres of 0.5% O_2, 0.5% CO_2 were imposed either immediately upon loading or after 2 weeks. Scald development, internal ethylene concentration (IEC), room ethylene, and SRAB levels are being evaluated monthly as well as room ethylene and other volatile compounds including volatile SRABs.

Year 3
At-harvest scald risk assessment
Delicious apples will be harvested multiple times from a location with a history of scald susceptibility. Apples will be stored in air for up to 6 months. Peel tissue will be sampled at harvest and 2 and 4 weeks following harvest. Scald incidence and severity will be assessed monthly.

RESULTS & DISCUSSION

Year 1

Scald risk assessment for Delicious apples
Scald risk assessment biomarker (SRAB) levels monitored in Scarlet Spur Delicious apples stored in air began to increase between 1 and 2 months. SRAB levels in CA fruit increased between 2 and 3 months, then the O_2 was decreased to 0.5% in one of the 2% O_2 chambers. Increased SRAB levels preceded scald development on fruit stored in air or 2% O_2. Apples stored in 0.5% O_2 did not develop scald and SRAB levels did not increase. Reducing O_2 after scald risk was detected in fruit

stored at 2% O_2 did not prevent scald. These results are, in part, consistent with previous results for Granny Smith.

Impacts of delayed CA on scald development of Granny Smith apples
A one week CA delay lead to higher IEC, room ethylene, and SRAB levels (Fig. 1) but significant scald incidence was not observed in any of the treatments after 10 months storage. IEC continued to increase as did room ethylene for the storage period indicating that rapid CA imposition is key to controlling this event. SRAB levels were considerably lower in all of the treatments than those that preceded scald development in previous years' trials. In year 2, CA imposition was delayed for 2 weeks and fruit from 3 orchards will be moved to air storage at 3 months to simulate long-term transport and retail. Again, SRAB and IEC are already considerably higher in the room where CA was delayed (Fig. 3).

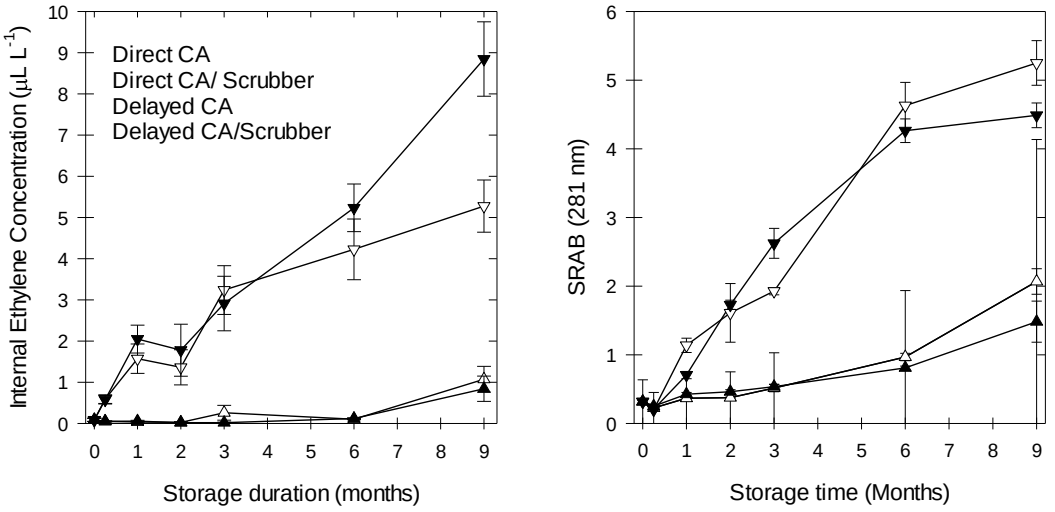

Fig. 1. (Year 1) Fruit internal ethylene concentration (left) and SRAB levels (right) over storage period in research CA rooms (30 bins, 1 orchard) set at 0.5% O_2/0.5% CO_2 immediately or following a 1 week delay. SRAB levels were higher in rooms with delayed CA imposition as were IEC values, although SRAB levels were lower than those recorded in past experiments in fruit at high risk for scald development. Error bars represent standard error (n=3 for SRAB evaluation; n=18 for IEC assay).

Scald risk assessment of Granny Smith and Delicious apples stored in commercial rooms
SRAB levels increased in Granny Smith apples stored in 2 organic commercial rooms (8 lots total) but levels (281 nm) were considerably lower than those associated with high scald risk in past experiments. There was a difference in overall SRAB levels between the two rooms after 2 months of storage, although the difference disappeared following 3 months storage. Scald was detected only after 8 months+7 days at room temperature. When the room was opened for processing, sample fruit was removed and placed in an RCA room set at 0.5% O_2 possibly impacting the scald outcome as previous experiments in our test chambers and in RCA rooms have suggested, where lowering storage oxygen later in storage can positively impact scald outcome.

In the AgroFresh experiment, SRAB levels were much higher than our previous results in all fruit except for those treated with SmartFresh where no scald was detected at the end of the trial (Fig.2).

