			APPLE HORTICULTURE AND POSTHARVEST RESEARCH REVIEW
			Wed January 25, 2017
Time	Page	PI	
8:00		Hanrahan	Welcome - Introductions and housekeeping
			Final Reports
8:15	1	Combs	Validation of Honeycrisp and Granny Smith pollen tube growth models
8:30	8	Combs	Pollen tube growth model validation & utilization for flower thinning
8:45	16	Peace	After RosBREED: Developing and deploying new apple DNA tests
9:00	28	Mattheis	Id of procedures to extend Honeycrisp storage life: No-cost extension
9:15	37	Rudell	Commercial testing of early scald risk assessment tools: No-cost extension
9:30	49	Hanrahan	Assessment of overhead cooling practices for apple food safety
9:45	60	Kalcsits	Effectiveness of foliar calcium applications in bitter pit management
10:15	73	Kalcsits	Effects on physiology of apple under photoselective anti-hail nets
10:30	87	Lewis	Mechanical pruning in apple, pear & sweet cherry: No-cost extension
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			Continuing Projects: 2:45 - 4:45
1	96	Ganjyal	Improving food safety by hot air impingement drying: 4th year report
1	103	Zhu	Ozone in apple storage: microbial safety and decay management
1	111	Evans	Apple scion breeding program
1	118	Musacchi	WA 38: evaluation of rootstocks and training systems: No-Cost Extension
1	126	Rudell	Reducing scald after long-term CA storage
2	132	Rudell	Risk assessment for delayed sunburn and sunscald
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2	142	Amiri	Improved risk assessment and management of apple postharvest diseases
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3	159	Mazzola	Managing rhizosphere/soil microbiology via apple rootstock chemistry
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FINAL PROJECT REPORT

Project Title: Validation of Honeycrisp and Granny Smith pollen tube growth models

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

Other funding sources: None

Total Budget: Year 1: **$20,840**
Other funding sources: None

WTFRC Collaborative expenses:

Item	2016
Salaries	4000
Benefits	1200
Wages	2000
Benefits	500
Shipping	100
Supplies	100
Travel	500
Total	**$8,400**

Budget:
Organization Name: Virginia Polytechnic Institute and State University (Va. Tech)
Contract Administrator: Eric James Dinwiddie, Pre-Award Administrator
Telephone: 540-231-9368 **Email address:** EricJD@VT.edu

Item	2016
Salaries*	8000
Benefits	4080
Supplies	360
Total	**$12,440**

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

RECAP OF ORIGINAL OBJECTIVES

Validation Testing of Honeycrisp and Granny Smith Pollen Tube Growth Models in Washington Orchards. (Virginia Tech & WTFRC)

Pollen tube growth model validation included criteria from three tests:

Test 1: Commercial use of the pollen tube growth models. In this test, grower-participants use the models made available to them through the AgWeatherNet website. These growers (beta-testers) trained in the use of the models then monitor the blocks start times and bloom thinning application timings. At the end of harvest, the beta-test participants rate their actual crop relative to their ideal expected yield. Comparing the desired yield with the actual harvested yield demonstrates that the beta-test participants understand the principles of the model and that it is working to their satisfaction. This harvest data will be cross-referenced with application timings as done with other models in previous years.

Test 2: Validation test 2 includes flower samples collected in Washington orchards after thinning chemicals were applied, by comparing model-predicted pollen tube growth versus actual growth in flowers. Sampling of flowers from beta-test blocks and evaluating them microscopically will determine if fertilization occurred on the segment of the flower population that was intended to be the harvested crop. Bloom thinning applications can then be re-applied to reduce unwanted additional cropping.

Test 3: We will request harvest data from selected Washington orchard blocks that were bloom thinned using the pollen tube growth models in the 2015 growing season. This data, if available for validating Honeycrisp and Granny Smith models, will come from selected beta-testers who had access to the beta tests models for the 2015 growing season.

Once 2015-16 research findings are complete, they will be combined with 2013-14 data and evaluated as a whole. Only after validation tests 1, 2, and 3 are completed will the Honeycrisp and Granny Smith models be endorsed for release to all growers beginning in 2017.

Table 1. Chronology of beta-testing and release of the pollen tube growth models.

Pollen Model	Began field beta-testing using Excel spreadsheet models (Year)	Began field beta-testing using AgWeatherNet website models (Year)	Released for public use (Year)
Gala	2007	2012	2014
Golden Delicious	2007	2012	2014
Fuji	2009	2012	2014
Pink Lady	2011	2012	2014
Honeycrisp	**2013**	**2013**	**2017 (projected)**
Granny Smith	**2014**	**2014**	**2017 (projected)**

SIGNIFICANT FINDINGS

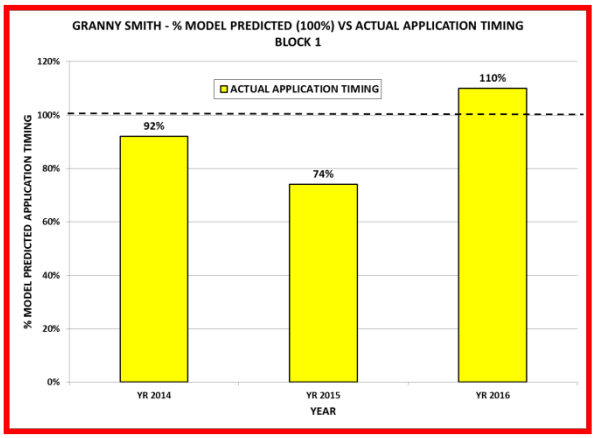
- Developed cultivar-specific equations for Honeycrisp and Granny Smith pollen tube growth and interfaced these models with real-time and forecasted weather data on the AgWeatherNet website.
- Created web-based interface to make these models user friendly and the output results easy to understand.

- The AgWeatherNet interface allows for site and cultivar specific information to be generated.
- By using forecast data through the AgWeatherNet site, pollen tube growth is projected 48 hours into the future, which allows growers to more easily schedule bloom thinning sprays in advance.
- Microscopic evaluation of the model in the laboratory included sampling flowers from the field to determine the percent of flowers that had been fertilized helps to verify predicted fertilization by the models.
- Comparing average style length determined in the field and in the laboratory is an integral part of evaluating and refining the models to actual field conditions. These comparisons confirm that grower averages of style lengths measured in the field were comparable to those of samples in the microscopic evaluation.
- Results to date have shown that, overall, the pollen tube growth models are helping growers achieve their targeted crop load by better timing of applications of bloom thinning sprays.
- Beta-testers using the models say biennial bearing can be reduced when model applications are properly applied.

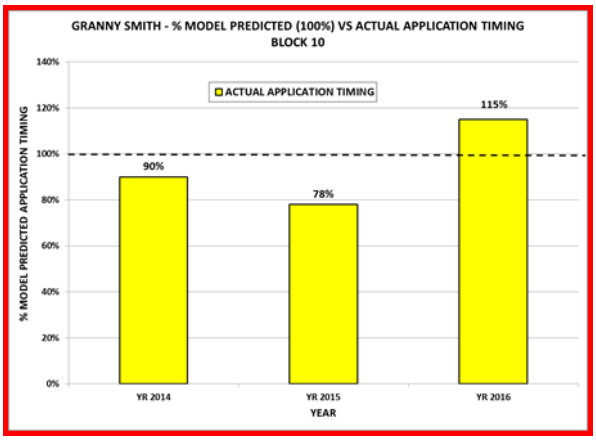
RESULTS AND DISCUSSION

In developing new models, there will always be issues with how best to adapt new models to circumstances that arise on any given day at any specific site. If all things were equal and there were no variables to consider, then all application timings would be at 100% of model predicted fertilization of desired crop-load, resulting in a perfect crop load. As shown below in Graphics 1-8 applications go on at various stages and timings are generally due to factors that the grower/ orchard manager or someone else in charge at that specific site deems relevant to change or adjust spray timings which is exactly the way it should be. The application timing in the same blocks over a 3-year period can vary greatly as shown in these graphics.

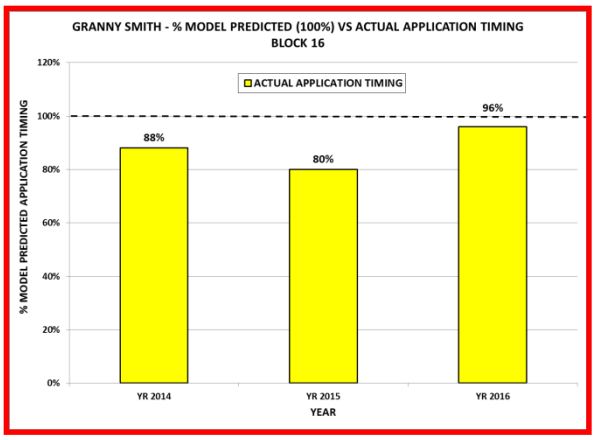
The models are tools to help reduce crop load at bloom, but are not the only tool that will be needed to help with crop load management. Judgment and working knowledge of the orchard blocks will help maximize the effects of the pollen models you are using to bloom-thin your crop.



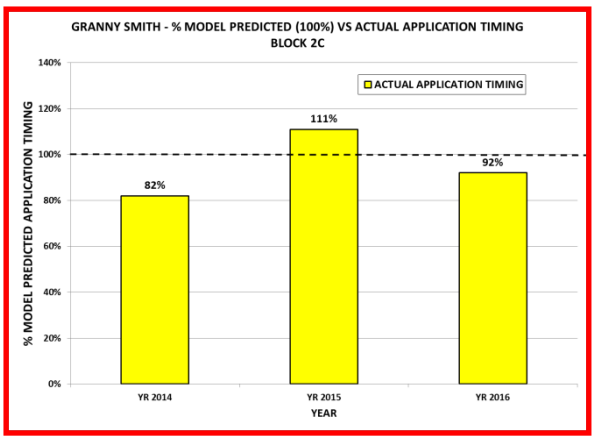
Graph 1



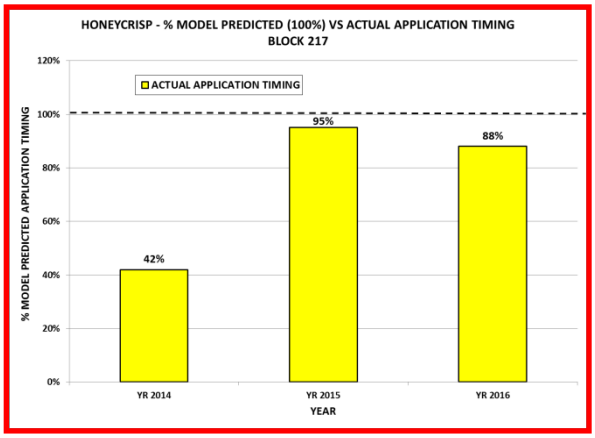
Graph 2



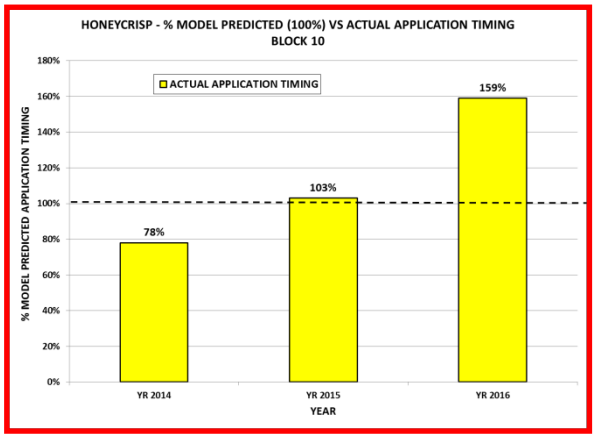
Graph 3



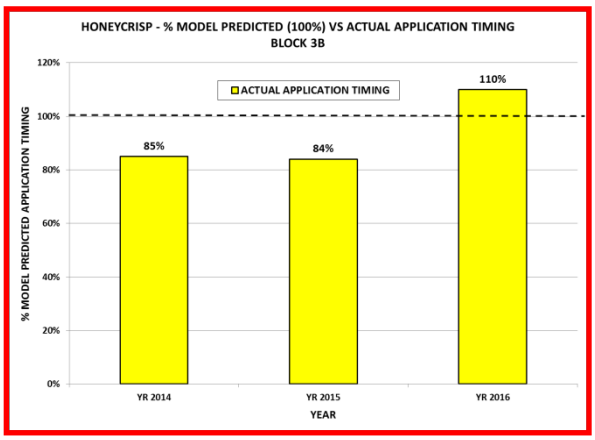
Graph 4



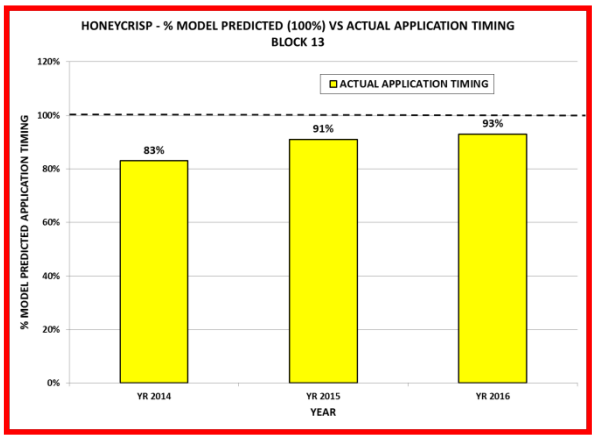
Graph 5



Graph 6



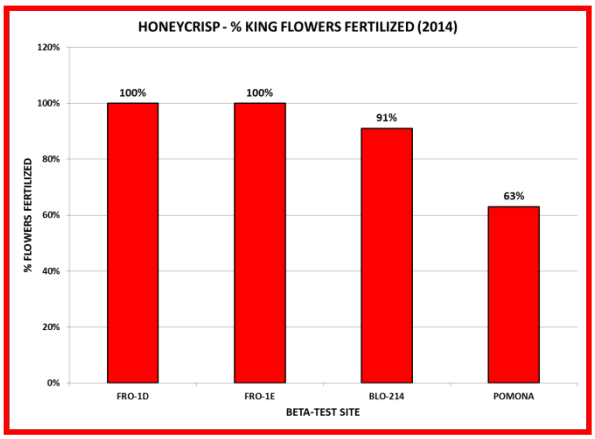
Graph 7



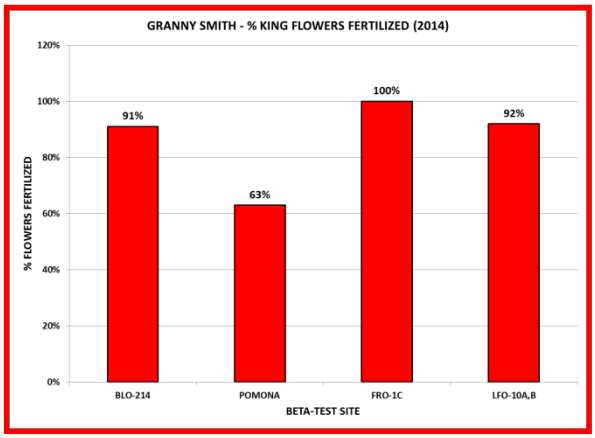
Graph 8

By microscopic examination of flowers sampled at beta-test sites for fertilization, we can best track how well the models are predicting fertilization. Flower samples taken from test sites are sent

for evaluation of percent of flowers fertilized as predicted by models. Graphs 9 and 10 show results of tests from flowers evaluated at 8 different orchard sites in the Washington apple growing regions in 2014.

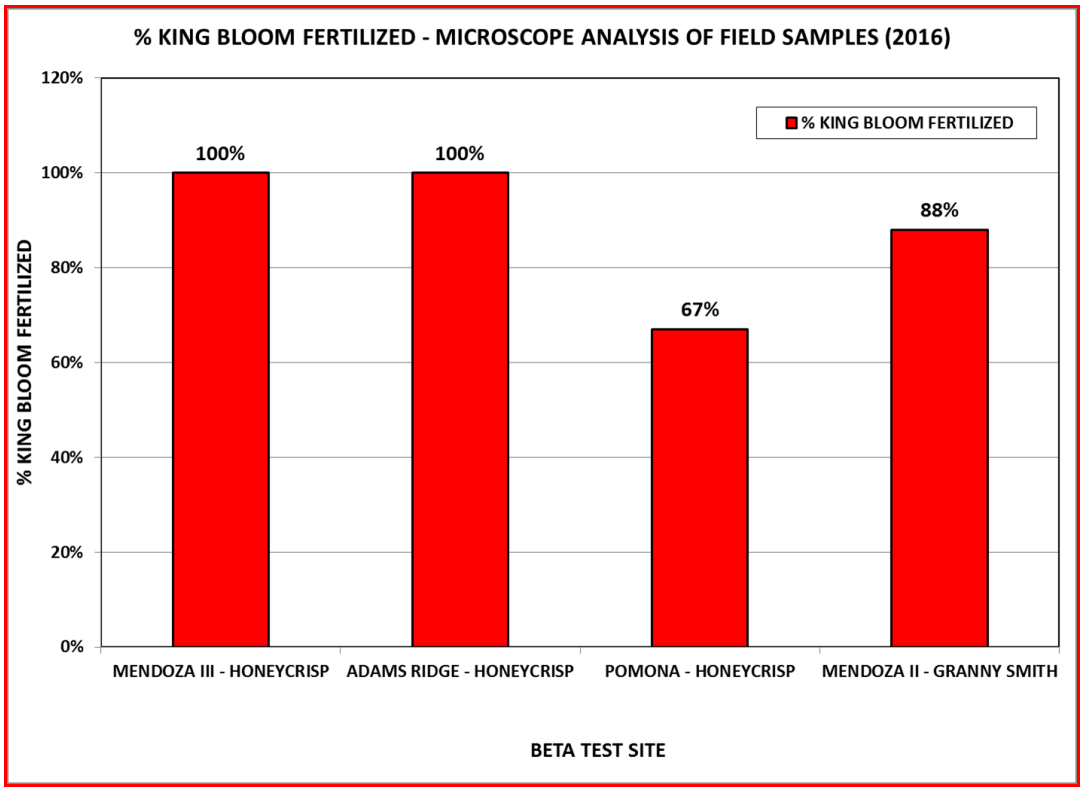


Graph 9

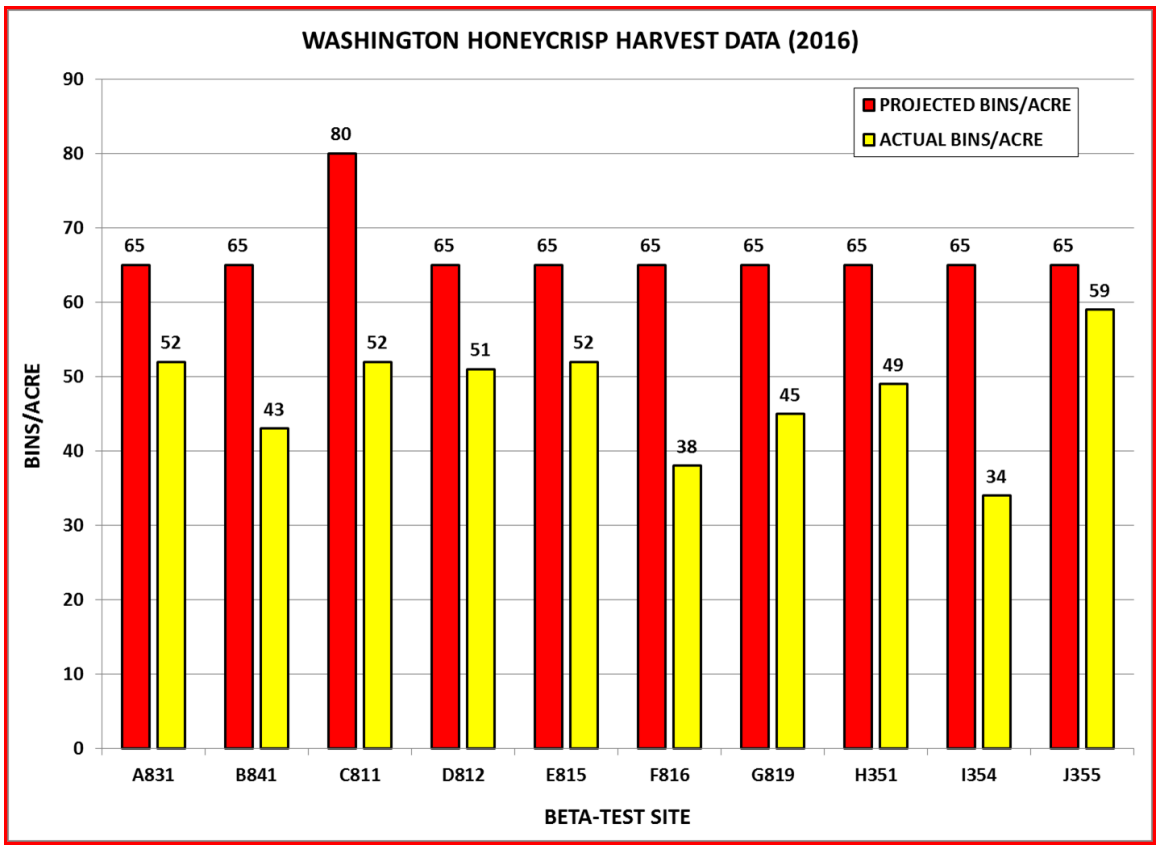


Graph 10

Graph 11 shows results of flower fertilization testing carried out in 2016. Graph 12 shows Honeycrisp crop load data (Projected Bins/Acre vs Actual Bins/Acre) at harvest. Table 1 shows 2016 crop load data from various beta-test sites across the Washington growing region for Honeycrisp and Granny Smith.



Graph 11



Graph 12

Farm	Block	Variety	Desired Bins/Acre	Actual Bins/Acre	% Desired Yield
Frog	4, 7, 8	Honeycrisp	73	60	82%
Fox	1, 2	Honeycrisp	78	58	74%
Squirrel	12, 13, 15	Honeycrisp	75	53	73%
Squirrel	1, 5, 9	Granny	77	71	92%
Hound	11, 12, 13, 14	Granny	45	51	113%

Table 1

EXECUTIVE SUMMARY

In summary, we tend to agree with our colleague Tory Schmidt in a statement he made recently regarding the models, "it seems clear that the model has some fans, some skeptics, and just about everything in between". For some it is seen as a useful tool to help them better control their crop load. For others, it seems unreliable because predicted timing vs what growers are seeing in the field that do not match. What the models can do exactly versus what users expect them to do may be where the problem lies. In working with beta-testers for many years we have come to respect their opinions and recommendations for how they use the model and what they expect it to do.

The following are the opinions and insights of one of the beta-testers in regards to what he expects the models to do for him and how he adapts them to his own situations.

Darin Case - Dovex Fruit Company:

> "In my opinion the Pollen Tube models are an exceptional tool for the apple industry and for us as growers here in Washington State. The trend in our industry has been that we have fewer and fewer materials that work effectively, or if a material works, MRL's or restrictions have an impact on markets, so this model helps us better use chemicals as well as timings related to blossom thinning. As I have always said, if we have models to help guide us to make better decisions, we will be better at what we do and have a better handle on bi-annual bearing habits.
>
> In regards to the Honeycrisp and Granny Smith models;
>
> 1. Honeycrisp – Can be a difficult variety to thin and have return bloom. Use the model as a guide, but be aware of the amount and type of cross pollinizers you have. If you have heavy cross pollination, go on the early side, if you are weak on cross pollination, go on the later side. I did use on some blocks, the 2016 model, especially on younger blocks with low cross pollination and the model worked very well. One also has to take into account if they are blossom thinning by hand as well, and what they really want out of their bloom thinning programs.
> 2. Granny Smith – Again, as in all the pollen tube growth models, really understand what your cross pollination is like. To me, Grannies can be easy to thin so watch temperature trends and use the model accordingly. I used both models in 2016, but mostly stayed with the older model that was first developed.
>
> As far as using the 2016 models versus the older model, I don't think it will make too much of a difference. One has to remember this is a great tool and you have to understand what you are wanting out of the blossom thinner you are applying along with the variety and cross pollination percentage one has, bee activity and number of hives per acre used. By looking at style lengths each year, one gets a greater feel for if you have winter injury, frost damage, difference in style length between cultivars, or locations in the orchard. The more one grasps this model, the better they can use it as a decision aided tool and can set trends in your own farm related to how early or later you are vs. the nearest Ag Weather Net Weather site, how easy or difficult your varieties are to thin".

This is the final report for the work completed on the Honeycrisp and Granny Smith models that was funded for one year.

FINAL PROJECT REPORT

Project Title: Pollen tube growth model validation & utilization for flower thinning

PI:	Keith Yoder	**Co-PI**:	Greg Peck
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City:	Winchester	**City:**	Winchester
State/Zip:	VA 22602	**State/Zip:**	VA 22602

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

Total Budget for Virginia Tech:
Year 1: $43,591 Year 2: $43,591

Other funding sources: The Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, USDA, provide partial funding through salary support for Yoder and Peck, as well as the Virginia Tech facilities. Indirect support is also provided through the AgWeatherNet Program of Washington State University and its 177 automated weather stations.

BUDGET

Item	**2015**	**2016**
Salaries*	27,000	27,000
Benefits	13,298	13,298
Wages (4 wks, 20 hr/wk @ $15	1,200	1,200
Benefits	93	93
Equipment		
Supplies	1000	1,000
Travel	0	0
Contractual services & repairs	1,000	1,000
Plot Fees		
Total	$43,591	$43,591

***Note: Salary for Research Specialist Leon Combs; Wage person TBD.**

RECAP OF ORIGINAL OBJECTIVES:

Develop low-temperature pollen tube growth rates to allow for more precise pollen tube growth models for all seven varieties (Golden Delicious, Gala, Fuji, Pink Lady, Honeycrisp, Granny Smith and Red Delicious models) (Virginia Tech).

During our 2014 stakeholder meetings with the pollen tube growth model beta-testers, it became apparent that we needed to further explore the effects of low temperatures on the pollen tube growth model. In particular, the beta-testers felt that the model was underestimating the amount of pollen growth that occurs at temperatures below 55ºF. In developing the models, our earlier focus had been on temperatures that are more typical during bloom. When the model was brought into field situations, we extrapolated the empirically derived curves for the pollen tube growth that occurred below 55ºF. However, it is possible that the actual curve does not follow the predicted trajectory, and thus, empirical data is needed to develop more precise low-temperature pollen tube growth rates. These data will be extremely important in years when there are cooler than normal temperatures during bloom.

We will conduct these low-temperature tests on all of the cultivars for which we have developed pollen tube growth models, including Golden Delicious, Gala, Fuji, Pink Lady, Honeycrisp, Granny Smith, and Red Delicious. Better understanding of the effects of temperature on these processes and more attention to actual temperatures during the bloom period will improve the accuracy of post-fertilization application timing, thereby providing more reliable bloom thinning results. By comparing temperature data from various beta-test sites from across the Washington apple growing regions, we can better evaluate the effects of low temperatures on model parameters.

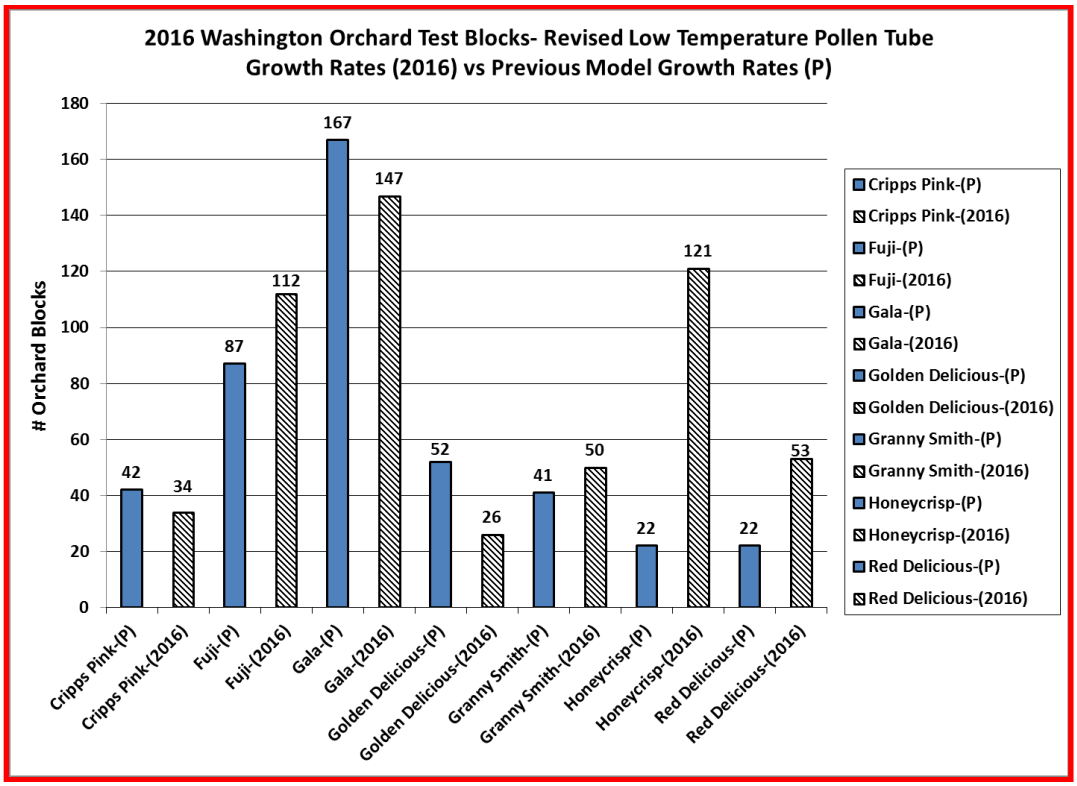


Figure 1. Number of test sites in 2016 using present model (P) vs Low temperature growth rate models (2016).

SIGNIFICANT FINDINGS

Low-temperature pollen tube growth rates from the first year of low temperature testing were compared to present model parameters on all models. The following graphics show hourly differences

between presently used models prediction of timing of first bloom spray vs predicted spray timing using the 1st year preliminary growth rate for each model. Also shown is the percent of hours that temperatures were below 55°F from start of model to application of first bloom thinning spray at each location in 2015. As shown in charts 1, 3, 5, 7, 9, and 11, hours below 55°F during bloom varied significantly across different growing regions and cultivars as shown above below from data taken during usage of pollen models at selected locations during the 2015 bloom thinning season.

Golden Delicious

- Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray at four locations in 2015 ranged from 43% to 88% (Chart 1).
- Differences in application timing (hours) comparing present model versus first year low temperature test data ranged from 4 hours to 22 hours (Chart 2).
- First bloom thinning spray would have been applied earlier than present model predicted application timing if first year low temperature research testing parameters were used.

Chart 1

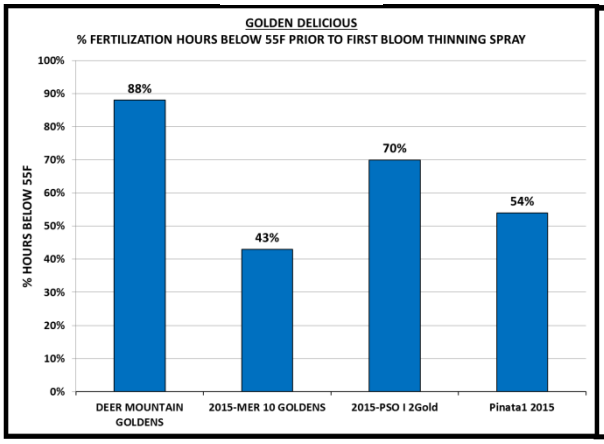
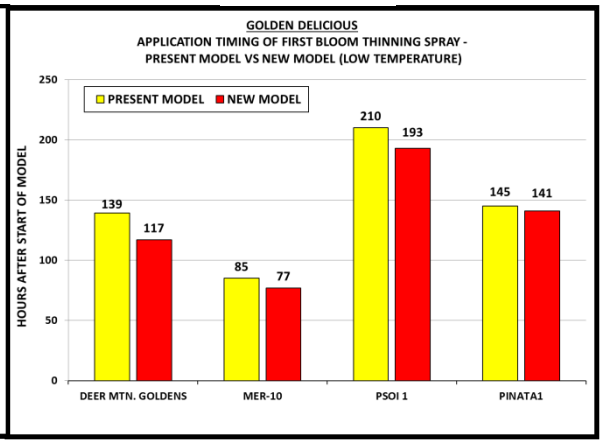


Chart 2



Gala

- Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray at four locations in 2015 ranged from 30% to 88% (Chart 3).
- Differences in application timing (hours) comparing present model versus first year low temperature test data ranged from 3 hours to 18 hours (Chart 4).
- First bloom thinning spray would have been applied later than present model predicted application timing if first year low temperature research testing parameters were used.

Chart 3

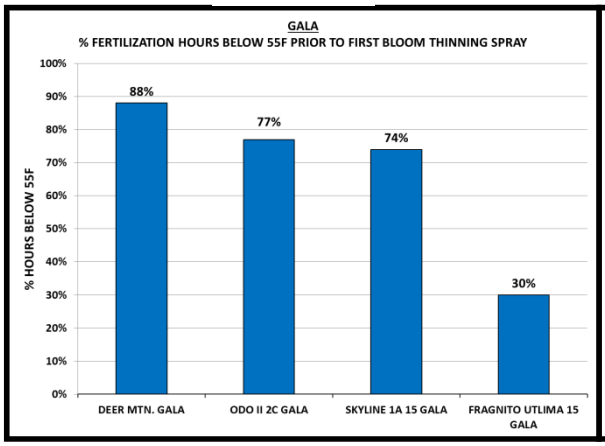
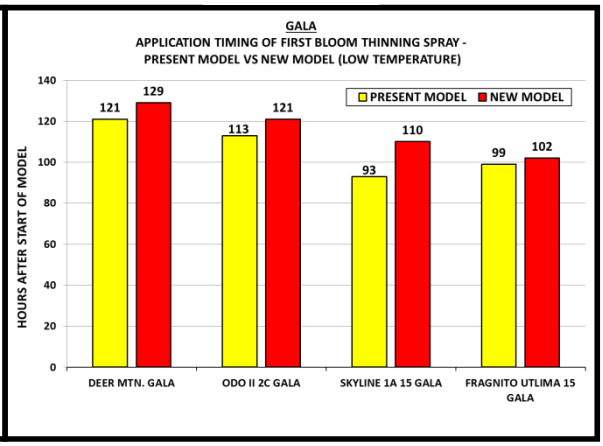


Chart 4



Fuji

- Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray at four locations in 2015 ranged from 48% to 89% (Chart 5).
- Differences in application timing (hours) comparing present model versus first year low temperature test data ranged from 5 hours to 32 hours (Chart 6).
- First bloom thinning spray would have been applied earlier than present model predicted application timing if first year low temperature research testing parameters were used**.**

Chart 5

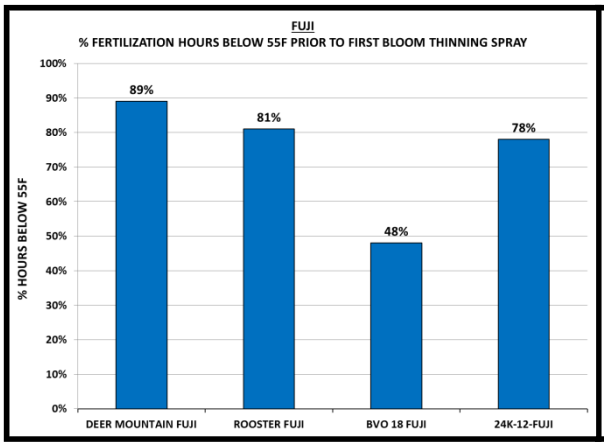
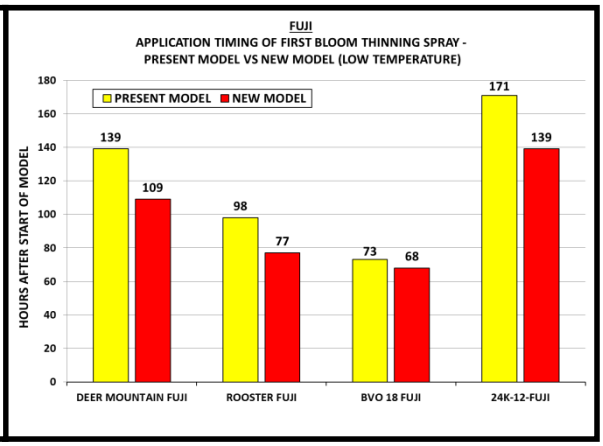


Chart 6



Cripps Pink

- Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray at four locations in 2015 ranged from 68% to 78%.
- Differences in application timing (hours) comparing present model versus first year low temperature test data ranged from 18 hours to 25 hours.
- First bloom thinning spray would have been applied earlier than present model predicted application timing if first year low temperature research testing parameters were used.

Chart 7

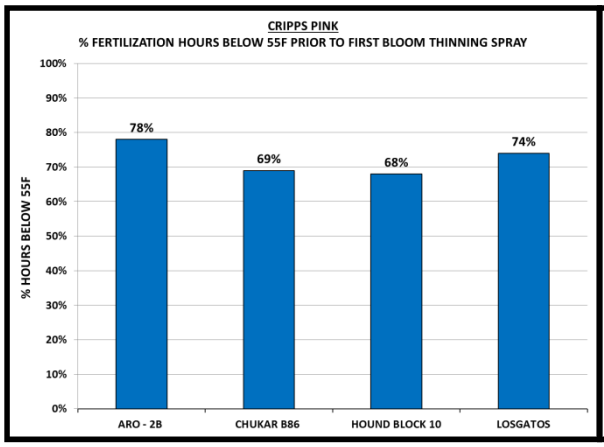
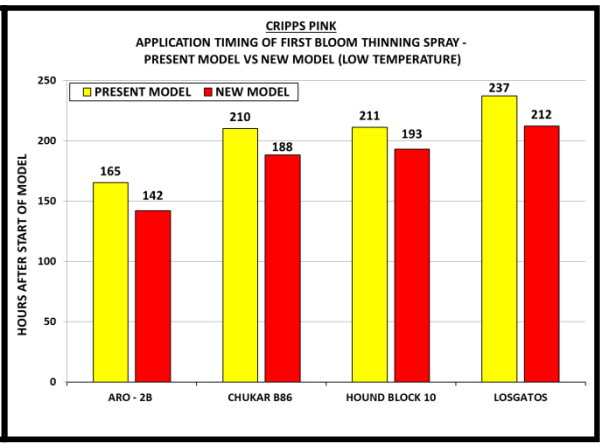


Chart 8



Honeycrisp

- Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray at four locations in 2015 ranged from 37% to 64% (Chart 9).
- Differences in application timing (hours) comparing present model versus first year low temperature test data ranged from 1 hour to 30 hours (Chart 10).
- First bloom thinning spray would have been applied earlier than present model predicted application timing if first year low temperature research testing parameters were used**.**

Chart 9

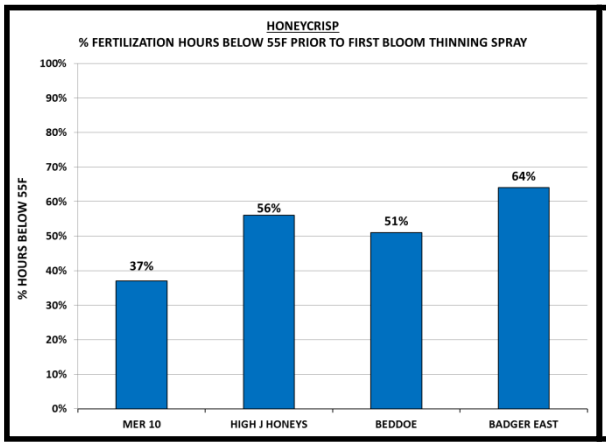
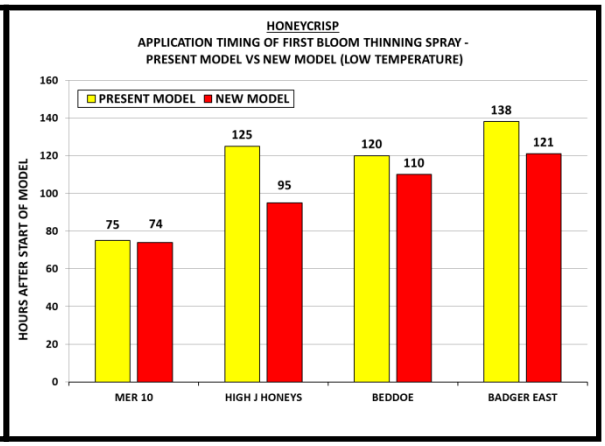


Chart 10



Granny Smith

- Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray at four locations ranged from 37% to 74% (Chart 11).
- Differences in application timing (hours) comparing present model versus first year low temperature test data ranged from 2 hours to 53 hours (Chart 12).
- First bloom thinning spray would have been applied earlier than present model predicted application timing if first year low temperature research testing parameters were used.