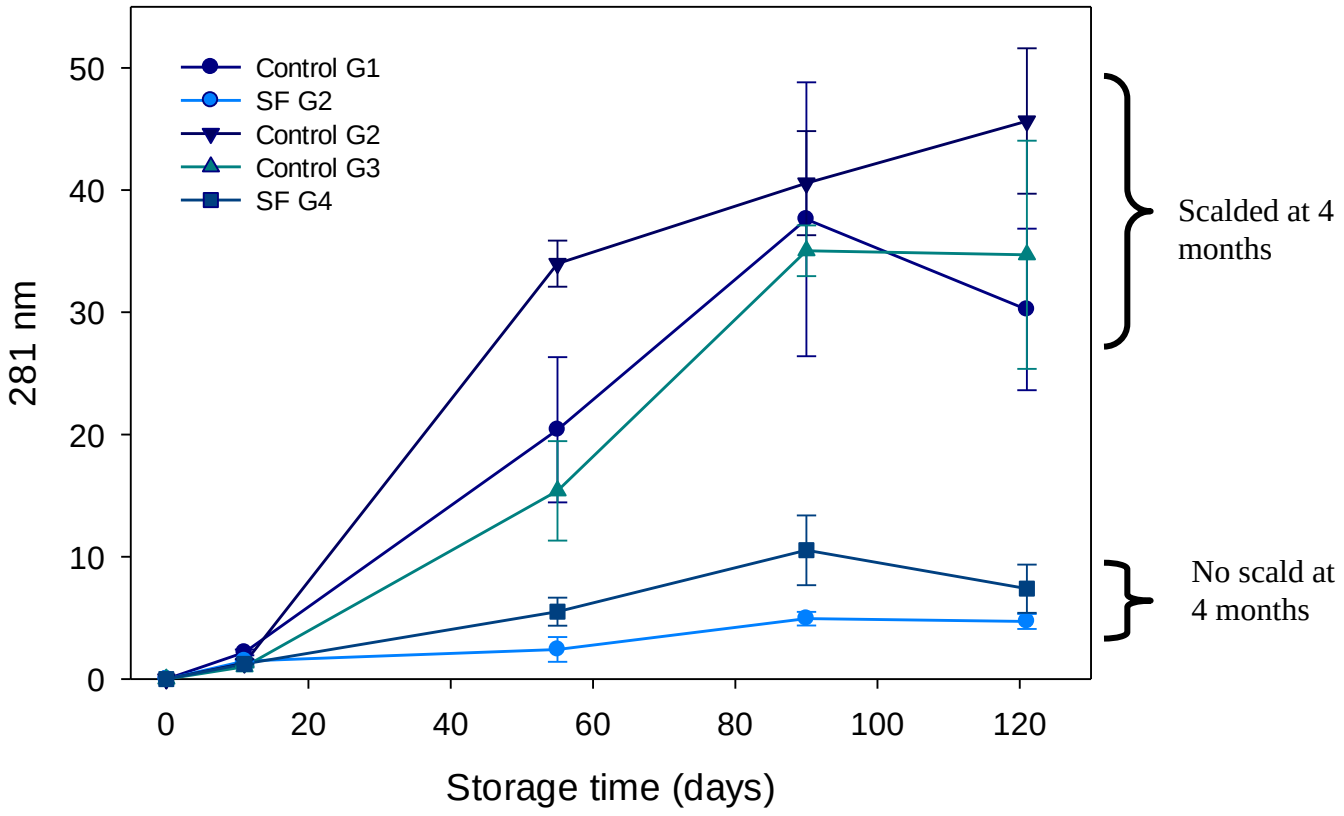

Fig. 2. Granny Smith SRAB levels and final scald incidence after 4 months commercial CA storage in an AgroFresh study using ARS methodology. Apples were chosen from a total of 4 growers treated with SmartFresh (SF) after harvest. Error bars represent standard error (n=3).

Year 2

To improve upon the year 1 trials, year 2 (2014) trials employ fruit from additional Granny Smith and Delicious orchards and commercial CA rooms. Granny Smith average SRAB levels were already considerably higher in one of the rooms after 1 month indicating a higher risk in this room (Fig. 3, left). Average values among orchards are very similar within the same CA room indicating factors contributed by the room (ie. room loading time, other fruit in the room, oxygen concentration) had a greater impact on SRAB levels than factors brought in from the orchard. Samples from one of the orchards are stored in 2 commercial rooms and are also used in the delayed CA imposition trial this year (Fig. 2, right). SRAB levels at 1 and 2 months were nearly equal in Room 4 and the RCA room with 2 week CA imposition while they remain relatively the same as the initial values in the other rooms. SRAB levels in one of the commercial Delicious rooms are slightly higher (not shown). While IEC levels were higher in Delicious than Granny Smith at harvest, indicating fruit was more mature, SRAB levels remained very similar to similarly stored Granny Smith. SRAB levels across all of the trials this year do not increase alongside IEC indicating there are other factors contribute to their generation (not shown). Fruit from each of the commercial and RCA trials will be moved into air to simulate long term transport and 0.5 % O_2 storage to assure the contributions to scald development of these first three months of storage are evaluated equally.