Chart 11

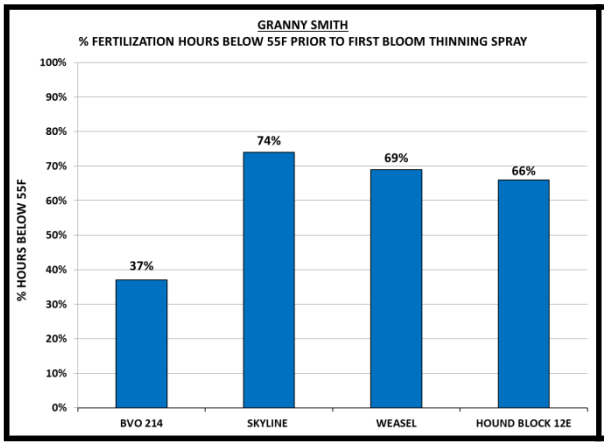
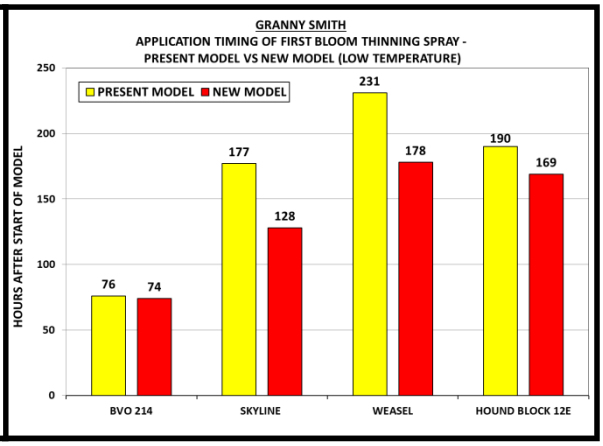


Chart 12



Red Delicious

- Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray at four locations ranged from 78% to 81% (Chart 13).
- Differences in application timing (hours) comparing present model versus first year low temperature test data ranged from 6 hours to 22 hours (Chart 14).
- First bloom thinning spray would have been applied later than present model predicted application timing if first year low temperature research testing parameters were used.

CHART 13

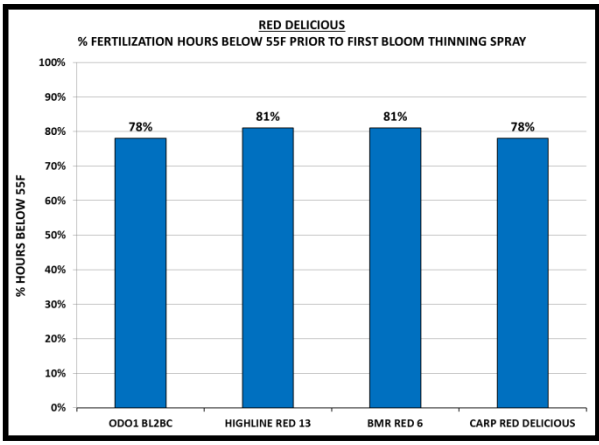
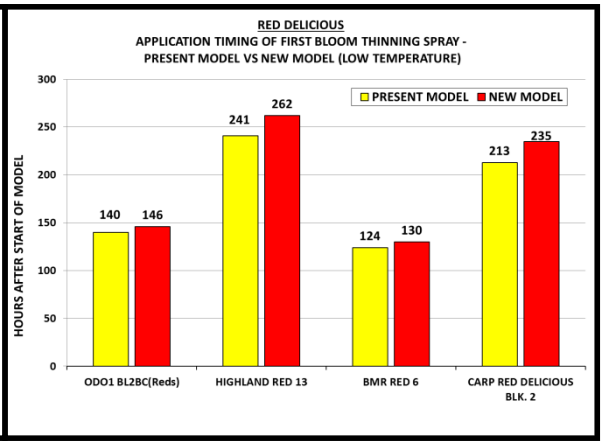


CHART 14



RESULTS AND DISCUSSION

The purpose of this research was to evaluate the effect of lower temperatures during bloom on the rate of pollen tube growth projected by the present pollen tube growth models presently available on the AgWeatherNet site. Prior to 2016, hourly growth rates for modeled pollen tube growth were extrapolated by using 35°F as base for zero hourly growth of pollen tube. Previous experimental growth chamber work had concentrated only on hourly growth rates starting at 55°F. Concerns raised at meetings with beta-testers, growers, and industry representatives prompted the WTFRC to request research covering the lower temperature growth rates from 55°F to 35°F.

As shown in Figure 2, by implementation of the new growth rates on the Honeycrisp pollen tube growth model, the first application of bloom thinning sprays would have been applied earlier than when using the 2015 model growth rates. In 2015 first year low temperature tests in Gala and Red Delicious dictated bloom thinning application timing would have been later than predicted application timings using presently available 2016 models. In all other cultivar models, bloom thinning application timing would have been earlier using first year low temperature tests data.

In reviewing the use of the models by beta-testers it has become apparent that as the growers get more comfortable with using the models the more they are adapting them to fit the individual needs of the specific blocks they are using the models on. As we have emphasized at our training sessions and in personal contact with growers that modifying the models to adjust on-site conditions is their decision to make. Figure 3 shows the high degree of variation in temperatures from year to year below 55°F. It also shows the high variation in overall temperature which occurs from season to season that makes tracking the pollen tube growth rates and proper application timing of king bloom thinners so challenging.

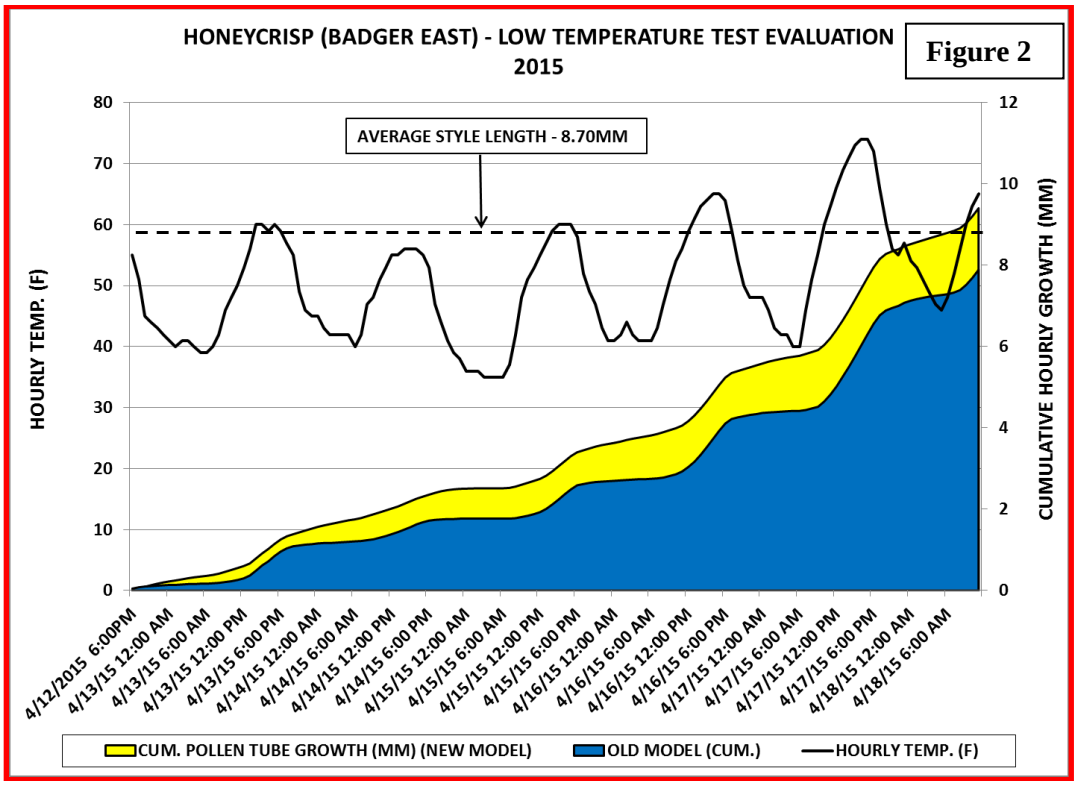


Figure 2. Comparison of effect of implementation of revised low temperature pollen tube growth rates vs previous model growth rates. The high amount of hours below 55°F shown in Figure 2 illustrates the need to re-consider the lower range of pollen tube growth below 55°F.

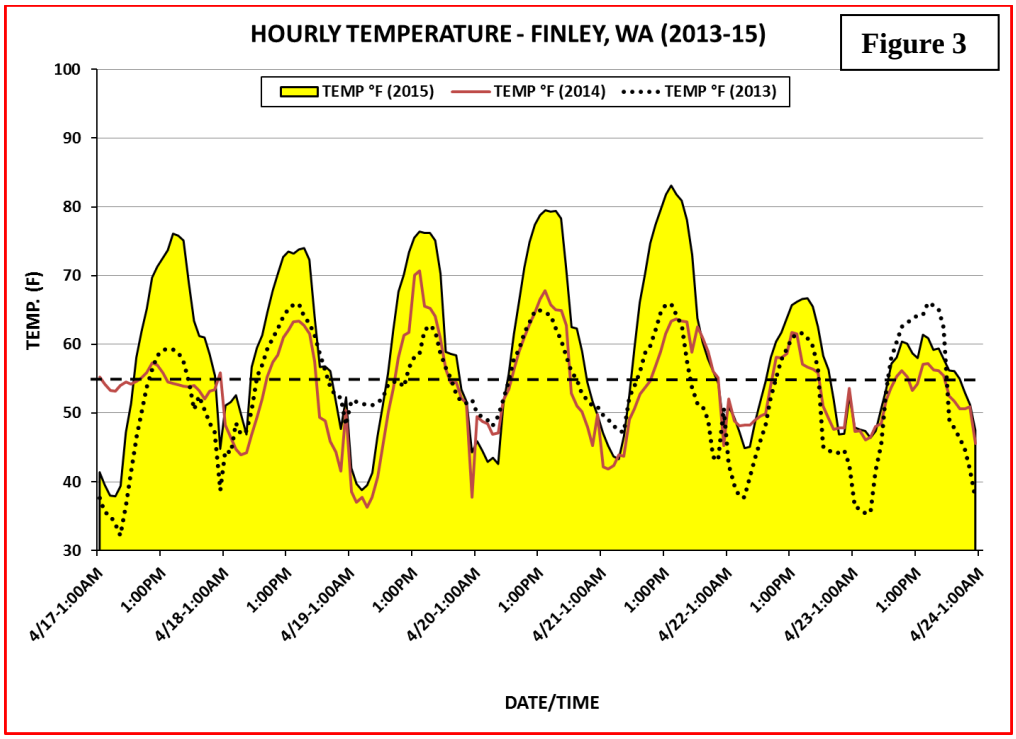


Figure 3. This graphic depicts the hourly temperature for Finley, WA for the same weekly period of April 17 to April 24 for 2013, 2014 and 2015.

EXECUTIVE SUMMARY

Temperature tests showed that in 2015 first bloom thinning application timing would have been later for Gala and Red Delicious than what is predicted by presently available 2016 models. In all other cultivar models, bloom thinning application timing would have been earlier using first year low temperature tests data.

As one beta-tester said when using the models "Don't assume anything". The most important part of using the models is to have as thorough an understanding of the specific block history as possible when preparing to use the model. The model can only tell you when it recommends applications to be applied. The information you input into the modeling program is the key to success or failure. Don't assume that just because you have an average style length of 10.5mm in Honeycrisp Block A (Adams Ridge) that Honeycrisp Block B (Pomona) will be the same. The more specific details you can input to the models, the more successful you will be at achieving the desired crop load goal. The primary goals of the models are to help reduce crop load, produce a volume of fruit that requires less hand thinning and also grows the desired fruit size for optimum pack-out. The model should also help to reduce biennial bearing the following year. The models enable the grower to schedule application timing in advance by using the 48 hour predicted growth and temperature data feature integrated into the model parameters.

These models are tools that can help the grower with crop load management, but they are only one tool and not a silver bullet that answers all the mysteries of bloom thinning. In talking with beta-testers, not all users follow all the steps laid out in the models' applications. How each user applies the models to their specific situation is up to their discretion. As was said earlier, the grower's knowledge of the block they are using the models on is the final deciding factor on how it will be used by them for applying bloom thinning applications at the proper time. The models cannot see what is happening at these locations, so final decisions rest with the people on the ground at the site. The decision of using these models in any form, or not using them at all, rests with the owners/growers, farm managers and field consultants.

We - suggest that training sessions should be conducted on how to use the models. In the past several years (2012-2015) we conducted training sessions at different locations throughout the Washington apple growing regions. As more new users sign up to access the models, repeated training sessions would be of great benefit to them in understanding the process of how to use the models properly. In working with the beta-testers, the one thing we have heard them say is, the more they use the models, the more comfortable they are with them. As for new users, we don't want them to try to use the models without proper training and then make a mistake that could have been avoided with better guidance. A bad experience using the models without proper training could result in new users doing a "one and done" test of the models and never using them again or having a negative opinion of the modeling program and passing that opinion on to others.

FINAL PROJECT REPORT

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

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Cooperators: Paul Sandefur, Sushan Ru, and Feixiong Luo (PhD students, WSU Pullman), Daniel Edge-Garza, Julia Piaskowski, and Sook Jung (WSU Pullman), Jay Norelli (USDA-ARS Kearneysville), Fred Bliss (Davis, California)

Other funding sources

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

Agency Name: WTFRC Apple Review
Amount awarded: $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: $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.

Total Project Funding: $269,000

Budget History:

Item	**Year 1:** 2014	**Year 2:** 2015	**Year 3:** 2016
Salaries	51,008	52,249	53,540
Benefits	16,127	16,965	17,850
Wages			
Benefits			
Equipment	9,865	9,786	9,610
Supplies	2,000	2,000	2,000
Travel	5,000	8,000	8,000
Plot Fees			
Miscellaneous	5,000		
Total	89,000	89,000	91,000

RECAP ORIGINAL OBJECTIVES

Overall goal

Improve prospects for apple breeding efficiency, accuracy, creativity, and pace by developing and strategically deploying predictive DNA tests targeting valuable traits for the WSU Apple Breeding Program (WABP)

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

Objectives 1a, 1b, and 2a were accomplished in this three-year project (Figure 1). Objective 2b was partially accomplished. For 1a, at least two new trait-predictive DNA tests were developed each year and some previous DNA tests were refined. For 1b, software was developed for determining robust estimates of DNA test effects. For 2a, strategies for optimal deployment of multiple DNA tests were determined that account for various influencing factors. For 2b, working software was developed to support the critical cross-planning stage. Remaining elements of this software will be developed in 2017 with remaining funds.

Objective 1a

- New DNA tests developed or refined that account for <u>some</u> of the genetic influences variable in <u>most</u> WABP families and target <u>essential</u> thresholds for the following traits: **fruit firmness** (two genomic regions) and **crispness** (second region), **fruit acidity** (second region), **fruit texture** (combined test)
  Previously available DNA tests in this category: **fruit ethylene/storability** (two genomic regions), **fruit crispness** (first genomic region) and **juiciness**, **fruit acidity** (first genomic region), **fruit bitter pit incidence**

- New DNA tests developed or adapted that account for <u>most/all</u> of the genetic influences variable in <u>some specific</u> WABP families and target <u>essential</u> thresholds (just for those families) for the following traits: **powdery mildew resistance ('White Angel' source)**, **pink flesh color ('Pink Pearl' source)**

- New DNA test developed that accounts for some of the genetic influences variable in some specific WABP families and targets essential threshold for the following trait: **blue mold resistance (a *M. sieversii* source)**

- New DNA test developed that accounts for most/all of the genetic influences variable in most WABP families and targets enhancing threshold for the following trait: **fruit fructose content**
  Previously available DNA test in this category: **skin overcolor amount**

- New DNA test adapted for the WABP that reveals important allelic information for parents and elite selections/new cultivars: ***S*-genotyping for cross-compatibility**

- DNA test development for several other traits is still underway: harvest timing, fire blight resistance, and soft scald

- Note that there are no DNA tests available accounting for most/all of the genetic influences for essential attributes in most/all WABP germplasm. This situation reflects the biological nature of WABP goals and germplasm – numerous genetic factors contribute to the most important attributes considered to be essential for commercial success in Washington. Therefore, use of DNA tests in the WABP should enhance efficiency and accuracy of selection but is not expected to lock in particular attributes.

Objective 1b

- *DNA Test Effects*, software to calculate DNA test effects from datasets of multi-family replicated trials, developed. Provides a streamlined statistical approach to predict performance from DNA test outcomes. Currently being used to update all DNA test effect predictions

Objective 2a

- Workshop "DNA Test Deployment Strategies for Rosaceae Crop Breeding" hosted in 2014. Well attended by international researchers and affiliated scientists, keeping us on the cutting edge

- Framework developed for DNA test deployment strategies that consider essential vs. enhancing trait levels, cost and genetic gain efficiencies, operational logistics, and which particular germplasm is relevant; scientific papers published

Objective 2b

- *Multi-Trait Family Planning*, software to predict numbers of seedlings and their distributions of trait levels for hypothetical families, developed. Provides a streamlined statistical approach for preparing DNA test deployment during cross-planning and greenhouse-stage seedling selection

- An interim DNA test deployment strategy is available for the WABP, prior to more sophisticated software. The major deployment decisions are whether to use a DNA test only for parent selection or for both parent selection and seedling selection, and for most or for very specific families

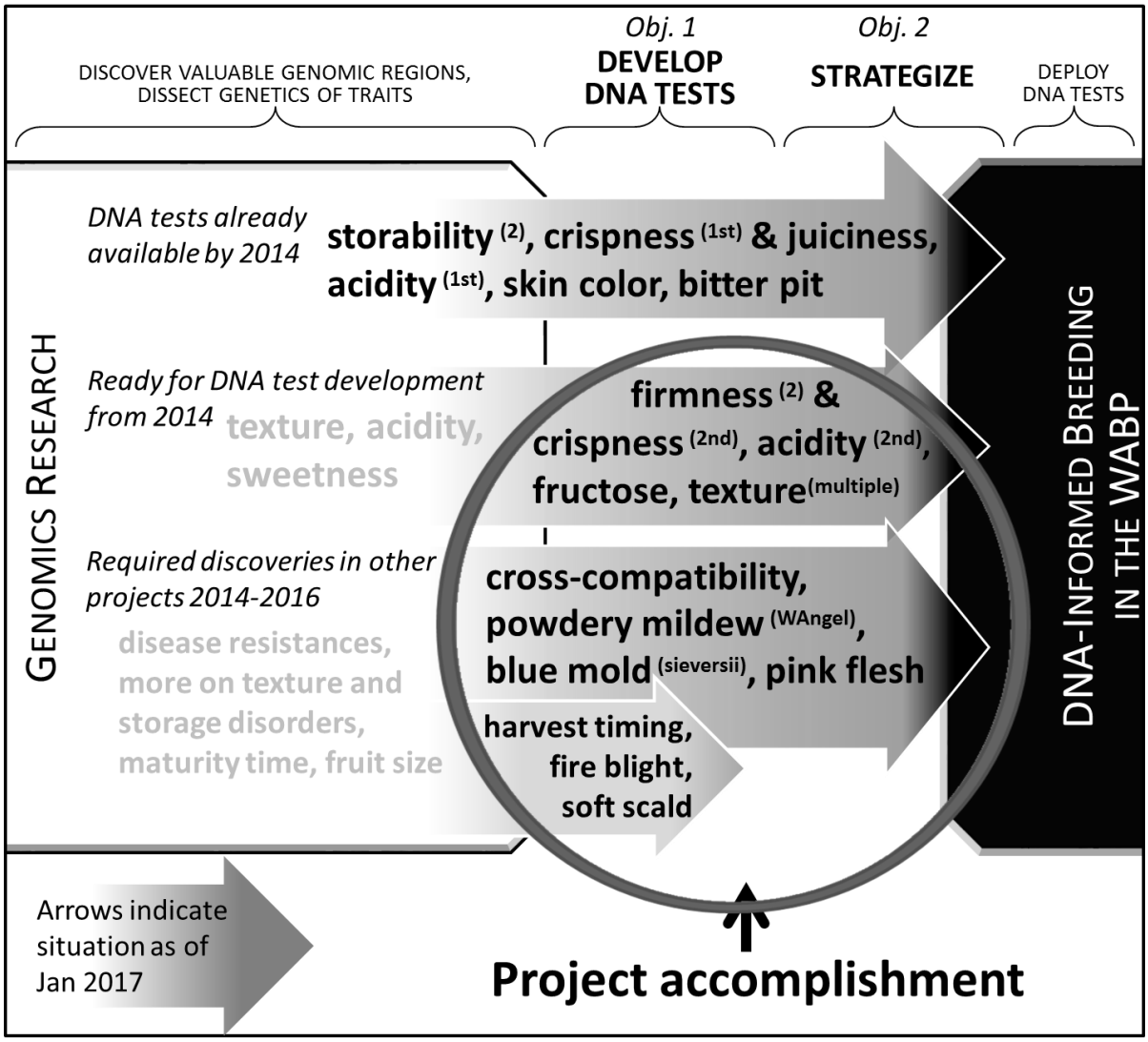

Figure 1: Progress made in 2014-2016 to develop, adapt, and refine DNA tests and establish strategies for their deployment in the WSU Apple Breeding Program (WABP).

RESULTS AND DISCUSSION

Activity 1a: DNA test development

Nine DNA tests were newly developed, adapted from publications, or refined for availability to the WABP. Previously available DNA tests were for fruit ethylene/storability (Md-ACS-indel and Md-ACO-indel), fruit crispness and juiciness (Ma-indel), fruit acidity (Ma-indel), and fruit bitter pit incidence (Bp16-SSR). Below are the 2014-2016 additions and refinements.

Fruit texture and storability (firmness, crispness, juiciness, especially after storage): A combined DNA test as well as individual tests are now available for four genomic regions that influence components of fruit texture and are variable within WABP germplasm. The first two genomic regions

are the genes encoding the ethylene biosynthesis enzymes ACS1 and ACO1 (targeted by DNA tests Md-ACS1-indel and Md-ACO1-indel). These DNA tests detect a small but significant effect on firmness especially at harvest. The third genomic region is the *Ma* locus (targeted by the DNA test Ma-indel). Among descendants of 'Honeycrisp', this DNA test detects a large difference in firmness at any storage duration and a large difference in crispness especially after storage. The fourth genomic region is the gene encoding the cell wall metabolizing enzyme of PG1 (targeted by the DNA test Md-PG1$_{SSR}$10kdb). This fourth DNA test is associated with large differences in firmness, crispness, and juiciness after long storage. These DNA tests can be run in a single assay all at once (we call it the texture "Wonder test") or individually. In addition, the Ma-indel test is predictive for some other traits (below).

Fruit acidity: The marker LG8A-SSR was developed for the second of two genomic regions associated with large genetic differences among WABP individuals for fruit acidity. LG8A-SSR when combined with the previous Ma-indel DNA test enhances prediction of apple acidity. Ma×A Acidity is the new combined DNA test that targets both genomic regions.

Fruit fructose content: A new DNA test, Md-LG1Fru-SSR, was developed for prediction of fruit fructose content. This DNA test differentiates almost all of the genetic differences observed in WABP seedlings for fruit fructose content (and some other sugars).

Disease resistances (foliar powdery mildew, blue mold) from particular sources: New DNA tests were developed for the 'White Angel' source of foliar powdery mildew resistance (presence of resistance allele is associated with strong resistance), Md-Plw-SSR, and a *Malus sieversii* source of resistance to the postharvest disease, blue mold (presence of resistance allele is associated with some resistance), Md-Pe3-SSR. The powdery mildew and blue mold DNA tests are only relevant for "pre-breeding" families that have used the wild sources in recent generations. These DNA test advances have been achieved by Pullman-based PhD student Feixiong Luo, supported by the China Scholarship Council, with guidance from pathologist and geneticist Dr. Jay Norelli (USDA-ARS Kearneysville, WTFRC- and RosBREED-funded) and PIs Peace and Evans. New progenies will be generated and inoculated with powdery mildew to validate the Md-Plw-SSR test.

Pink flesh color: Md-S3-indel, a DNA test for "Type 2" pink flesh, which identifies the allele associated with pink derived from 'Surprise' and 'Pink Pearl', was adapted from an existing marker for the S3 allele of the apple *S* locus. The *S* locus is closely linked to the *MYB110a* gene associated with flesh color, and the S3 allele is associated with the pink-flesh *MYB110a* from 'Pink Pearl'. This DNA test is only relevant for WABP families descended from 'Pink Pearl'.

Cross-compatibility: A recently reported "universal" DNA test for the *S* locus was adapted for the WABP. This DNA test, Md-S-universal, reveals most of the *S*-alleles present in the WABP, especially the common ones. Several allele-specific tests have also been adapted to detect further alleles. However, several WABP alleles have not yet been determined. In most cases, the test(s) can determine if two parents or selections carry the same two *S*-alleles and therefore would not be able to cross with each other.

Others in development: DNA tests are currently in development for two sources of fire blight tolerance and a genomic region for blue mold tolerance (tolerance alleles for all of these are present in elite WABP parents). DNA tests are also in development for major-effect genomic regions reported in the literature or detected by RosBREED collaborators to be associated with some of the genetic influences on harvest timing and soft scald.

Activity 1b: DNA test effects calculations

Software that we named *DNA Test Effects* was developed in R programming language implemented in ASReml. This software calculates the trait levels and variability associated with DNA test outcomes (i.e., genotypes, aka allelic combinations). Input data are from multi-family replicated trials – specifically for our current use is the dataset of RosBREED 1 in which performance data for many traits were recorded and genome scans were obtained on WABP germplasm (and two other U.S. apple breeding programs) for many hundreds of seedlings, selections, and cultivars. *DNA Test Effects* uses a statistical genetics model to estimate not only the relative effects on a trait of a DNA test of interest but also the "genetic background" (the cumulative effects of all the other genetic influences on the trait, large and small), fixed external effects such as year and location, statistical interactions between the DNA test and external effects (such as certain alleles whose influence is only manifested in some years, for example cold wet ones), and residual effects (effectively noise). This software provides a streamlined statistical approach to predict performance from DNA test outcomes, and is currently being used to update estimates of all DNA tests on their target trait(s). Next, the software will be used to estimate effects of our DNA tests on dozens of other traits of WABP interest that were measured in the RosBREED 1 project.

Activity 2a: Devising DNA test deployment strategies

Available DNA tests cannot simply be used all at once on all germplasm – it's much more complicated than that. The four major factors underlying deployment are value of the trait levels differentiated (trait levels), how well genetic differences among breeding program individuals are captured (predictiveness), which particular families or other germplasm are relevant (germplasm), and how much any given test is associated with another test or other traits (genetic complexity). The strategies associated with variations in these factors were discussed, modeled, calculated, and described in scientific papers. We developed a conceptual framework for capturing the above features of available DNA tests.

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. The workshop outcomes, including subsequent presentations and scientific papers on the topic, are keeping our fruit breeding programs on the cutting edge.

Detailed considerations of cost-, time-, and genetic gain-efficiency, as well as logistical feasibility, were described in detail in this project's second-year continuing report. Scientific papers arising from this work are listed below. More than a dozen professional meeting presentations have also been made on this topic over the last there years.

- Edge-Garza D, Luby J, and Peace C (2015). Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Molecular Breeding 35:223
- Ru S, Hardner C, Carter PA, Evans K, Main D, and Peace C (2016). Modeling of genetic gain for single traits from marker-assisted seedling selection in clonally propagated crops. Horticulture Research 3:16015
- Evans K, and Peace C. Advances in marker-assisted breeding for apple. In (ed. K. Evans) Achieving Sustainable Cultivation of Apples, Burleigh Dodds (in press)

- Peace C. DNA-informed breeding of rosaceous crops: Promises, progress, and prospects. Horticulture Research (submitted)

Two examples of DNA test deployment strategies considering trait levels, predictiveness, germplasm, and genetic complexity are described below.

Example 1: Ma-indel for multiple traits

The Ma-indel DNA test is in a hotbed for trait influences of importance to the WABP. The test itself lies inside a gene strongly influencing acidity content – in fact, in some families it is possible to know the Ma-indel genotype by tasting fruit (of course it is more efficient to figure this out by DNA testing seedlings many years ahead of actual fruiting). Individuals with two copies of the allele associated with low acidity are often too bland, while those with two high-acidity alleles are often too acidic. A second genomic region influencing acidity determines whether the double-low or double-high Ma-indel seedlings will be pushed over the edge beyond acceptable WABP thresholds. Ma-indel, especially when incorporated into the more comprehensive Ma×A Acidity test, therefore differentiates essential trait levels, indicating that it warrants use in parent selection (to help choose crosses likely to result in seedlings with fruit acidity not bland and not too high) and seedling selection (to cull any seedlings generated with extreme acidity genotypes). The predictiveness of the Ma×A Acidity test is medium, whereby the DNA test accounts for about half of genetic influences on acidity in WABP germplasm. Combining that predictiveness with the fact that the influence on acidity of all genetic factors variable in WABP germplasm is high means that, according to the framework of Ru et al. (2016), the DNA test should be used to avoid and cull the extreme genotypes in parent and seedling selection, respectively. The extreme alleles are common in WABP germplasm, and so most families deserve attention when considering Ma×A Acidity.

Finally, the genetic complexity of Ma-indel is in two main ways. The first is its connection with the second genomic region influencing acidity, as described above. The second is that genes influencing several other traits – crispness, firmness, bitter pit incidence, phenolics content, fruit size, sweetness, and others – are located adjacent to the acidity gene that Ma-indel targets. Because Ma-indel reveals numerous alleles (several associated with high acidity, several with low, and one medium), each allele can be associated with certain levels of those other traits. For example, one of the Ma-indel alleles from ‘Honeycrisp’ is associated with medium acidity, lower crispness after storage, lower firmness at any point, lower bitter pit incidence, slightly increased size, and slightly decreased sweetness. The other ‘Honeycrisp’ allele (also inherited by ‘WA 38’) is associated with lower acidity, higher crispness after storage, higher firmness, higher bitter pit incidence, slightly decreased size, and slightly increased sweetness. We believe the second allele is an essential component to the ultra-crisp texture of ‘Honeycrisp’ and ‘WA 38’, and that case the Ma-indel DNA test targets an essential trait level for some families. The other traits influenced can be mitigated by alleles at other genomic regions (and the second Ma-indel allele carried by any individual), as attested by the high acidity, lack of bitter pit, and large size of ‘WA 38’. Therefore, the information provided by Ma-indel for those other traits can be considered as targeting enhanced trait levels, and weighed up as part of many contributors to those traits during parent and seedling selection.

Example 2: Pink flesh

Pink or red flesh color vs. white flesh color of apple fruit is conditioned by genetic variants at just two genomic regions. What is more commonly called “red flesh”, or “Type 1 red flesh”, is due to a rare allele (originally from a subtype of *M. sieversii* called *M. niedzwetzkyana*) at the same gene as conditions skin overcolor amount. Pink flesh color, or “Type 2 red flesh”, is conditioned by a different gene on another chromosome, and the pink-flesh allele is from ‘Surprise’, an old cultivar, and some of its offspring including ‘Pink Pearl’. The DNA test for pink (vs. white) flesh, Md-S3-indel, is only relevant for WABP families descended from ‘Pink Pearl’. Therefore, this DNA test

targets a trait level that is essential but only in particular germplasm being purposely advanced for combining pink flesh with other elite attributes, and has high predictiveness for the trait. The Type 2 flesh color gene's genetic complexity is its genetic linkage with the *S* locus such that individuals with pink flesh will also carry the S3 allele – which can be exploited by making crosses that allow fertilization only by the S3-carrying pollen, most of which should also carry the pink flesh allele. These features indicate that effective deployment of Md-S3-indel in the WABP would be to use the DNA test's information fully during parent and seedling selection but only for those families intended to introduce the pink flesh attribute.

Activity 2b: Implementing DNA test deployment strategies

Software is needed to capture the many variables in these considerations. A software tool, *Multi-Trait Family Planning* was developed to target the most critical deployment stage: cross planning and greenhouse-stage seedling selection. Given known DNA test genotypes of pairs of parents being considered to create a hypothetical family with a user-chosen initial number of seedlings, this Microsoft Excel-based tool predicts and graphically displays genotypic outcomes. Such outcomes are in terms of the numbers of seedlings and their distributions of trait levels. (Trait effect estimates are determined by results from *DNA Test Effects* (Activity 1b) and can be updated as desired.) The user can examine the predicted effect of selecting for/against certain seedling genotypes on the trait level distribution of remaining seedlings. Because some DNA tests are known to influence traits other than what they were developed for, the tool calculates and displays the predicted outcomes of selecting with one DNA test on up to four other traits. If there are DNA tests available underlying those other traits, the user can continue the exploration of predicted outcomes of selecting with the next DNA test, and the next, and the next. With information on certain DNA tests already pre-loaded, all of these applications of the Excel tool could also be used in real time during marker-assisted seedling selection operations each spring. In this case, rather than predicted proportions of seedlings in each genotypic class, actual data from the DNA testing lab is used (Figure 2).

With remaining funds, in 2017 the Excel tool will be improved in 2017 with additional functions and possibly conversion to stand-alone software in a programming language such as R or with R-based functions connected to the Excel tool. An additional functions will be the ability to consider seedlings with unintended parentage, which DNA testing of seedlings reveal in most families usually at low levels. Another function will be the ability to automatically populate genotypic information on parents when their cross number entered, rather than currently having to enter such information by hand.

Prior to more sophisticated software, an interim DNA test deployment strategy in place for WABP. The major consideration is whether to use the DNA test only for parent selection (P) or for both parent selection and seedling selection (P+S). Decision factors are whether the trait level is essential (P+S) or enhancing (P), the DNA test is highly predictive (P+S) or somewhat predictive (P), and which families are relevant (deployment only for families expected to carry both good and bad alleles).

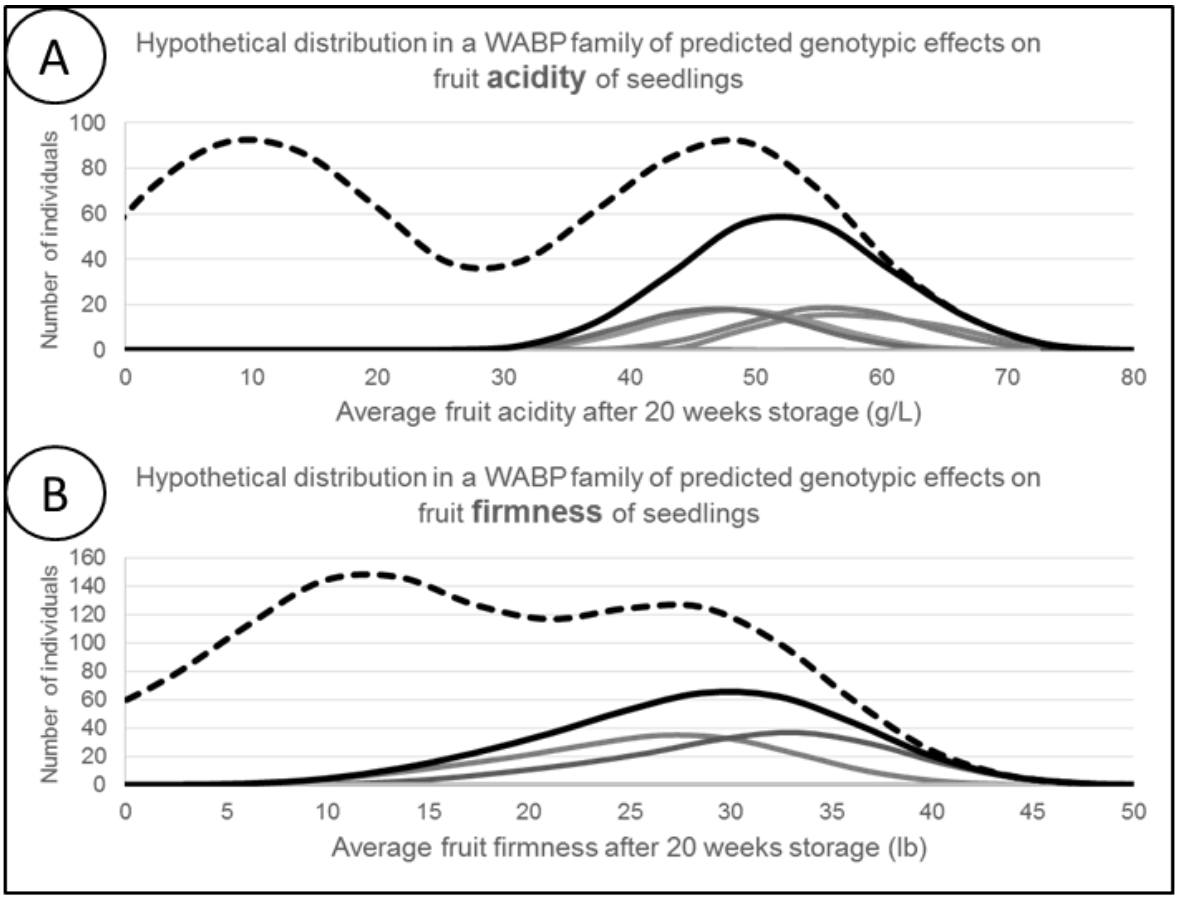

Figure 2: Example output of software Multi-Trait Family Planning*, Excel-based software to support cross-planning and seedling selection. Dotted lines represent estimated distributions of trait performance potential of seedlings prior to any culling. Thick continuous black lines represent the estimated distributions of seedlings after culling. Lighter gray lines represent estimated seedling distributions within specific genotypic classes for DNA tests underlying the trait. Note that input data in all cases here is hypothetical, created for the purposes of demonstration; our true estimated effects of DNA tests on these traits are not exactly the same.*

(A) Acidity distribution of a family after culling for low-acid genotypes associated with the DNA test Ma-indel. Note a substantial shift to the right (higher acidity) as well as a large reduction in total number of seedlings.

(B) Effects on seedling distribution for firmness in the same family as above after the culling for low-acid genotypes. Note that as well as fewer total seedlings there is a shift to the right – indicating that in this case the alleles associated with higher acidity were also associated somewhat with higher firmness. The software can model alternative situations too. The two gray lines represent estimated firmness distributions for two seedling genotypes of the next DNA test that could be considered by the breeder, Md-PG1SSR10kdb.

Continued next page.