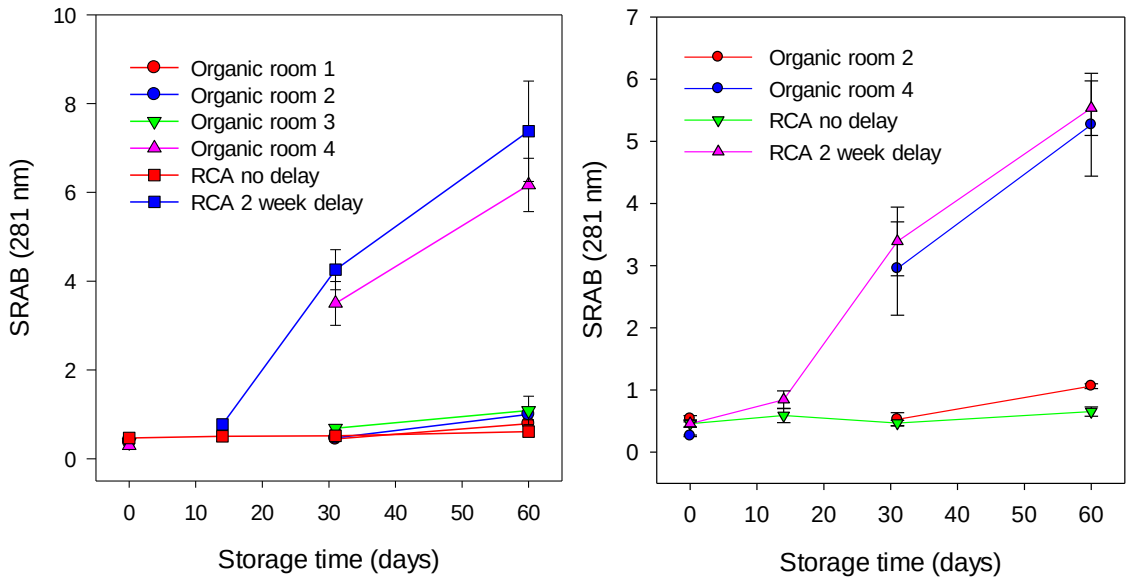

Fig. 3. (left) Average SRAB levels for samples from organic Granny Smith storage rooms and organic research CA room trials (Year 2). Averages represent samples from 3 orchards in each room. The low variability of SRAB levels among orchards in a particular room indicate factors evoked by the room have a greater impact on SRAB levels than those from the field. (right) SRAB levels of the same orchard lot stored under 4 different storage conditions including 2 commercial rooms and RCA rooms pulled down immediately or after 2 weeks to 0.5% O_2:1% CO_2. Error bars represent standard error (n=3).

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-13-105

Project Title: Effect of crabapple pruning on Sphaeropsis and speck rot incidence

PI: Yong-Ki (Richard) Kim
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Cooperators: Growers: Jeff Cleveringa (Oneonta/Starr Ranch Growers), Bob Bossen (Northern Fruit Company) , and Teah Smith (Zirkle Fruit Company)

Total Project Request: **Year 1:** $20,984 **Year 2:** $26,756 **Year 3: $15,451**

Other funding sources: None

WTFRC Collaborative Expenses:

Item	2013	2014	2015
Salaries			
Benefits			
Wages[1]	137	137	
Benefits	66	66	
RCA Room Rental[2]	2,100	2,100	
Shipping			
Supplies			
Travel[3]	190	190	
Plot Fees			
Miscellaneous			
Total	$2,493	$2,493	

Footnotes: [1]Wages and Benefits @ $15/hour (two trees pruned per hour); [2] Storage for approximately 50 cartons of fruit (1/3 of a RCA room@ $6,300 per room); [3]Travel @ $0.555 per mile for two trips each to 3 sites in central Washington.

Budget 1

Organization Name: Pace International **Contract Administrator:** Micah Dunstan

Telephone: 206-331-4777 **Email address:** micah.dunstan@paceint.com

Item	2013	2014	2015
Salaries			
Benefits			
Wages[1]	8,640	12,980	8,986
Benefits[2]	2,851	4,283	2,965
Equipment			
Supplies[3]	4,500	4,500	2,500
Travel[4]	2,500	2,500	1,000
Plot Fees			
Miscellaneous			
Total	$18,491	$24,263	**$15,451**

Footnotes: [1] Wages for a part-time research technician to work 720 hrs in year 1 and 3 and 1040 hrs in year 2 at $12/hr for performing sample collection, pathogen isolation, decay evaluation, and data management. The increase in wages for years two and three reflects a 4% rate increase. [2] Benefits 33%. [3]Supplies include culture media, chemicals, petri-dish plates for isolation of fungi. [4] Travel to orchards for sampling and harvesting is required for sampling and harvesting.

Objectives:

1. Generate practical information regarding the impact on commercial cultivars of removal of inoculum from infected 'Manchurian' crabapple pollinizers in commercial apple orchards as part of a postharvest decay IPM program
2. Understand the in-season incidence of fruit infection by *Sphaeropsis pyriputrescens* and *Phacidiopycnis washingtonensis* following winter pruning of adjacent crabapple trees
3. Evaluate the impact of crabapple pruning on the incidence of Sphaeropsis rot and speck rot in commercial cultivars following storage

Significant Findings:

- In 2013, pruning crabapple trees significantly reduced the incidence of speck rot in apples during cold storage compared to the unpruned control.
- Fruit infections monitored during the 2013 growing season were highly correlated to the development of speck rots in storage.
- All crabapple samples collected from orchard D with a history of Sphaeropsis rot harbored viable pycnidia of the fungus before and at the time of pruning, indicating that they are the primary source of inoculum for *Sphaeropsis* infections in apples.
- Regardless of the treatments, the fruit infections in orchards A and C during the 2014 growing season were numerically lower than those in 2013, probably due to that all crabapple trees that were not selected for the study were chainsaw pruned in these orchards.
- No or limited infections of *P. washingtonensis* were detected on apple fruit adjacent to crabapple trees that were detail-pruned in 2013 and 2014.
- No *Sphaeropsis* infections on apple fruit during the growing season were detected in orchard D, even though viable pycnidia of the fungus were observed in all crabapple trees sampled from the orchard.