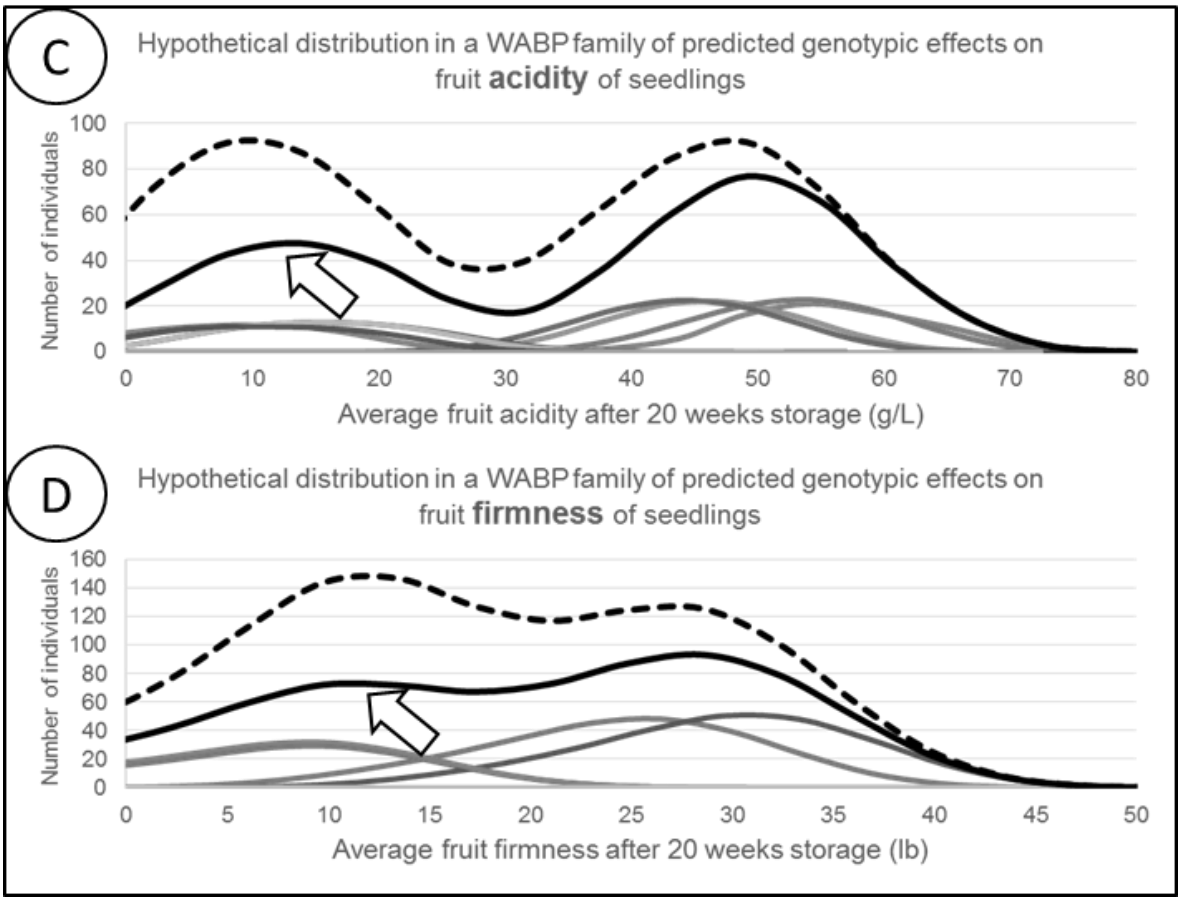

Figure 2 cont'd: (C) and (D) are the same as for previous (A and B), showing estimated distributions for acidity and firmness, except that there is retention of seedlings carrying a particularly desirable genotype at some other locus (in this case, high fructose content according to DNA test Md-LG1Fru-SSR). The main differences with (A) and (B) are that some seedlings with lower acidity and firmness are retained, pointed out by the two arrows, and more seedlings are retained overall.

EXECUTIVE SUMMARY

This project was about supporting the WABP with trait-predictive DNA information: New DNA tests were developed. Strategies to combine these new ones with already-available tests were devised. Software to improve both DNA test development and DNA test deployment were also developed.

New DNA tests were developed, adapted, or refined for the traits of: **fruit firmness** (two genomic regions) and **crispness** (a second region), **fruit acidity** (second region), **fruit texture** (combined test), **powdery mildew resistance ('White Angel' source)**, **pink flesh color ('Pink Pearl' source)**, **blue mold resistance (a *M. sieversii* source)**, **fruit fructose content**, and ***S*-genotyping for cross-compatibility**. DNA test development for several other traits is still underway: harvest timing, fire blight resistance, and soft scald.

The above DNA tests add to previous DNA-based tools for the traits of: fruit ethylene/storability (two genomic regions), fruit crispness (first genomic region) and juiciness, fruit acidity (first genomic region), fruit bitter pit incidence, and skin overcolor amount.

A new software tool, *DNA Test Effects*, was developed that calculates trait levels and variability associated with DNA test outcomes. This software provides a streamlined statistical approach to predict performance from DNA test outcomes, and is currently being used to update estimates of all DNA tests on their target traits. Next, the software will be used to estimate effects of our DNA tests on many other traits of WABP interest measured in the RosBREED 1 project.

Available DNA tests cannot simply be used all at once on all germplasm – it's much more complicated than that. The four major factors of each DNA test underlying their deployment in the WABP are value of the trait levels differentiated (trait levels), how well genetic differences among breeding program individuals are captured (predictiveness), which particular families or other germplasm are relevant (germplasm), and how much any given test is associated with another test or other traits (genetic complexity). The strategies associated with variations in these factors were discussed, modeled, calculated, and described in scientific papers. We developed a conceptual framework for capturing the above features of available DNA tests. The most critical components were distilled for objective DNA test deployment.

Software is needed to fully capture the many variables in the above considerations. A software tool, *Multi-Trait Family Planning* was developed to target the most critical deployment stage – cross planning and greenhouse-stage seedling selection. This software will be extended in 2017.

FINAL PROJECT REPORT

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

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Cooperator: Tory Schmidt, WTFRC

Other funding sources: CNPQ, Brazil (direct support to two graduate students)
Borlaug Foundation (direct support to one visiting scientist)

Total Project Funding: $210,142

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	2016
Salaries	$39,586	$39,586	$39,586	
Benefits	$19,498	$19,498	$19,498	
Supplies	$1,000	$1,000	$1,000	
Total	$60,084	$60,084	$60,084	$0

Footnotes: No cost extension for 2016.

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

Objective 1: Fruit from middle and low positions in v-trellis canopies displayed higher sensitivity to chilling injury in storage. Fruit position within the canopy influenced at harvest fruit quality to a greater extent than netting (except sunburn development). Netting of orchards led to changes in fruit quality after storage, most notably less bitter pit developed.

Objective 2: Ethylene green life varies with maturity at harvest and orchard lot. Fruit chlorophyll fluorescence changes during cooling but is not an indicator of chilling sensitivity. Fruit with high titratable acidity (TA) at harvest have relatively high TA after storage. The DA meter was able to track fruit maturation before picking, but did not correlate closely to other maturity indicators and did not pick up chilling injury development in storage. Dry matter is poorly correlated with soluble solids content or other quality and disorder indicators at harvest and after storage.

Objective 3: Conditioning less than 7 days can enhance chilling injury. Humidity during conditioning does not influence chilling disorder development.

Objective 4: High CO_2 during 1-MCP treatment the day of or after harvest does not cause CO_2 injury.

Objective 5: Bitter pit incidence can be reduced by CA and 1-MCP. Incidence is reduced the most by 1-MCP treatment the day of harvest followed by CA establishment the following day while fruit is at 50 ^{o}F. Total non-chilling disorder incidence is not enhanced by CA during conditioning.

RESULTS & DISCUSSION

1. Orchard factors: Fruit position in tree & light environment: Disorder incidence in storage was highly correlated by harvest sequence and location of fruit within the tree in all three years of the study. In particular, *soft scald* 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, at times beginning with the second pick, but at much lower overall levels (Figure 1). Generally the first symptoms were observed after four weeks of forced cooling (chilling temperatures of 33°F), preferentially in lower parts of the canopy (example in Figure 2). Netting delayed the onset of soft scald, diminished the total amount expressed over time and evened out the canopy effect, i.e. symptoms expressed throughout canopy. (Fig. 1&2)

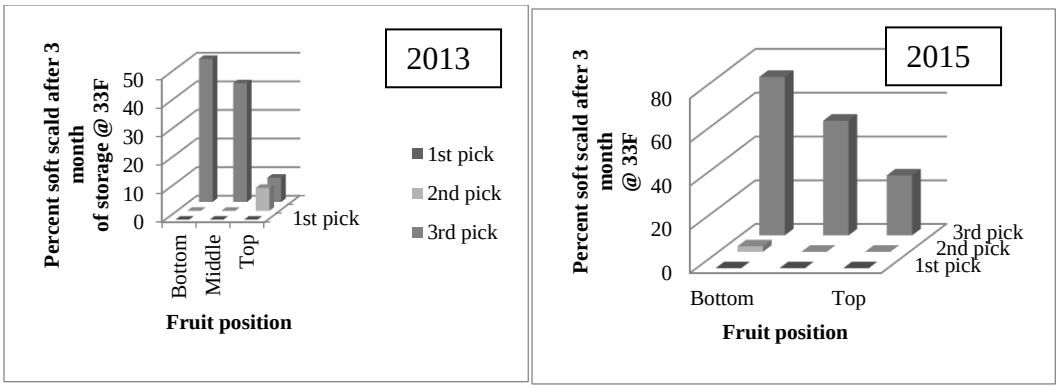

Figure 1: Development of soft scald in Honeycrisp apples stored for 12 weeks at 33°F. Fruit was harvested in 3 picks from three canopy positions from 2013 and 2015.

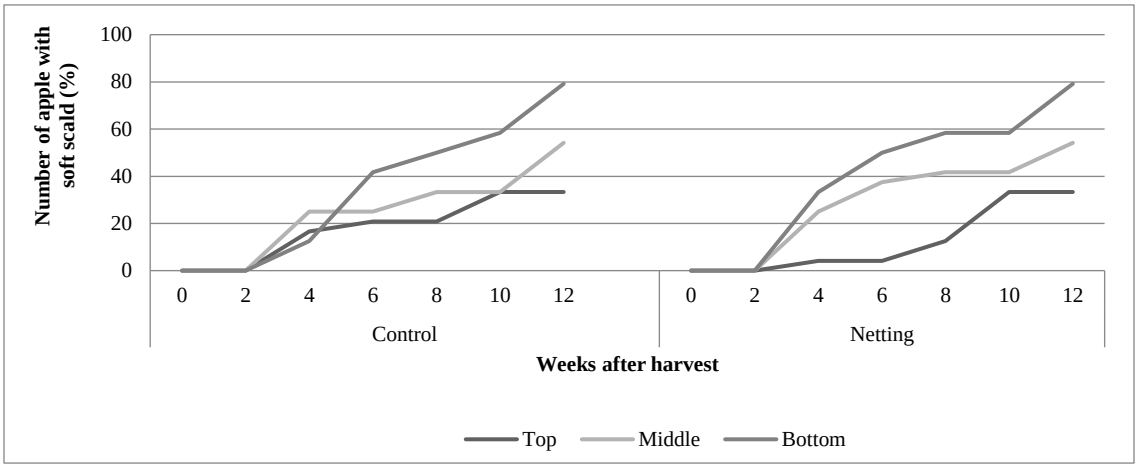

Figure 2: Time course of soft scald development in Honeycrisp apples stored for 12 weeks at 33°F in 2015. (Orchard 2, 3rd pick)

When storing fruit from netted and un-netted sections of Honeycrisp orchards, with or without 1-MCP application prior to CA establishment, we also found a marked *reduction of bitter pit* symptom expression in fruit grown under the 20% shade net, regardless of orchard or postharvest treatment (example in Figure 3).

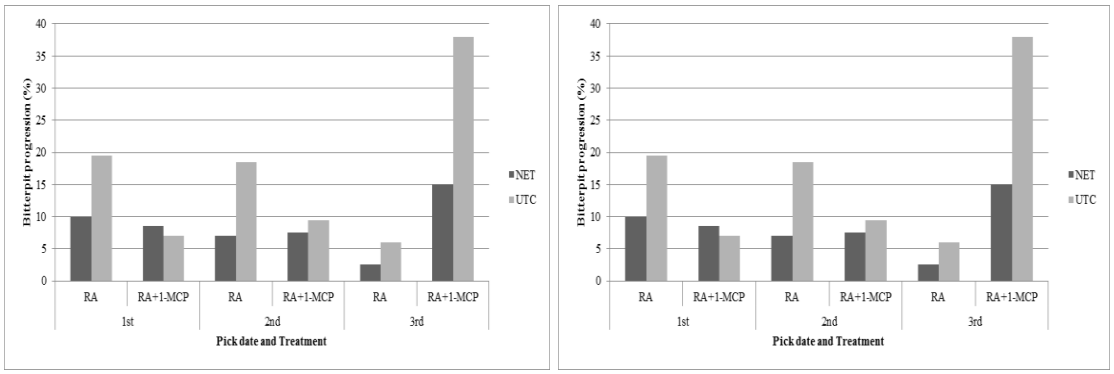

Figure 3: Bitter pit expression of Honeycrisp apples after three months of cold storage; fruit grown in two orchards near Gleed, WA in 2014 with and without shade netting

We utilized the DA meter in all three years of the study to determine it's utility to track maturity development, assess potential correlations to other common maturity indicators and non-destructively track fruit in forced chilling conditions to determine the DA meters capacity to detect chilling stress before visual symptoms appear on the fruit surface. As fruit matured on the tree, DA meter values decreased (as expected) and at harvest we typically observed a range of 1.2-0.5, depending on fruit position within tree (lower values in higher canopy positions). (Table 1) Fruit grown in the top section of the canopy was generally redder, sweeter and more acidic (2015 example in Table 1), while starch degradation rates, background color change, fruit size, and DA meter values appeared to be more independent of position of fruit within the canopy. Netting sometimes affected single maturity parameters depending on orchard location and year, but most often, fruit from netted sections expressed maturity similar to fruit from unnetted sections (Example in Table 1). Examples of effects of netting on at harvest maturity form 2014 include: the lone maturity parameter affected by netting was higher colored fruit in the upper netted section as compared to the lowest untreated section in the first pick (2014); netted fruit had lower sugar concentration (2nd and 3rd pick) and higher DA meter values (2nd pick) (data not shown).

Table 1: Selected at harvest quality parameters for fruit from the third pick of two orchards near Gleed, WA partially covered by netting in 2015.

Parameter	Location 1		Location 2	
	Control	Netting	Control	Netting
Diameter (inch)	3.3	3.4	2.9	3.0
Color (1-4)	3.8	3.7	2.9	2.8
Firmness (lbs.)	13.4	12.7	14.6	14.7
SSC (%)	13.3	13.1	13.3	13.3
TA (%)	0.377	0.397	0.473	0.529
Starch (1-6)	4.7	4.6	5.0	4.8

Netting of orchards consistently influenced the amount and severity of sunburn at harvest for all three years of the experiment (2015 example in Figure 4), thus significantly increasing the amount of packable fruit at harvest.

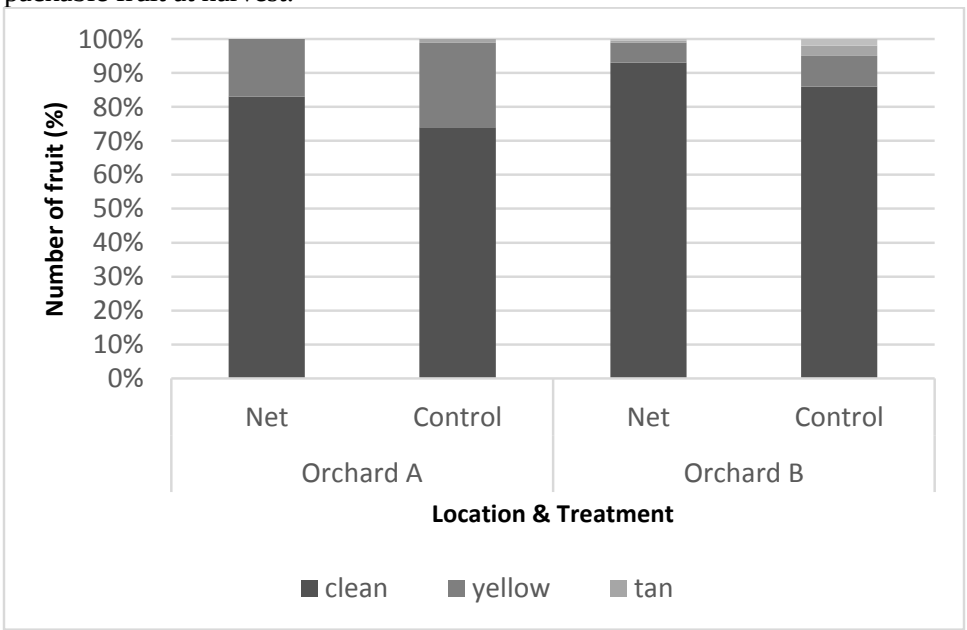

Figure 4: Sunburn incidence and severity in two Honeycrisp orchards at harvest in 2015.

2. Harvest and postharvest factors. Correlations among maturity and quality indicators at-harvest and at-harvest and after storage were evaluated particularly for dry matter and soluble solids content. Correlations were low for dry matter and soluble solids content at harvest and after 4 months air storage (Figure 5), however, a high correlation existed for soluble solids content at harvest and after storage. Results are for the first year of this comparison, additional results will be presented with the final oral report. Notable in the dry matter – SSC comparison are the low SSC/high dry matter values for late harvest, poor quality fruit. Harvest typically with most starch hydrolyzed may be a contributing factor to the relationships observed.

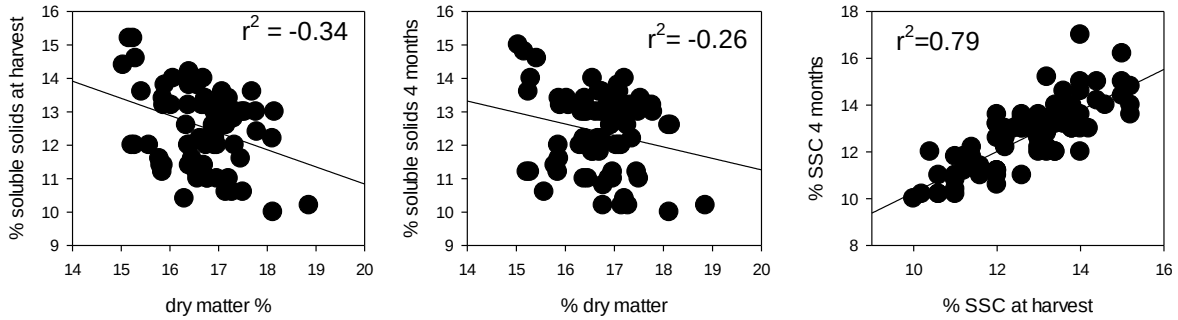

Figure 5. Relationship between fruit dry matter and soluble solids content at harvest and after storage. Fruit were stored 4 months in air then 7 days at 70 °F.

Initial ethylene production and rate of production increase may be indicators of storability. Lower ethylene production is associated with earlier harvest but the production increase during a week at 70 °F is not always reflective of initial values (Fuller harvests 1 and 2; Figure 6). Lower ethylene production is often associated with lower respiration rate and reduced utilization of titratable acidity, slower yellowing and greasiness development.

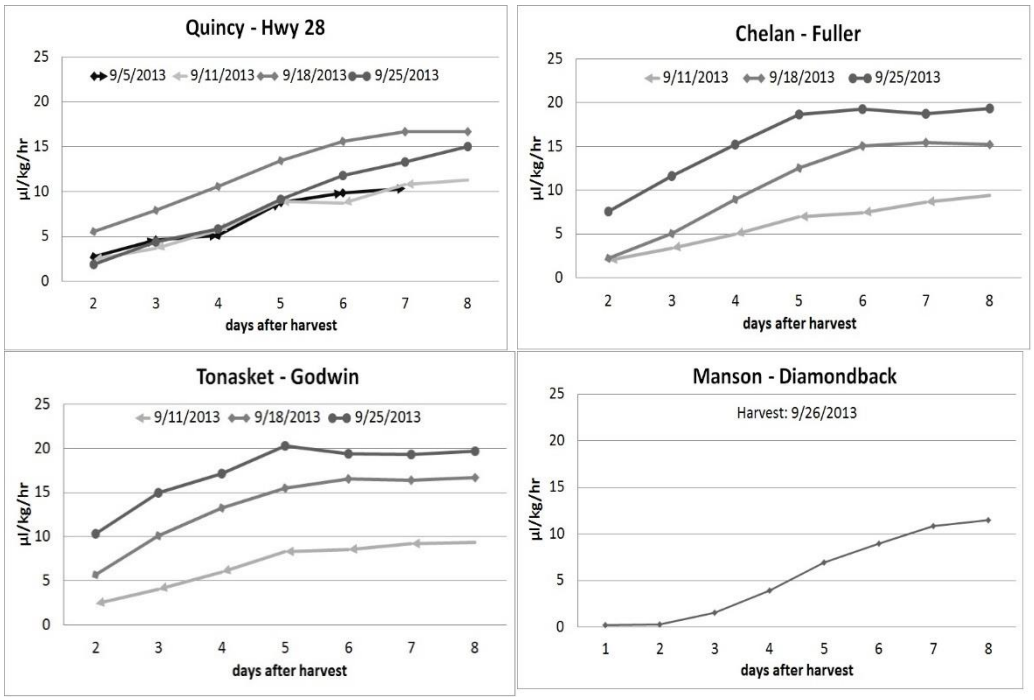

Figure 6. Ethylene production during 8 days at 70 °F following harvest.
Cold room humidity had no effect on subsequent development of chilling disorders (Figure 7). Fruit not conditioned but cooled to 37 °F in 40 or 85% relative humidity developed similar amounts of soft scald.

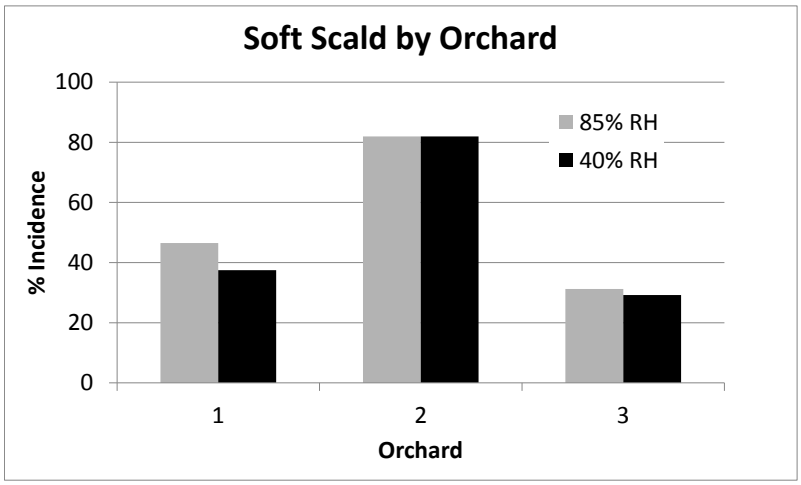

Figure 7. 'Honeycrisp' soft scald following 4 months cold storage in air.

The lack of difference in chilling sensitivity was in contrast to a difference in fruit chlorophyll fluorescence during cooling in the two relative humidities (Figure 8). The results indicate the hypothesis that water loss as provoked by low humidity during cooling can impact fruit chilling sensitivity appears to be invalid.

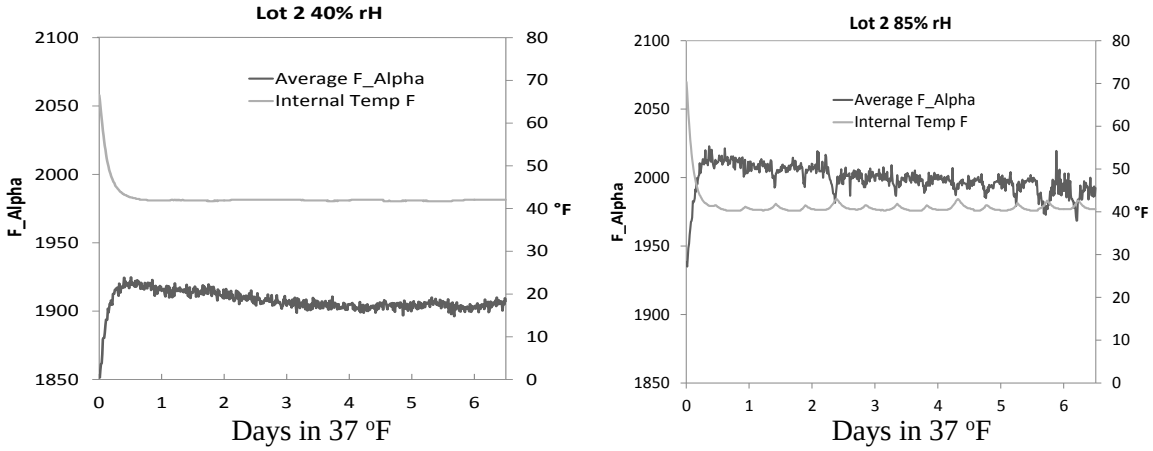

Figure 8. Honeycrisp chlorophyll fluorescence during cooling to 37 °F.

No relationship was observed between chlorophyll fluorescence at the initiation of chilling and subsequent development of soft scald or bitter pit (Figure 9).

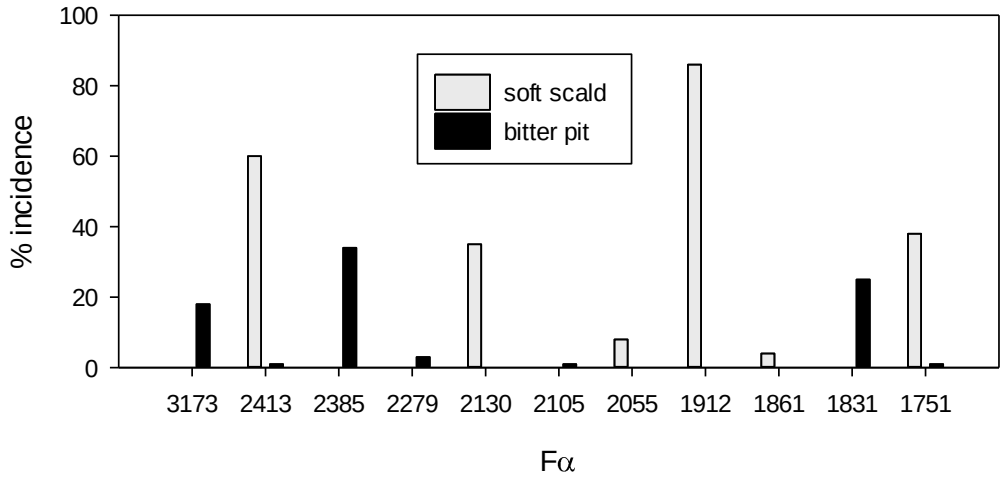

Figure 9. 'Honeycrisp' chlorophyll fluorescence Fα at harvest and soft scald and bitter pit development after storage at 33 °F in air for 3 months.

Managing conditioning rooms loaded over an extended period is a logistical challenge to meet the 7 day conditioning recommendation. We found that reducing the conditioning temperature by 5 °F after 2 and 4 days and then 3 °F at 7 days enhanced chilling injury (Figure 10). The results indicate risk of chilling injury can be enhanced by altering the conditioning protocol in this step down fashion. Additional research could be conducted to examine less rapid cooling during conditioning.

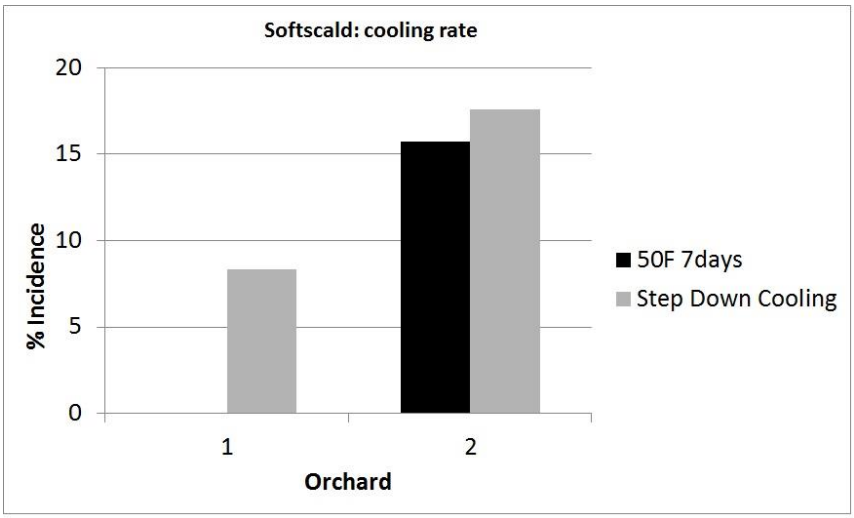

Figure 10. Soft scald incidence after 4 months cold storage in air.

Risk of CO_2 injury resulting from a 24 hour exposure resulting from CO_2 accumulating during 1-MCP treatment after harvest was examined over two years in three orchards. Fruit were conditioned for 7 days then held in air for 4 months. In no instance was a relationship observed between 1-MCP treatment and CO_2 injury in chambers where up to 4% CO_2 was present during 1-MCP treatment

(Table 2). Untreated fruit did not develop high amounts of CO_2 injury either. The results indicate a negligible risk for fruit CO_2 injury resulting from a 24 hour exposure within a day of harvest. As the risk of CO_2 injury decreases the longer fruit have been in storage, the results suggest a concern for CO_2 exposure should not limit the timing of 1-MCP treatment after harvest.

Orchard	% CO_2 initial	% cortex browning Control	1-MCP
A	0.0	0 (2.1)*	0 (1.9)
	2.0	0 (3.8)	0 (3.7)
	4.0	0 (4.8)	0 (5.2)
B	0.0	0 (2.6)	0 (2.4)
	2.0	0 (4.5)	0 (4.5)
	4.0	0 (5.1)	0 (5.8)
C	0.0	0 (2.1)	0 (2.0)
	2.0	2 (3.8)	0 (3.7)
	4.0	2 (5.1)	0 (5.5)

Table 2. Honeycrisp CO_2 injury incidence following 24 hour CO_2 exposure after harvest. Fruit were stored in air for 4 months plus 7 days at 70 ^oF.

Procedures to definitively establish orchard susceptibility to bitter pit remain unknown. As CA storage is known to reduce bitter pit development for other apple varieties, assessment of CA with and without the use of 1-MCP was assessed including initiation of CA during conditioning. In three years with 2 or 3 lots per year, both CA and 1-MCP were shown to reduce bitter pit development compared to untreated fruit stored in air (Figure 10). The best bitter pit reduction resulted from 1-MCP treatment the day fruit was received with CA established the following day. No evidence of enhanced incidence of other disorders due to CA during conditioning was observed. Some evidence of enhanced titratable acidity after storage was apparent from the 1-MCP/rapid CA treatment. The lack of damage from the rapid CA protocol used (3% O_2, 0.5% CO_2 after 1 day, 2% O_2, 0.5% CO_2 after 5 days) suggests additional research is needed to identify conditions where rapid CA establishment can cause fruit injury.

Atmosphere 1-MCP	Bitter Pit %	Peel Blotch %	Diffuse Browning %	Cavity %	Total non-chilling %	Soft Scald %	Soggy Breakdown %	Titratable Acidity %
Control:	26a	0.9b	3.0	5.1a	31a	2.3	3.3	0.387a
CA9d	15bc	2b	0.9	2.1b	17b	2.0	2.1	0.409a
CA 1d	30a	0b	1.6	0.6b	30a	1.3	0.4	0.294b
air	18bc	9a	1.7	5.3a	23ab	4.0	2.9	0.415a
1-MCP: CA	10c	7a	1.6	4.7a	15b	2.3	3.1	0.445a
9d	23ab	15a	0.7	2.0b	24ab	2.1	0.9	0.372ab
CA 1d								
air								

Table 3. "Honeycrisp' disorders and titratable acidity after 7 months storage 7 days at 70 ^oF. Summary of 7orchard years (2 years 2 lots per year, 1 year 3 lots). CA: 3% O_2 2 days, then 2% O_2, 0.5% CO_2 throughout. All fruit held 7 days at 50 ^oF, then at 37 ^oF. Means followed by different letters are significantly different, $p \leq 0.05$.

EXECUTIVE SUMMARY

Identification of orchard and postharvest factors that influence 'Honeycrisp' quality and disorder susceptibility provides information to enhance grower returns. Information developed in this project suggests pre- and post-harvest techniques that may reduce losses and enhance fruit quality. These include tree canopy management to reduce fruit numbers inside the canopy that tend to be highly susceptible to chilling injury. These fruit typically also are poorly colored and have low quality due to poor ripening and low soluble solids content. Reducing direct sunlight by netting also resulted in less bitter pit development while minimally impacting fruit quality attributes. Further research to more clearly define light environment impacts on fruit quality and postharvest disorders may provide additional benefits for field management.

Harvest and postharvest technologies continue to become available that assess additional components of fruit physiology and quality. The differential absorbance (DA) meter measures chlorophyll activity and changes in DA values have been related to fruit maturation. While the maturation tracking was confirmed for 'Honeycrisp', DA values did not correlate well with other indicators of maturity and quality. This may be due to a lack of physiological connection between chlorophyll metabolism and other aspects of fruit maturation that contribute to quality. Orchard as well as in-orchard variability also may compound the use of DA technology. However, individual growers may find utility for this instrument with repeated use over years and blocks that may enhance or confirm knowledge of fruit physiological progression in specific areas. Chlorophyll fluorescence (CF) is another property for which relatively new technology is available. This technology has been typically applied for use to establish CA oxygen content, and we found while CF values vary with fruit lots during cooling in air and in low and high humidity, utility of the CF values as a predictor for chilling injury was not established. Continued research evaluating CF as a tool during CA establishment during conditioning is warranted. Ethylene production during the immediate postharvest period can provide a means to indicate lot specific production patterns, however, utility of this information as an indicator of storage performance remains to be established. Technologies that reduce ethylene production and response (CA, 1-MCP) have been demonstrated to effectively extend Honeycrisp storage life. Conditioning to reduce chilling injury remains a necessary protocol in the absence of a means to identify lot susceptibility to low temperature. This protocol can enhance bitter pit development, and our results showing CA established during conditioning provide a means to reduce this disorder. Further work to define the optimal CA environment as well as CA conditions under which injury occurs would enhance the utility of this protocol as well as define the risk of rapid CA for Honeycrisp.

The continued profitability of Honeycrisp due in part to apparent lessening of chilling disorder risk due to adoption of conditioning throughout the industry is an example of research contributing to industry success. This project's results expand the knowledge of Honeycrisp produced under PNW conditions and may further enhance Honeycrisp management. The project participants thank the WTFRC for the opportunity to conduct these studies and look forward to continued work in this research area.

FINAL PROJECT REPORT

Project Title: Commercial testing of early scald risk assessment tools

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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.

SIGNIFICANT FINDINGS:

1. SRAB levels indicate which CA room will have the highest scald incidence for Delicious and Granny Smith as early as 1 month into storage.
2. Low and unchanging SRAB levels during CA indicate that apples will not scald while in those CA conditions.
3. When scald risk is high, room conditions can be checked and changed or fruit marketed according to assessed risk of each room.
4. A protocol to monitor SRAB (281 nm) could be used in the industry as part of a quality control regime.
5. SRAB levels increase with higher O_2 levels in CA storage.
6. Delaying CA imposition results in enhanced ethylene and SRAB levels.
7. CA conditions and room environment are the most important factors in scald control without crop protectants.
8. 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.
9. Metabolic pathways potentially associated with scald risk or tolerance at harvest were identified.
10. Natural apple wax components that are also accurate SRABs were identified.
11. Identification of additional chemistries linked with scald risk at-harvest.

RESULTS & DISCUSSION

Scald risk assessment for Delicious apples

In year 1, scald risk assessment biomarker (SRAB) levels monitored in Scarlet Spur Delicious apples stored in air began to increase between 1 and 2 months (Fig. 1). 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 by 9 months and SRAB levels did not increase. Reducing O_2 after scald risk was detected in fruit stored at 2% O_2 reduced, but did not prevent, scald. These results are, in part, consistent with previous results for Granny Smith, where scald incidence can be reduced if CA conditions (if not optimal) are remedied once increased SRAB levels are detected.

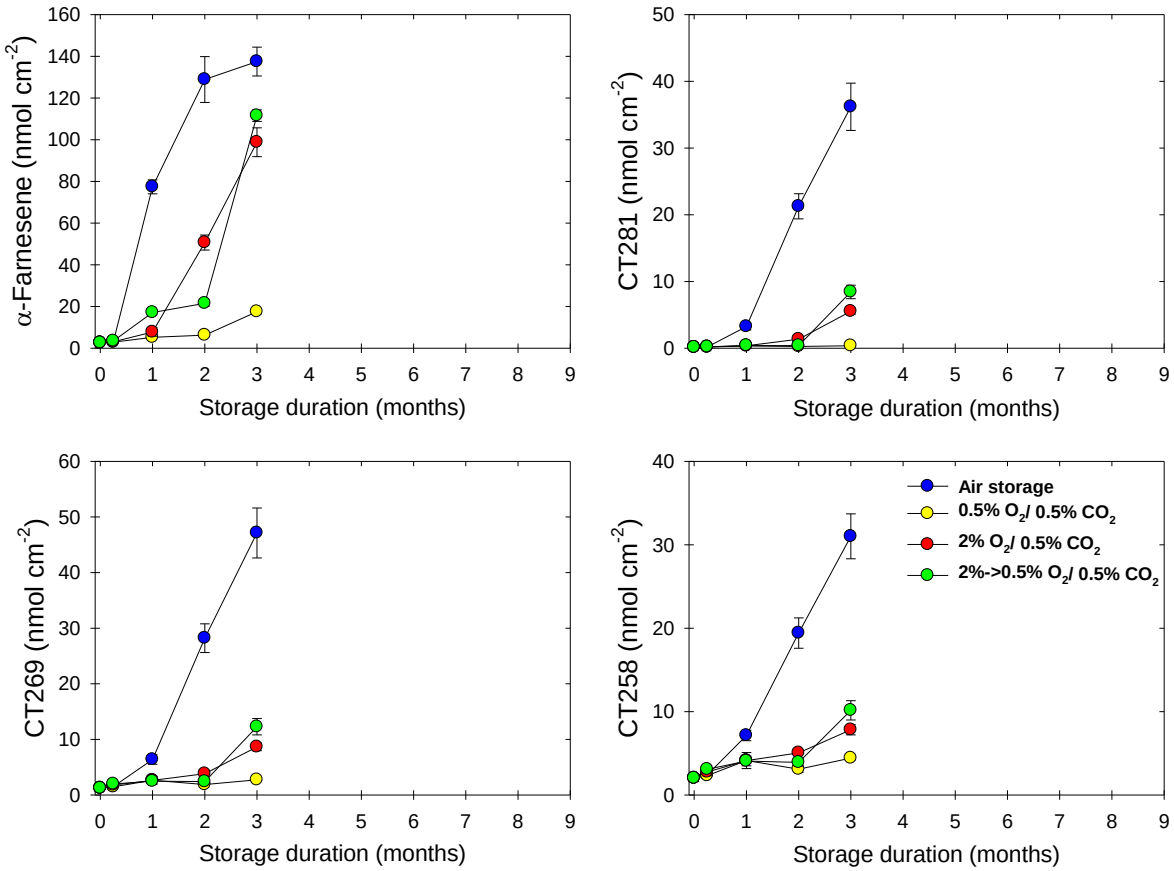

Fig 1. α-Farnesene (the "building block" of SRABs monitored in this study) and SRAB levels over the first 3 months in Delicious stored in test chambers at 33 ºF in air or 0.5% or 2% O_2. The oxygen was reduced to from 2% to 0.5% O_2 when SRAB levels were found to be increasing. Scald began to develop at 6 months in air and chambers held at 2% O_2 during the first part of storage. Scald incidence was less where the O_2 were reduced.

In year 2, three organic CA rooms containing samples from 3 orchards each were used to assess if the trial was still effective if scaled up to commercial sized. Average SRAB levels were relatively higher in rooms 1 and 3 and continued to increase in room 1 until 3 months (Fig. 2, top). At 3 months, all test samples were removed and half placed into a 36 F air room to simulate transit and retail supply chain or 33 ºF RCA room (0.6% O_2, 0.5% CO_2), monitoring scald monthly. Fruit stored in the RCA room was removed into the same air storage at 6 months to simulate a supply chain starting at 6 months. SRAB levels at 3 months accurately predicted relative scald incidence among rooms that was first detected at 4 months in air (Fig. 2, bottom left). Likewise, SRAB levels at 3 and 6 months predicted relative scald incidence among rooms after 6 months CA + 3 months air (Fig. 2, bottom right). Results indicate the test is scalable and SRAB levels were similar to those related to risk in our test chambers. SRAB levels suggested greater risk in 2 of the rooms and, although those rooms had more scald, all rooms developed at least some scald starting at around the same time with room 2 incidence around 10%. In this way, SRAB levels and the conditions that contributed to the relatively elevated levels, reflected only incidence and not when the disorder would actually develop. Consistent with previous results, SRAB levels most accurately predicted scald as influenced by the

storage conditions of a particular room rather than the orchard where the fruit was sourced (not shown). This is similar to results from Granny Smith (below).