Methods:

Orchard and plot design

In 2014, orchard B was excluded due to the similarity of the infection and decay development patterns as orchard C and ununiformed crabapple planting in the orchard; instead an orchard that had a history of Sphaeropsis rot (Orchard D) was added to evaluate the effect of crabapple pruning on Sphaeropsis rot caused by *S. pyriputrescens*. All crabapple trees used in 2013 were treated as the previous year in orchards A and C, but the trees that were chainsaw-pruned in 2013 were pruned as the detailed pruning before bloom in the 2014 season (March 27, 2014 in orchard A and March 25, 2014 in orchard C). For the orchard newly added in 2014 (Orchard D1), four replicated rows, three crabapple trees per replicate in the middle of the orchard with two buffer rows between the replicate were randomly selected. Treatments were unpruned control, detailed pruning before bloom, and detailed pruning after bloom. However, the entire block was pruned by an accident after petal fall. Therefore, another block with a history of Sphaeropsis rot (Orchard D2) was added to compare unpruned control vs. commercially pruned crabapple trees.

Decay evaluations of apples harvested in 2013

To evaluate the development of speck rot in cold storage, eighty apple fruit per replicate were harvested from trees adjacent to the crabapple trees with different treatments during commercial harvest dates. Fruit were placed on apple trays in cardboard boxes at 0-4°C cold storage. The development of decay symptoms were examined monthly for up to 10 months after harvest. To confirm the causal agent, decayed fruit were gently sprayed with 70% ethanol and allowed to dry in a hood. The skin of the diseased area on the fruit was peeled off using a sterile scalpel, and then small fragments of fruit flesh were cut and placed on acidified potato dextrose agar (APDA; 4 ml of a 25% solution of lactic acid per liter of medium). Plates were incubated at room temperature (22 ± 1ºC) for up to 7 days. The fungus was identified based on the morphological characteristics on APDA and confirmed according to the descriptions of *P. washingtonensis* (Xiao et al. 2005).

Identification of S. pyriputrescens in infected crabapple trees

Three twigs with dieback or canker symptoms and three mummified crabapple fruit per tree from ten trees in orchard D were randomly sampled before bloom (Block 1) or at the time of pruning crabapple trees (Block 2). Samples were examined under a dissecting microscope for the presence of pycnidia. Pycnidia similar to that of *S. pyriputrescens* were then crushed in sterile water and examined under a microscope. A drop of conidial suspension was streaked on APDA to establish cultures of the fungus. To confirm the pathogens on twigs with dieback or canker symptoms, samples were surface-disinfested by immersing 5 min in 0.6% sodium hypochlorite solutions, rinsed twice with sterile deionized water, and air-dried in a laminar hood. Small segments were cut from the margins between the diseased and healthy tissues of the twigs and embedded in APDA. If no pycnidia are formed, mummified fruit were then gently sprayed with 70% ethanol and allowed to dry in a hood. The skin of the fruit was peeled off using a sterile scalpel, and then small fragments of fruit flesh were cut and placed on APDA. Plates were incubated at room temperature for up to 7 days. The fungal identification was confirmed according to the descriptions of *S. pyriputrescens* (Xiao et al. 2004).

In-season monitoring of fruit infections in 2014

To monitor fruit infections among the treatments during the fruit growing season, ten apple fruit from trees adjacent to the treated (chainsaw and detailed) or control (unpruned) crabapple trees in replicate (four replications per treatment) were collected every 5-6 weeks from 3 weeks after petal fall to harvest. After being surface-disinfested in 0.6% sodium hypochlorite solutions for 5 min, rinsed twice with sterile water, and air-dried in a laminar hood, stems and sepals of the fruit were aseptically excised and embedded on APDA. The presence and absence of either *P. washingtonensis* or *S. pyriputrescens* were examined for up to 7 days. The identifications of the fungi were confirmed as described above.

Data analysis

All data in percentage were arcsine-transformed before performing analysis of variance using SAS (version 9.2; SAS Institute, Inc., Cary, NC). Since there was no significant interaction between the treatments and sampling times in fruit infection monitoring during growing season, data were pooled and the mean separation was conducted using the least significant difference ($P = 0.05$). Correlation analysis was performed using PROC CORR of SAS ($P = 0.05$) to compare the monitoring of fungal infections on stems and sepals of apple fruit during the fruit growing season and the decay development during cold storage on apple fruit harvested in 2013.

Results and Discussion:

Development of speck rots in storage

Pruning crabapple trees that involved removing infected twigs and branches significantly reduced the incidence of speck rot in apples during cold storage compared to the unpruned control, although the differences among the treatments were not statistically significant in orchard B (Table 1). Detailed pruning was numerically better than the chainsaw pruning in controlling speck rot in all three orchards. Although detailed pruning significantly decreased the fruit infections during growing season compared to the chainsaw pruning, the difference was not significant in the development of speck rots in storage. It is apparent that pruning crabapple trees in the apple orchard can reduce speck rot of apple during cold storage. However, we could not demonstrate a complete control of speck rot on apples by pruning crabapple trees since our study was conducted in the middle of the orchards where unpruned crabapple trees are surrounded. As we observed in the in-season infection monitoring, the incidence of speck rot in orchard A was higher than orchard B or C (Table 1), probably due to the use of overhead sprinklers in orchard A.