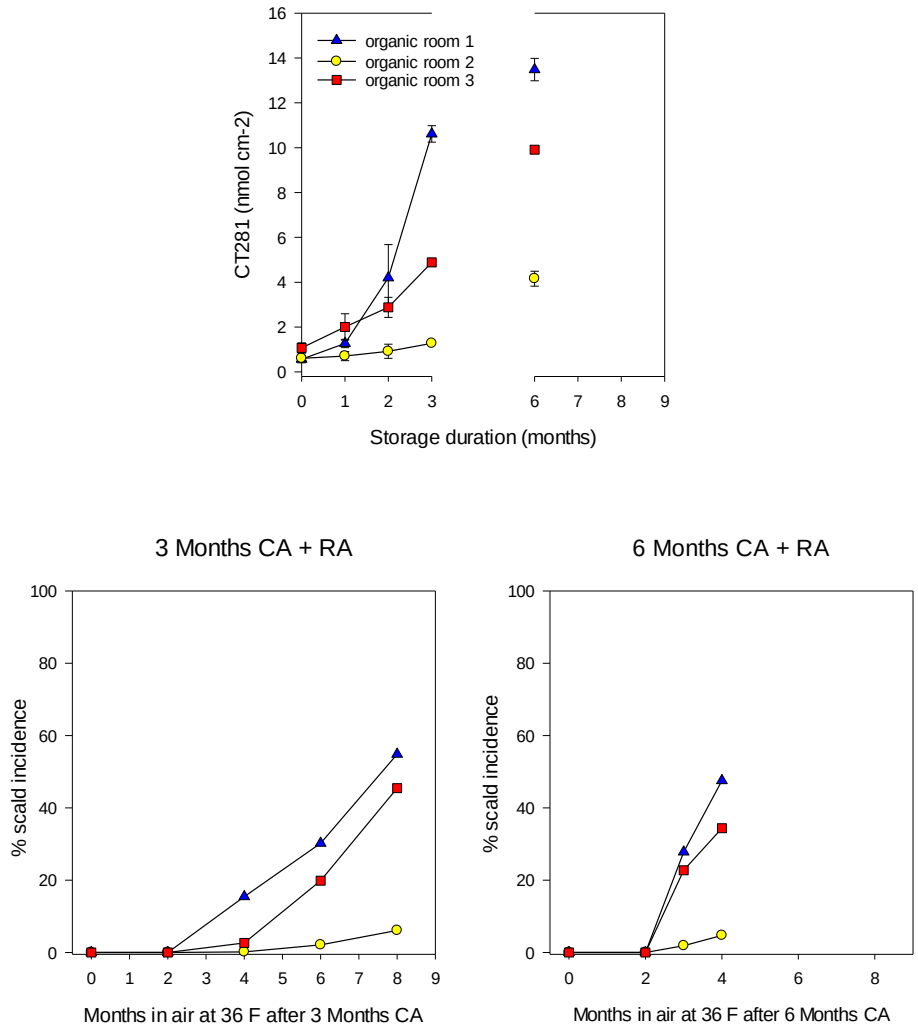

Fig. 2. (Top) Average SRAB levels measured in peel of Delicious apples stored in three commercial CA rooms containing fruit from 3 orchards each. Apples were removed from all CA storages and placed in air at 36 ºF or moved to an RCA room (0.6% O_2, 0.5% CO_2) at 3 months and, from here, moved to air at 6 months. Scald was monitored periodically up to 10 months storage to simulate a prolonged post-storage supply chain.

Scald risk assessment of Granny Smith apples

In year 1, 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 reduces scald.

In another 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). Scald was accurately predicted by monitoring SRABs.

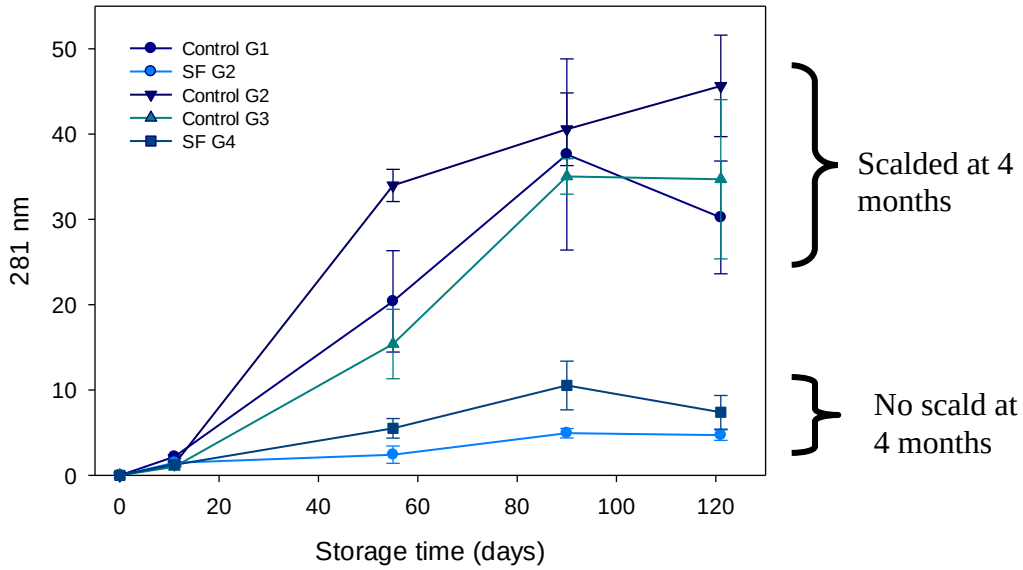

Fig. 3. Granny Smith SRAB levels and final scald incidence after 4 months commercial CA storage in an AgroFresh study using ARS methodology. Apples were chosen from a total of 4 growers treated with SmartFresh (SF) after harvest. Error bars represent standard error (n=3).

To improve upon the year 1 trials, year 2 (2014) trials employed fruit from additional Granny Smith orchards and commercial CA rooms. Average SRAB levels from all orchards within rooms were higher in one of the rooms after 1 month and continued to increase by 3 months indicating a higher risk in this room (Fig. 4, top). As in the Delicious trial, to simulate transit and retail supply chain, the samples were split and moved to air storage or the RCA room at 3 months and then from RCA to air at 7 months. Scald was first detected in all rooms at 4 months and continued to develop after removing apples after 3 months CA (Fig. 4, bottom). Scald began to develop in all rooms at 3 months following 7 months of CA. SRAB levels as early as 1 month reflected eventual scald incidence after 3 and, then, 7 months CA. Average values among orchards were 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.

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) and, therefore, IEC would not be an accurate method for assessing scald risk. Overall, results indicate that monitoring SRAB levels is an accurate means of assessing scald risk as influenced by CA conditions after as little as 1 month CA storage.

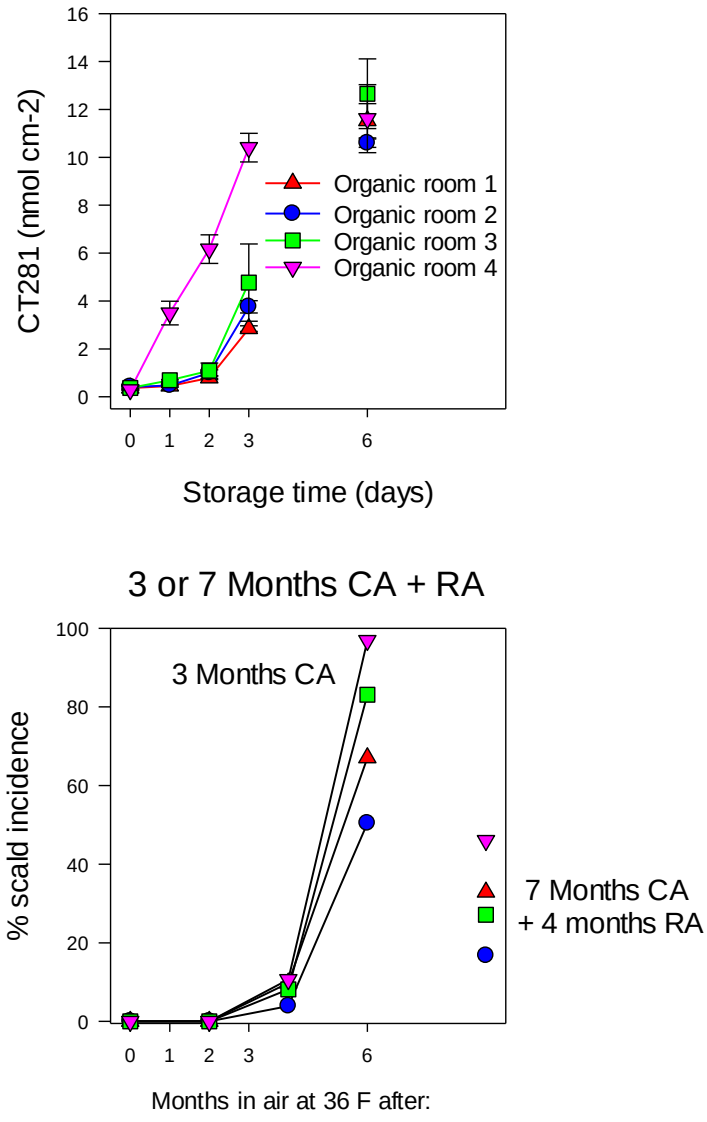

Fig 4. (top) Average SRAB levels for samples from 4 commercial organic Granny Smith storage rooms containing doorway samples from 3 orchards each. Fruit were moved at 3 months from commercial rooms into air at 36 ºF or RCA room (0.6% O_2, 0.5% CO_2, 33 ºF) and, then, to air at 7 months. Scald levels were evaluated periodically after until up to 11 months to simulate a prolonged post-storage supply chain.

Impacts of delayed CA on scald development of Granny Smith apples
In year 1, A one week CA delay lead to higher IEC, room ethylene, and SRAB levels (Fig. 5) 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 were moved to air storage at 9 months and scald incidence monitored for up to 2 months in 36 ºF air storage. As in year 1, SRAB and IEC are already considerably higher in the room where CA was delayed (Fig. 6) but scald did not develop until 6 weeks following removal from CA and was not different among orchards or between rooms. The influence of the delayed storage imposition was lessened by long-term storage under optimal CA conditions. Interestingly, samples from one of the orchards are stored in 2 commercial rooms and are also used in the delayed CA imposition trial this year. 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.

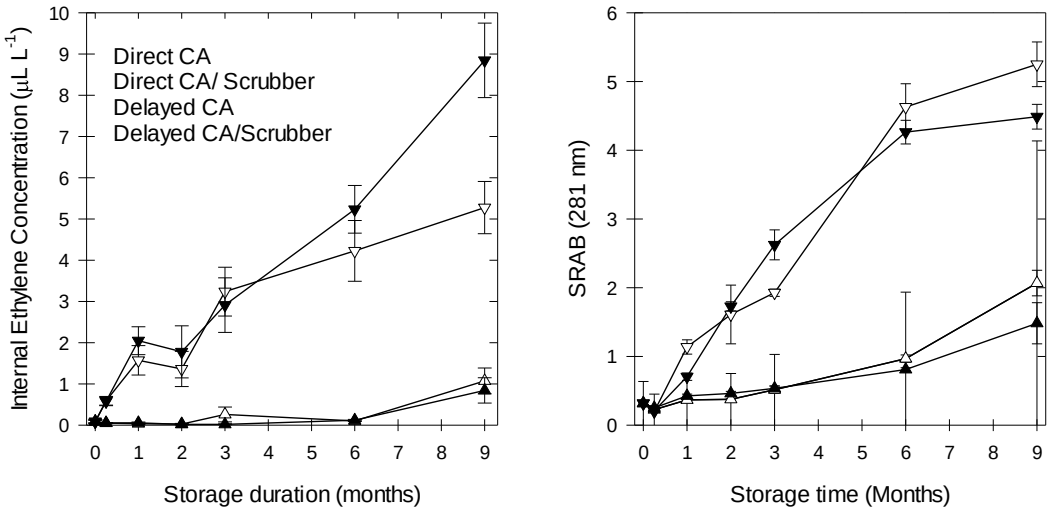

Fig. 5. (Year 1) Fruit internal ethylene concentration (left) and SRAB levels (right) over storage period in research CA rooms (30 bins, 1 orchard) set at 0.5% O_2/0.5% CO_2 immediately or following a 1 week delay. SRAB levels were higher in rooms with delayed CA imposition as were IEC values, although 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).

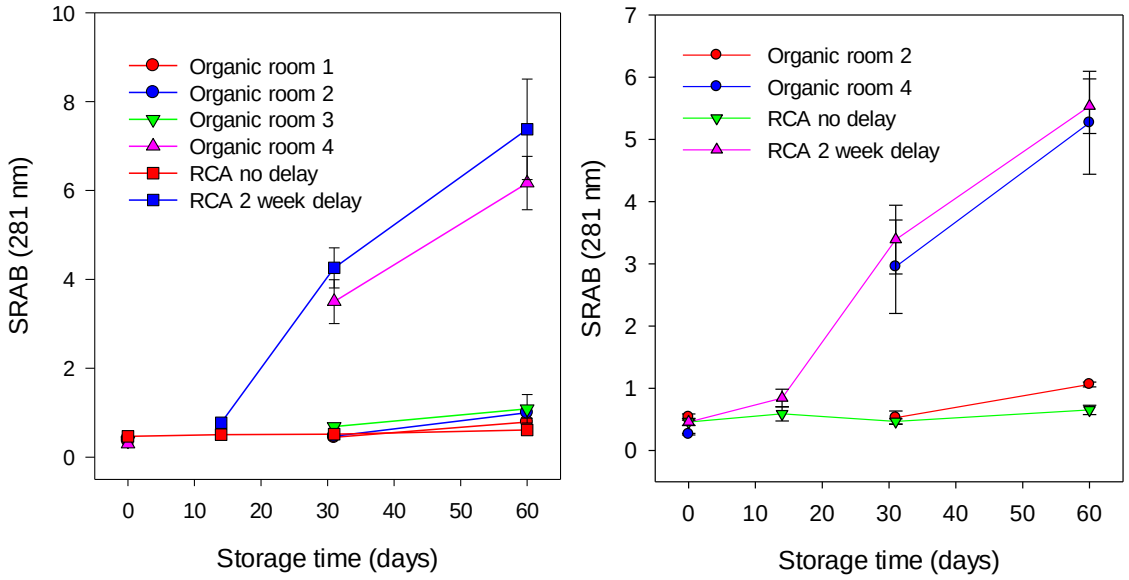

Fig. 6. (left) Average SRAB levels for samples from organic Granny Smith storage rooms and organic research CA room trials (Year 2). Averages represent samples from 3 orchards in each room. The low variability of SRAB levels among orchards in a particular room indicate factors evoked by the room have 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).

At harvest scald risk assessment

For Year 3, Granny Smith apples were harvested 2 weeks before, at, and 2 weeks after commercial maturity from 3 different locations (Basin and Wenatchee area). Orchards were relatively the same maturity across all three harvests with average starch index ranging from 1.2-1.5 at H1 and 2.1 – 2.6 by H3. IEC was low or undetectable as is typical of Granny Smith at harvest. Scald incidence, SRAB levels, and peel samples are taken after 0, 1, 2, 3, 4 and 6 months 33 ºF air storage and at 6 months (0.6 kPa O_2, 0.5 kPa CO_2) CA storage. Scald incidence continued to be monitored following 6 months CA on fruit kept in 36 ºF air. Peel samples were evaluated at-harvest for differences in peel chemistry (800+ natural peel chemicals screened) associated with scald risk. In air storage, scald symptoms began to appear between 3 and 4 months on apples from all orchards and harvest timings (Fig. 7). Scald incidence was less on fruit from the Mattawa location from all harvests. Scald incidence at 4 months decreased with harvest date only on fruit from the Mattawa orchard. Scald incidence at 6 months remained less on fruit from the Mattawa location for the final harvest. Scald levels were also lowest in the Mattawa orchard following 6 months CA+ 5 months air (not shown). Scald incidence was not reflected by the relative harvest maturity and, instead, was related to other undetermined factors.

SRAB levels did not entirely reflect differences of scald incidence among orchards and harvest maturities after air and CA storage but not earlier (not shown). This is consistent with our earlier work which indicates that this test does not consistently reflect scald risk among orchards held under the same storage conditions. Instead, we suggest that, when using only CA storage to control scald, the CA conditions have the greatest influence over whether a lot will scald or not, regardless of its

susceptibility going into storage and this test shows its greatest value in monitoring SRAB levels during the first 3 months to indicate whether storage conditions are controlling scald. Six month SRAB values using this method also did not accurately assess scald risk and, as we have reported before, should not be used to assess scald risk.

Evaluating natural peel chemicals at harvest yielded compounds may be positively or negatively associated with scald risk at 4 months. To find out which natural chemicals are associated with scald at harvest, 800+ peel chemicals were evaluated. A consensus of results from different statistical modeling techniques found those chemicals most associated with the Mattawa orchard at any harvest compared to the other two orchards (Fig. 7). Chemicals most associated with fruit that were at the highest risk were of a particular origin, the isoprenoids, while those associated with fruit having the lowest risk were primary oils and fats, many of which reside in the peel wax. These results are supported by an earlier preliminary study. As mentioned above, like superficial scald incidence, these associations apparently also have very little relationship with standard estimations of harvest maturity such as starch index and internal ethylene.

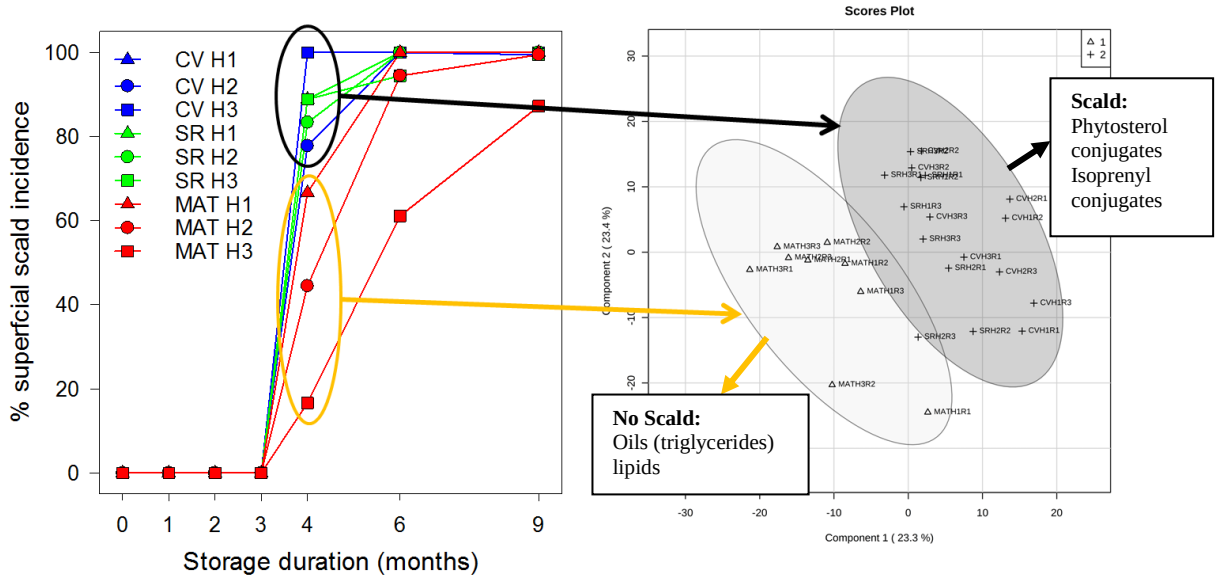

Fig. 7. (A) Superficial scald incidence on Granny Smith during 33 ºF air storage from Columbia View (CV), Sunrise (SR), and an orchard located near Mattawa (MAT). (B) Screening of ~800 peel chemicals at harvest indicates the low scald orchard had levels of a number of these metabolites that were different. These included higher levels of oils and lipids in peel less likely to get scald and a class of other non-polar compounds, called isoprenoids, in peel more likely to develop scald.

There is considerably more work required to devise a test that could use these sorts of metabolites as for scald risk assessment. Challenges potentially include difficulty of analyzing these compounds, likely requiring an agricultural services company's expertise, and additional validation required to determine accuracy. The existing SRAB storage monitoring protocol actually interrogates the fruit once the stress has been imposed, "asking" the fruit whether the storage conditions are sufficiently controlling the biological processes caused by chilling that lead to scald. However, it is likely that at-harvest risk assessment could be included as one basis for diagnosis of scald risk very early in the storage and evaluation of compounds like these may be one mean for achieving this goal.