Table 1. Incidence of infection during the fruit growing season and speck rot in storage caused by *Phacidiopycnis washingtonensis* on apples adjacent to crabapple trees that were unpruned (control), chainsaw pruned, and detail pruning that involved the removal of all infected twigs, branches, and fruit mummies in three commercial 'Red Delicious' orchards in 2013

		Incidence of *P. washingtonensis* (%)[z]	
Orchard	Treatment	Infection in-season	Speck rot in storage
A	Unpruned	25.0 a	23.5 a
	Chainsaw	10.0 b	16.6 ab
	Detailed	5.6 c	7.8 b
B	Unpruned	10.6 a	8.1 a
	Chainsaw	4.4 ab	5.9 a
	Detailed	3.8 c	3.1 a
C	Unpruned	10.6 a	11.6 a
	Chainsaw	5.6 b	3.4 b
	Detailed	1.9 c	2.5 b

[z] Values within an orchard and column, when followed by a common letter, are not significantly different according to the analysis of variance and least significant difference ($P = 0.05$).

Correlation between fruit infection and speck rot development in storage

To analyze the relationship between the fruit infection monitored during the fruit growing season by isolating *P. washingtonensis* on stems and sepals of apple fruit and speck rot development during cold storage, Pearson correlation analysis was performed. The result indicated that there was significant correlation between the fruit infection by *P. washingtonensis* during growing season and the decay development in storage in all three orchards ($P < 0.05$) (Fig. 1). Therefore, monitoring fruit infections during growing season can predict the incidence of speck rot on apples in storage.

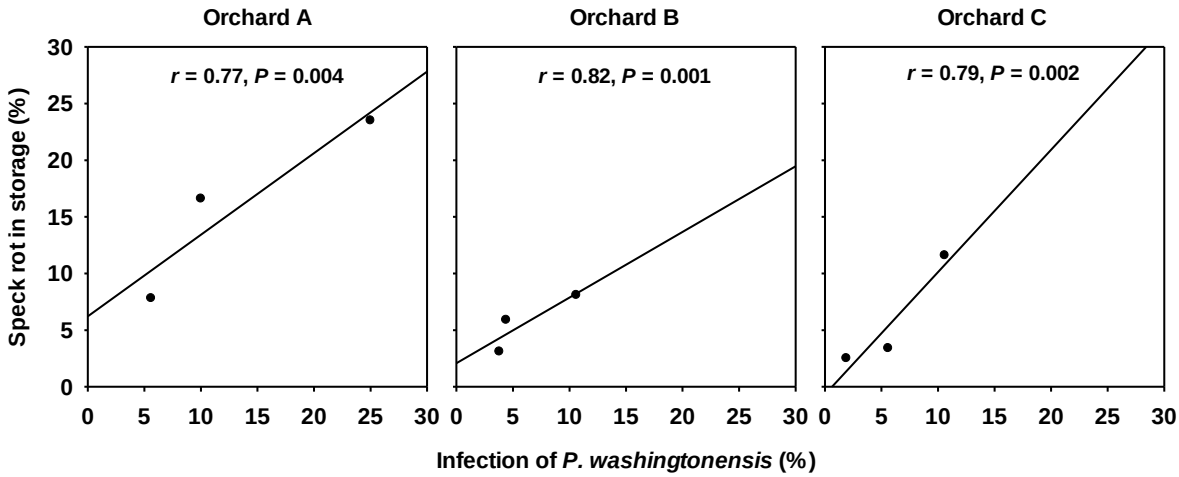

Fig. 1. Correlation between the infection of *Phacidiopycnis washingtonensis* on stems and sepals of apple fruit adjacent to crabapple trees during fruit growing season and speck rot development during cold storage harvested from three commercial 'Red Delicious' orchards.

Identification of S. pyriputrescens in orchard D

The presence of *S. pyriputrescens* was confirmed by sampling crabapple twigs with dieback and canker symptoms and mummified crabapple fruit in two 'Red Delicious' blocks of orchard D. Pycnidial spores from all samples were microscopically examined, and the identification of the cultures isolated from diseased tissues was confirmed according to the descriptions of *S. pyriputrescens* (Xiao et al. 2004). All crabapple samples harbored viable pycnidia of the fungus before and at the time of pruning (Data not shown). This result indicates that infected crabapple trees are the primary source of inoculum for *Sphaeropsis* infections in the orchard.

In-season monitoring of fungal infections on apples

Regardless of the treatments, the fruit infections in orchards A and C during the 2014 growing season were numerically lower than those in 2013 (Tables 1 and 2). This might be due to that all crabapple trees in these orchards that were not selected for the study were pruned by the growers after bloom in 2014. No or very few infections of *P. washingtonensis* were detected on apple fruit adjacent to crabapple trees that were detail-pruned in 2013 without additional pruning in 2014, while a limited infection (1.3%) was detected in the chainsaw-detailed treatment, of which crabapple trees were chainsaw pruned in 2013 and detail-pruned in 2014 (Table 2). However, the difference was not statistically significant in orchard A. In the 2014 trial, an orchard with a history of Sphaeropsis rot was added and detail-pruned before and after bloom. However, the entire block including unpruned controls was accidentally pruned by the grower after bloom. Therefore, an additional block with similar crabapple planting in the orchard was added to compare unpruned vs. commercially pruned. Even though viable pycnidia of *S. pyriputrescens* were observed in all crabapple trees randomly sampled in this orchard, no infection of the fungus on apple fruit during the growing season was detected (Table 2).

Work next year

All fruit harvested in 2014 have been monitoring monthly for the development of speck rot or Sphaeropsis rot in cold storage. This coming year, no further in-season monitoring in the orchards is proposed.

Table 2. In-season monitoring of *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* on stems and sepals of apple fruit adjacent to crabapple trees that were unpruned (control), chainsaw pruned, and detailed pruning that involved the removal of all infected twigs, branches, and fruit mummies in commercial 'Red Delicious' orchards in 2014

Orchard	Pathogen	Treatment[y]	Infection in-season (%)
A	*P. washingtonensis*	Unpruned	6.3 a[z]
		Chainsaw-detailed	1.3 a
		Detailed	0.0 a
C	*P. washingtonensis*	Unpruned	4.4 a
		Chainsaw-detailed	1.3 ab
		Detailed	0.6 c
D1[x]	*S. pyriputrescens*	Unpruned	0
		Detailed before bloom	0
		Detailed after bloom	0
D2	*S. pyriputrescens*	Unpruned	0
		Chainsaw	0

[x] All crabapple trees in orchard D1 were accidentally pruned by the grower after pruning treatments.
[y] Crabapple trees that were not selected for the study in all orchards, but D2 were chainsaw-pruned by the growers after bloom. Trees in chainsaw-detailed treatment were chainsaw-pruned in 2013 followed by detailed pruning in 2014.
[z] Values within an orchard, when followed by a common letter, are not significantly different according to the analysis of variance and least significant difference ($P = 0.05$).

Literature Cited:

Xiao, C. L., Rogers, J. D., and Boal, R. J. 2004. First report of a new postharvest fruit rot on apple caused by *Sphaeropsis pyriputrescens*. Plant Disease 88:223.

Xiao, C. L., Rogers, J. D., Kim, Y. K., and Liu, Q. 2005. *Phacidiopycnis washingtonensis* – a new species associated with pome fruits from Washington State. Mycologia 97:464-473.

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

Project Title: Crop load and canopy management of apple

PI: Tory Schmidt
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 Coffey
Telephone: (509) 665-8271 **Email address:** kathy@treefruitresearch.com

Year	2014	2015	2016
Salaries	35,000		
Benefits	10,000		
Wages	50,000		
Benefits	17,000		
Equipment			
Supplies	1,000		
Travel	3,000		
Stemilt lab fees	2,000		
Statistical consulting	1,000		
Total gross costs	119,000		
Reimbursements	(119,000)		
Total net costs	0		

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 PGRs, chemical thinners, and mechanized thinning technologies for apple
2) Refine practical PGR programs to manipulate floral initiation and promote annual bearing
3) Document horticultural effects of synthetic materials deployed as reflective ground covers or overhead shade/wind/bird protection
4) Expand collaborative efforts with other research programs working on crop load and canopy management

2014 HIGHLIGHTS:

Aggressive use of metamitron during and after bloom provided desirable thinning results without damaging fruit or trees (Tables 2 & 4)

Several postbloom thinning programs were effective on Honeycrisp and Golden Delicious (Tables 2 & 4) but relative contributions of calcium phosphite remain unclear

Oil + lime sulfur programs remain the best option for chemical bloom thinning (Table 3); programs using BA generally outperform other postbloom thinners (Table 5)

Multiple applications of 100 ppm GA_3 have been proven effective at reducing return bloom over several years of trials (data not shown) including 2014 results from 2013 applications

New formulations of prohexadione calcium continue to effectively inhibit shoot growth in Fuji (data not shown)

Shade netting draped directly on Granny Smith trees & trellis reduced sunburn and promoted fruit size without sacrificing total tree yields (Table 6)

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. Most of our current trials are funded in part or whole by third party companies that contract our services to independently evaluate their products alongside industry standards. 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.

2014 chemical thinning programs are listed in Table 1; for 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 received the same programs.

Table 1. Chemical thinning programs evaluated. WTFRC 2014.

BLOOM THINNERS (applied 100 gal water/A @ 20-40 &/or 80-90% bloom)
2% Crocker's Fish Oil (CFO) + 3% Lime Sulfur (LS)
2% Wilbur Ellis Supreme Oil (WES) + 10% lime sulfur

5-10% lime sulfur
4 gal ammonium thiosulfate (ATS)/A

POSTBLOOM THINNERS (applied 100 gal water/A at PF & 10mm & 15mm)
48 oz Carbaryl 4L/A
48 oz Carbaryl 4L + 5 oz Fruitone L (NAA)/A
122 oz benzyladenine (BA)/A
122 oz BA + 36-48 oz Carbaryl 4L/A
122 oz BA + 5-6 oz Fruitone L/A
24 oz BA 5XL + 36 oz Carbaryl 4L/A
400-1000 ppm metamitron
400 ppm metamitron + 6 oz Fruitone L (NAA)/A
9.6 oz Apogee (Apo) + 2.8 oz ProVide (Pro)/A
64-96 oz Sysstem-Cal (SC) + 122 oz BA + 48 oz Carbaryl 4L/A
64-96 oz Sysstem-Cal + 122 oz BA/A
100 gal water + 16 oz Regulaid/A
100-200 gal water/A

BLOOM THINNING:
Although we did not conduct any dedicated bloom thinning trials in 2014, two studies evaluating overall chemical thinning programs did include a few bloom thinning treatments; while none of those treatments produced significant results, they tended to reduce fruit set compared to controls (Table 2). A trial on Golden Delicious demonstrated that aggressive use of the sugar beet herbicide metamitron could reduce fruit set and improve harvest size without damaging trees or fruit. This chemistry remains of interest primarily as a postbloom thinner, but showed modest potential when applied during bloom as well. A second contract trial for ENZA to evaluate thinner effects on crop load and fruit finish of Scilate (Envy) once again showed no major concerns regarding russet from chemical thinners aside perhaps from treatments which used very aggressive rates of lime sulfur (10%) with and without spray oil which showed slight increases in shoulder russet.

Table 2. Crop load and fruit quality effects of bloom + postbloom chemical thinning programs. WTFRC 2014.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		%	%	g		%
Golden Delicious / M.9 – Rock Island						
CFO + Lime sulfur	78 ab	51 b	28 ns	185 b	98	nd
Exilis Plus + Fruitone L	61 bc	60 ab	25	207 ab	88	
Metamitron 500 ppm bloom	75 abc	58 ab	22	206 ab	88	
Metamitron 500 ppm postbloom	84 ab	54 b	20	200 ab	91	
Metamitron 1000 ppm bloom	75 abc	55 ab	23	209 ab	87	
Metamitron 1000 ppm postbloom	53 c	65 a	23	207 ab	88	
Metamitron 1000 ppm bloom & postbloom (4 total apps)	64 bc	59 ab	25	221 a	82	
Control	90 a	50 b	24	199 ab	91	

Scilate/M.9 Nic 29 - Prosser						
ATS	60 ns	51 ns	40 ns	218 ns	83	14 ns
Carbaryl 4L	59	52	38	234	78	nd
Carbaryl 4L + Fruitone L	57	52	40	236	77	20
Lime sulfur 5 gal	55	54	38	235	77	12
Lime sulfur 10 gal	62	49	41	224	81	26
Water + Regulaid	61	48	43	231	79	14
Water only 100 gal/a	61	52	36	226	80	14
Water only 200 gal/a	63	52	35	222	82	9
WES + lime sulfur	54	55	37	236	77	25
Control (hand thin)	69	46	41	220	83	10

Table 3 reflects the cumulative success rates of our most frequently tested chemical bloom thinners over time at achieving our three main criteria for effective thinning and demonstrates the overall superiority of programs featuring lime sulfur.

Table 3. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials. WTFRC 1999-2014.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	15 / 59 (25%)	10 / 62 (16%)	4 / 53 (8%)
NC99	15 / 32 (47%)	7 / 34 (21%)	2 / 28 (7%)
Lime sulfur	25 / 56 (45%)	12 / 50 (24%)	9 / 48 (19%)
CFO + LS	62 / 113 (55%)	27 / 104 (26%)	22 / 102 (22%)
JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	14 / 29 (48%)	4 / 28 (14%)	4 / 27 (15%)
ThinRite	7 / 22 (32%)	0 / 23 (0%)	0 / 12

[1]Does not include data from 2014 trials.

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

POSTBLOOM THINNING:

After three years of study, we have yet to document improved performance of standard postbloom thinners like carbaryl and BA from the addition of calcium phosphite (Sysstem-Cal) to the spray tank (Table 4). We are currently evaluating Honeycrisp fruit from these trials which have been stored since harvest in order to assess potential calcium phosphite benefits to stored fruit quality, especially bitter pit incidence. Unlike in 2013, a tank mix of Apogee and Provide did not improve thinning or fruit size; if this unlikely combination fails to impress in 2015 trials, we will likely abandon further pursuit of this program. As mentioned earlier, we see enough potential with metamitron to warrant further investigation. Based on our experience to date, this product, which should soon be registered as a postbloom thinner of apple in the EU, can also be effective in Washington conditions. However, much more assertive rates and timings will be required than those employed by research colleagues in the Eastern US and Europe, where fruit trees tend to endure higher levels of photosynthetic stress during typically warm, cloudy conditions during chemical thinning season.

Table 4. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2014.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		%	%	g		%

Honeycrisp/MM.111 - Manson						
Carbaryl + Fruitone L	51 c	55 a	39 b	187 a	97	nd
Exilis Plus	70 a	43 b	45 ab	147 b	127	
Exilis Plus + carbaryl	65 abc	43 b	49 a	166 ab	109	
Exilis Plus + Fruitone L	54 bc	52 ab	42 ab	150 b	121	
Sysstem-Cal 64 oz + Exilis Plus	70 a	45 ab	42 ab	162 ab	112	
Sysstem-Cal 96 oz + Exilis Plus	63 abc	46 ab	46 ab	157 b	116	
Sysstem-Cal 64 oz + Exilis Plus + carbaryl twice	64 abc	46 ab	45 ab	155 b	117	
Sysstem-Cal 64 oz + Exilis Plus + carbaryl thrice	58 abc	49 ab	45 ab	167 ab	109	
Sysstem-Cal 96 oz + Exilis Plus + carbaryl twice	60 abc	49 ab	43 ab	168 ab	108	
Control	67 ab	45 ab	44 ab	154 b	118	
Honeycrisp / MM.106 - Gleed						
Carbaryl + Fruitone L	61 bc	47 ab	46 ns	268 ns	68	58 a
Exilis Plus	73 abc	41 bc	47	244	74	65 ab
Exilis Plus + carbaryl	56 c	53 a	40	249	73	57 a
Exilis Plus + Fruitone L	64 bc	44 abc	47	261	70	79 ab
Sysstem-Cal 64 oz + Exilis Plus	74 ab	41 bc	45	243	75	60 ab
Sysstem-Cal 96 oz + Exilis Plus	75 ab	40 bc	47	247	74	85 b
Sysstem-Cal 64 oz + Exilis Plus + carbaryl twice	60 bc	51 ab	41	259	70	70 ab
Sysstem-Cal 64 oz + Exilis Plus + carbaryl thrice	61 bc	48 ab	44	262	69	63 ab
Sysstem-Cal 96 oz + Exilis Plus + carbaryl twice	63 bc	46 abc	46	307	59	77 ab
Control	85 a	34 c	50	255	71	64 ab
Gala / M.26 - Quincy						
Apogee + ProVide	74 a	47 ab	35 ns	197 ab	92	60 a
Exilis Plus + carbaryl	53 ab	57 ab	34	199 ab	91	66 ab
Exilis Plus + FAL 850	46 b	61 ab	33	203 ab	89	75 abc
Exilis Plus + FAL 860	75 a	44 b	39	208 ab	87	76 abc
Exilis Plus + Fruitone L	48 ab	63 a	29	218 a	83	99 c
Exilis 5XL + carbaryl	57 ab	57 ab	30	194 ab	94	67 ab
Metamitron + Fruitone L	42 b	64 a	29	203 ab	89	77 abc
Metamitron 400 ppm	54 ab	55 ab	37	192 ab	95	56 a
Metamitron 800 ppm	48 ab	59 ab	34	197 ab	92	87 bc
Control	60 ab	53 ab	36	185 b	98	66 ab

Cripps Pink / Nic.29 – Prosser						
Apogee + ProVide	43 ns	61 ns	34 ns	197 ns	92	14 c
Exilis Plus + carbaryl	48	57	38	199	91	0 a
Exilis Plus + FAL 850	44	61	35	199	91	2 ab
Exilis Plus + FAL 860	54	52	43	190	96	7 bc
Exilis Plus + Fruitone L	52	52	43	198	92	14 c
Exilis 5XL + carbaryl	44	60	36	198	92	4 abc
Metamitron + Fruitone L	51	54	41	201	90	2 ab
Metamitron 400 ppm	58	50	42	192	95	10 bc
Metamitron 800 ppm	45	61	34	201	90	2 ab
Control	44	62	32	190	96	15 c

Our most effective postbloom thinning programs (Table 5) continue to feature BA, especially in combination with NAA, which is good news in light of the potential loss of registration for carbaryl as a postbloom chemical thinner.

Table 5. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2014.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	3 / 23 (13%)	0 / 24 (0%)	0 / 21 (0%)
Carb + BA	33 / 90 (37%)	10 / 89 (11%)	11 / 80 (14%)
Carb + NAA	16 / 59 (27%)	10 / 59 (17%)	5 / 55 (9%)
BA + NAA	13 / 31 (42%)	6 / 30 (20%)	3 / 21 (14%)

[1]Does not include data from 2014 trials.

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

2014 PLANT GROWTH REGULATOR PROGRAM HIGHLIGHTS:

Gibberellic acid (GA) for biennial bearing: Our latest results are consistent with prior findings in that we demonstrated significant 2014 bloom reduction from 2-4 applications weekly of 100 ppm GA_3 applied starting at 10mm fruitlet size (data not shown). Other formulations of GA have also proven effective in our trials and we continue to lobby PGR manufacturers to pursue necessary registrations or label amendments to accommodate this use pattern for apple growers seeking to promote annual cropping.

Naphthaleneacetic acid (NAA) for bloom promotion: Despite lackluster results from similar approaches in the past, we are currently evaluating new formulations of NAA that were applied in the spring/summer of 2014 in hopes of promoting 2015 bloom density. These results will be available next year.

NAA to inhibit preharvest fruit drop: Although very little fruit fell before typical harvest timing, two new formulations of NAA effectively delayed abscission of Jonagold fruit for several weeks after harvest (data not shown).

Prohexadione calcium to inhibit shoot extension: For the third consecutive year, a potential competitor for Apogee demonstrated its ability to significantly reduce Fuji shoot length in WTFRC

trials (data not shown). This product should be available to industry soon, hopefully driving down market prices for all prohexadione products.

PROTECTIVE MATERIAL TRIALS:
Unfortunately, our protective netting trials on Granny Smith at the WSU Sunrise Research Orchard have been significantly compromised by poor horticultural management, most obviously manifested by diminishing yields for the past few seasons. A combination of poor pruning and extensive cutworm damage rendered the 2014 bloom so light that a joint decision was made by WTFRC and Extenday USA to abandon the pod net trial established in 2012. Bloom was also light in the drape net trial block, but still sufficient to continue with that study which is comprised of 4 replicated plots (30' x 60') of drape nets laid directly on tree and existing trellis structures of three adjacent tree rows (Figure 1).

Figure 1. Installed drape shade netting at WSU Sunrise orchard.

As in 2013, we observed no clear treatment effects this year on yields or fruit set efficiency, but mean fruit weight was improved by approximately one box size under the drape nets (Table 6). While preliminary, these results suggest that apples may be produced in Washington's high light environment under nets which reduce photosynthetically active radiation (PAR) by up to 20% without having to sacrifice yields or fruit size. Once again, we documented a significant reduction in sunburn incidence by the nets and an increase in shoot growth, although that effect was not statistically meaningful. Unlike 2013, the trial site did not suffer any hail damage in 2014 and fruit finish, color, and internal quality were not significantly affected by netting (data not shown).

Table 6. Key preliminary results of protective drape netting studies. Granny Smith/M.9 – Rock Island. WTFRC 2013-2014.

Treatment	Yield efficiency	Fruit set efficiency	Harvest fruit weight	Sunburned fruit	Hail-damaged fruit	Mean shoot growth
	kg/cm^2 TCSA	fruit/cm^2 TCSA	g	%	% (est.)	cm
2013						
Drape net	0.7 ns	3.9 ns	179 ns	42 b	3	71 a
Control	0.7	3.6	183	64 a	80	63 b
2014						
Drape net	0.4 ns	1.2 ns	268 a	32 b	None	76 ns
Control	0.3	1.2	232 b	58 a	None	64