Identification of SRABs that previously uncharacterized natural wax components in plants

We identified some of the SRABs that are collectively estimated using the spectrophotometric method revealed new components of apple wax not previously reported. The novel components are fatty acyl esters of secondary and primary farnesols. Two of these esters are SRABs and are ostensibly synthesized by an active process. All of these components, including farnesene, are mostly or entirely found in the apple wax. It has been widely accepted that the oxidative process is abiotic, or occurs during storage as a result of exposure to air rather than metabolically. This new evidence indicates that this process, which is closely linked with scald development, may be an actual component of metabolism where farnesene is enzymatically oxidized and then esterified. We have also found evidence that high levels of these compounds at harvest are associated with soft scald risk in Honeycrisp. The role of these compounds in wax structure is unknown as is where in the cell these compounds are synthesized and how they arrive at the surface and are incorporated into the wax layer. However, given their association with scald and soft scald, understanding the role and biosynthesis of these novel wax layer components may be critical to understanding why apples scald and may even be found to be a useful target for phenotyping.

Executive Summary

Background: Our previous work screening hundreds of natural chemicals in apple peel during scald development has revealed many with potential for use as biomarker-based scald risk assessment tools. Around 25 scald risk assessment biomarkers or "SRABs" were initially discovered and validated using a wide variety of conditions known to impact scald development including crop protectants, harvest maturity, temperature conditioning, harvest maturity, and CA oxygen level. Initial tests using test chambers and research CA rooms indicated that monitoring SRABs may aid in commercial storage and supply chain management decisions by monitoring whether CA storage conditions or crop protectant usage is sufficient to prevent superficial scald. We chose a relatively inexpensive and easy means of monitoring a few of these SRABs that correlated well with more costly and rigorous analyses. It was unknown whether the tests would remain accurate in full sized, loaded CA rooms or with other cultivars. Accordingly, our current work addressed these issues and other issues related to the practicality of SRAB monitoring.

Project outcomes:

1. An effective means to verify if postharvest crop protectant and CA controls are effectively controlling scald during storage.
2. Scald risk assessment tools validated for Granny Smith and Delicious.
3. Scaled down monitoring method that could be incorporated into industry QC protocols.

Significant Findings:

1. Monitoring SRAB levels indicates which CA room will have the highest scald incidence for Delicious and Granny Smith as early as 1 month into storage.
2. Low and unchanging SRAB levels while in CA indicate that apples will not scald while in those CA conditions.
3. When scald risk is high, room conditions can be checked and changed or fruit marketed according to assessed risk of each room.
4. We devised a scaled-down protocol to monitor SRAB (281 nm) that could be used in the industry as part of a quality control regime.
5. SRAB levels increase with higher O_2 levels in CA storage.
6. Delaying CA imposition results in enhanced ethylene and SRAB levels.
7. CA conditions and room environment are the most important factors in scald control
8. 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.
9. Identification of previously unidentified natural apple wax components that are also accurate SRABs.
10. Identification of additional chemistries linked with scald risk at-harvest.

Future Directions:

1. Treatment approaches that diminish scald incidence where apples are unprotected during post CA storage distribution and retail, especially for organic apples.
2. Continued validation of storage monitoring SRAB-based tools and defining their utility.
3. SRABs that provide scald risk assessment at harvest and for all points in the supply chain.
4. Similar risk assessment systems for other disorders such as Honeycrisp soft scald.
5. Biomarker-based tools for other fruit production uses.
6. New, better storing cultivars, with reduced postharvest disorder risk.

FINAL PROJECT REPORT

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

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*Dr.'s Zhu and Hanrahan served as CO-PI's of this study after Dr. Killinger took a new position with the FDA in July 2015

Cooperators: This study involved 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. We acknowledge the generous donation from Wilson Irrigation.

Acknowledgements: Manoella Mendoza utilized data from year 1 of the study in her MSc. thesis and her contribution to the success of the project is acknowledged. WSU staff with major project contribution: Tonia Green, Lauren Walter, Kyu Ho Jeong, Andy Liao. WTFRC seasonal staff has contributed greatly to the success of this project and the effort is highly appreciated.

Total Project Funding: **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 provided funds for additional testing of irrigation water.

Budget History:

WTFRC Collaborative expenses (projected):

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 as originally proposed. Actual numbers vary, depending on year. Permanent staff time was not included into original budget. Total WTFRC collaborative expenses for 2016, incl. all staff costs, benefits, travel and equipment amounted to $ 48,867.

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:
[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

Objective 1 (inoculation method development):

- For the field study, highly sensitive methods and generic *E. coli* strains were selected, optimized and utilized to align with studies having similar objectives in other regions and with other commodities. Rifampicin resistance to regionally acquired *Salmonella* spp. and *E. coli* O157:H7 was developed for growth curve analysis. Growth curve methods were optimized.

- This project developed field inoculation methods coupled with specific staff training programs to generate a consistent method for application of generic *E. coli* surrogates in an orchard setting.

Objective 2 (surrogate survival under field conditions):

- Additional treatment with overhead, evaporative cooling did not appear to impact survival of generic *E. coli* on apples within the first four days after inoculation, compared to the response on control apples that did not receive overhead cooling application.
- Experimental data on mature Gala and Golden Delicious suggests *E. coli* were reduced at a rate greater than or equivalent to the 0.5 log per day reduction proposed by FDA for overhead evaporative cooling (EC) treated varieties for at least four days after inoculation when applied within one week of commercial harvest.
- Generally, the greatest reduction rate of generic *E. coli* on apples occurred within the first 8-10 hours of inoculation, with additional reduction at a slower rate between 24 – 106 hours. In some cases, increases in generic *E. coli* levels were observed between 24 hours and 176 hours.
- Based on initial analysis (without statistical analysis for all three years), the following factors did not appear to consistently impact reduction of generic *E. coli* levels at all time points: type of EC system (traditional vs. misting), and inoculum level (approximately 10 million/31,000,000 CFU, 7.5 log) vs. a lower inoculum level (approximately 3,100 CFU, 3.5 log).
- Some factors appeared to have an effect at certain time points within an experiment and warrant more detailed statistical analysis and continued investigation in future studies, including canopy location of fruit, training system of the orchard, weather conditions, fruit developmental stage (mature vs. immature), and yearly variability.
- The reduction of generic *E. coli* varied dramatically among individual apples within the same variety at any given time point. Typical standard deviations for most time points after 2 hours were greater than 1 log. Therefore, at any given time point, some individual apples had higher generic *E. coli* levels observed than the overall averages reflect (in some cases between 3.5 – 6.0 log CFU/apple, or approximately 3,000 – 1,000,000 generic *E. coli* remaining at the end of the sampling periods in the experiments.

- Reduction of generic *E. coli* was influenced by apple varieties. In general the reduction in generic *E. coli* on Fuji apples during the first 10 hours after inoculation was lower (slower rate) than for other varieties examined in the study (Gala and Golden Delicious). Fuji apples showed a 1.1 – 1.8 log reduction compared to 2.1-2.9 log for Gala/Golden Delicious, possibly due to the different harvest season of this variety, mid-October versus late August to early September.

RESULTS & DISCUSSION

Objective 1 (inoculation method development): Following is a description of the field inoculation protocol developed by the team as part of the project in Year 1:

Rifampicin-resistant generic *E. coli* strains (TVS 353, TVS 354, TVS 355, LJH 1238) were obtained from UC Davis and used for the inoculum cocktail. For the cocktail, lawns of each strain were grown on MacConkey agar with 50µg/ml rifampicin and removed by adding 0.1% peptone water followed by carefully dislodging the lawn. The liquid inoculum was collected from each plate, combined into a cocktail and transported to the orchard on ice for final inoculum preparation. Immediately prior to inoculation, the cocktail was combined with 9.6L 0.1% peptone water in a backpack sprayer and mixed.

Preliminary field experiments were performed in all years to optimize the field inoculation method and ensure consistent inoculum levels on apples. Individuals with the most consistent technique for inoculation were identified through preliminary experiments to perform inoculation during the field experiment.

Inoculation was performed after sunset to reflect the last application of potentially contaminated water prior to harvest as well as the highest risk for bacterial attachment and survival. Apple harvest time points reflected industry harvesting practices (e.g. start of picking at dawn). For inoculation, an individual navigating a ladder in the dark with a backpack sprayer of inoculum sprayed individual apples on each tree. Teams of "spotters" with flashlights communicated with the "sprayer" to ensure thorough inoculation. Emphasis was given to crew training and quality control throughout the study, by strictly adhering to a detailed experimental plan to optimize sampling consistency at each time point, performance assessment and corrections of all personnel, de-briefing after each experiment, and in general maintenance of a constant feedback loop between lab and field team members.

Enumeration of rifampicin-resistant generic *E. coli* (flow diagram provided in Figure 1) was performed at the WSU Pullman campus. For each apple, 10ml of 0.1% peptone water was added then rubbed for 1 minute, shaken for 30 seconds, and rubbed 1 minute. Media for enumeration involved CHROMagar™ ECC with 50µg/ml rifampicin with and without filtering as well as pre-enrichment in tryptic soy broth (TSB). Time 0 samples were plated onto CHROMagar™ ECC with rifampicin (Figure 1) for quantification. Samples at later time points were also plated onto CHROMagar™ ECC with rifampicin for enumeration as well as pre-enriched in TSB and the remainder of the sample was filtered. If samples had counts below the countable range, the TSB pre-enrichment was plated onto CHROMagar™ ECC with rifampicin. The detectable limit was 1 CFU/apple.

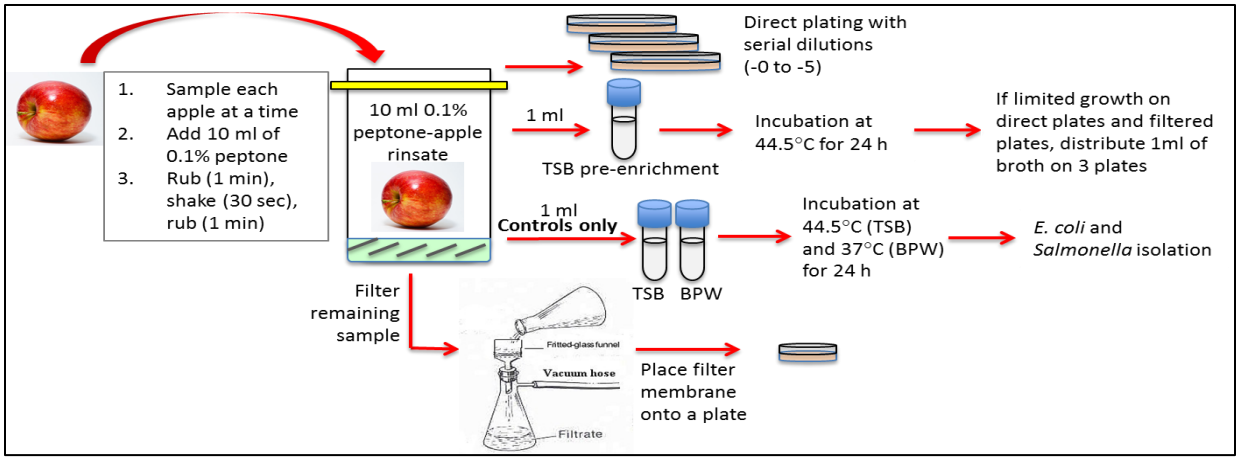


Figure 1: Flow diagram summary of enumeration method

Objective 2 (surrogate survival under field conditions):

WSU research orchards located in two regions representing a significant segment of the tree fruit growing region in Washington were utilized. Several apple varieties (Fuji, Gala, Golden Delicious) were examined with inoculation near harvest and earlier in the season in the Wenatchee and Prosser region, following the general inoculation protocol described in Objective 1. Prior to inoculation, trees were carefully selected for moderate crop load, all damaged fruit was removed prior to inoculation.

The primary objective, to examine the impact of EC versus untreated fruit on generic *E. coli* levels was examined over 2-3 years in replicated field blocks for 3 different varieties that represented different harvest seasons; mature Gala and nearly mature Golden Delicious apples were examined near harvest over three years, and mature Fuji were examined over two years. Each treatment (untreated control (UC), standard evaporative cooling (EC) and mist (Prosser, Fuji only)) included two field replications. The impact of canopy location was included as a variable in each year of these studies (approach differed slightly in 2015, see below for more detail). Notes on weather related variables during and following inoculation were documented by field staff. Additionally, WSU Ag WeatherNet data will be included in final data analysis.

During the course of the project, smaller scale studies were also performed to examine the following variables: canopy location of fruit, training system of the orchard, type of EC system used (traditional vs. misting), a high inoculum level (approximately 10 million/31,000,000 CFU, 7.5 log) vs. a lower inoculum level (approximately 3,100 CFU, 3.5 log), and fruit developmental stage (mature vs. immature) (more details in Table 1). As a result, an extensive data set including 25 experiments over three years was developed.

At the onset of each experiment, untreated control apples (20 of each variety and treatment) were collected from buffer rows between treatment blocks and examined for total coliforms, generic *E. coli* as well as pathogenic *E. coli* and *Salmonella*. For inoculated apples, immediately after an entire tree was inoculated, apples were randomly picked at each canopy position 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. Time points differed slightly between years. Generally, fruit were sampled for up to one week after inoculation at the following time points: 0, 2, 10, 18, 34, 42, 58, 82, 106, 154 hours.

Table 1: Experimental variables included into the assessment of overhead cooling practices for apple food safety

	2014			2015			2016		
	Gala	**Golden**	**Fuji**	**Gala**	**Golden**	**Fuji**	**Gala**	**Golden**	**Fuji**
Mature fruit	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Immature fruit				**X**	**X**				
Region(s)/tree architecture/training system						**X**			**X**
Misting system						**X**			**X**
Fruit position									
Fruiting wall (top/bottom)	**X**	**X**					**X**	**X**	
Fruiting wall (top/bottom inside/bottom outside)				**X**	**X**	**X**			
Traditional training system (full sun vs. shade)									**X**
Lower inoculum level (3-4 log)*									**X**

*typical inoculum levels for all other experiments: ~7.5 log

General observations: The reduction of generic *E. coli* varied dramatically among individual apples within the same variety at any given time point (detailed descriptions shown in year 1 & 2 reports). It is important to note that the standard deviations associated with each mean is typically around 1 log (detailed discussion provided in year 2 continuing report). Statistical analysis will be performed to fully evaluate project results. All results should be considered preliminary; for example, it is likely that seasonal influences impacted differences between varieties, and the statistical analysis will be useful in assessing this observation.

A total of 465 apples of untreated control fruit were examined between 2014 and 2016. For detection of foodborne pathogens, *E. coli* O157 was never detected, but 2 apples were positive for *Salmonella spp*. (Fuji, Oct. 3, 2016; Prosser). In addition, 37 (8%) apples had detectable levels of generic *E. coli* (32 Fuji, 3 Gala and 2 Golden Delicious, majority from Prosser) and 174 (37%) apples had detectable levels of total coliforms.

Mature fruit, Wenatchee region, Sunrise orchard: Averaged over three years of data for untreated **Gala** apples, at 10 hours, the average reduction in generic *E. coli* was 2.5 log and for treated Gala with evaporative cooling at 10 hours, the average reduction was 2.9 log (Figure 2). For untreated Gala apples, average values increased between 18 and 24 hours; a slight increase was also observed in treated Gala apples. The greatest reduction in generic *E. coli* levels on mature Gala apples inoculated near harvest were observed within the first 8-10 hours of inoculation, with additional reduction at a slower rate between 34 – 106 hours.

Averaged over three years of data for untreated **Golden Delicious** apples, at 10 hours, average reduction in generic *E. coli* was 2.1 log and for treated Golden Delicious (EC) at 10 hours, average reduction was 2.8 log (Figure 3). The greatest reduction in generic *E. coli* levels was observed within the first 8-10 hours of inoculation, with additional reduction at a slower rate between 34 – 106 hours for generic *E. coli* inoculated near harvest. For almost all time points, average generic *E. coli* levels for treated Golden Delicious apples tended to be slightly lower than corresponding

values for untreated Golden Delicious apples.

Figure 2. Generic *E. coli* levels on inoculated Gala apples with (EC: treated) and without (UTC: untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA from 2014 to 2016. Values reported in $\log_{10}$ colony forming units/apple.

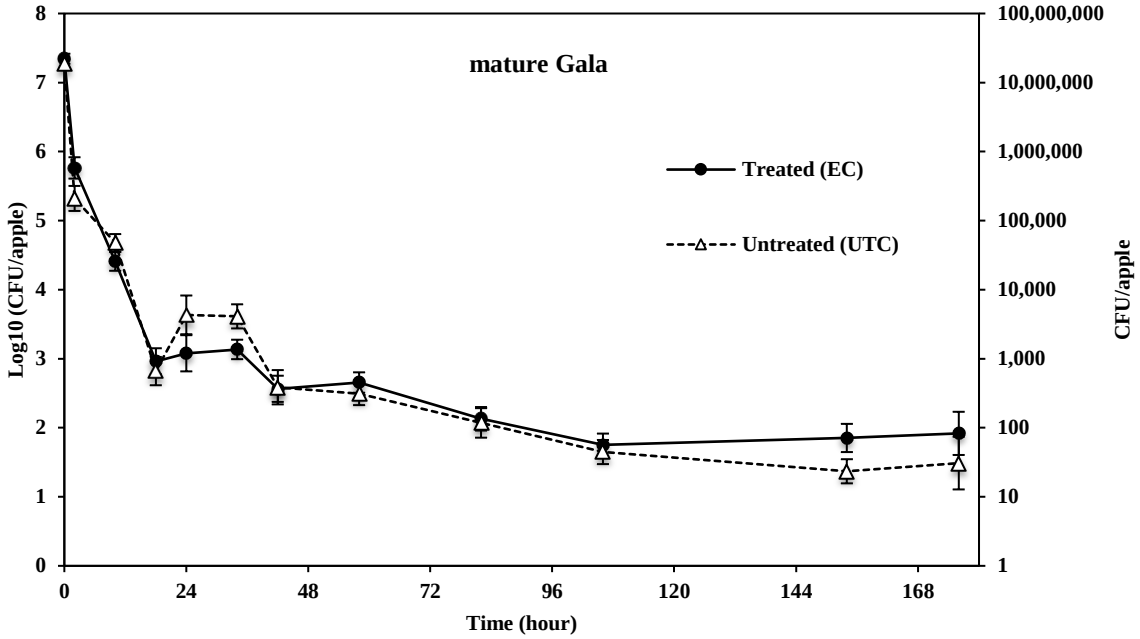
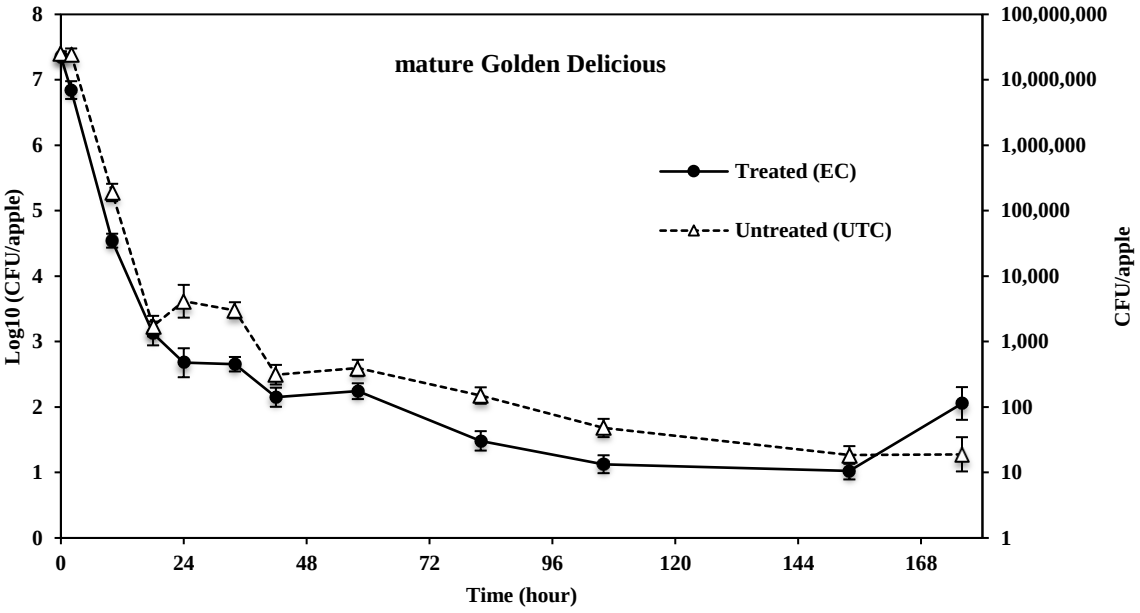
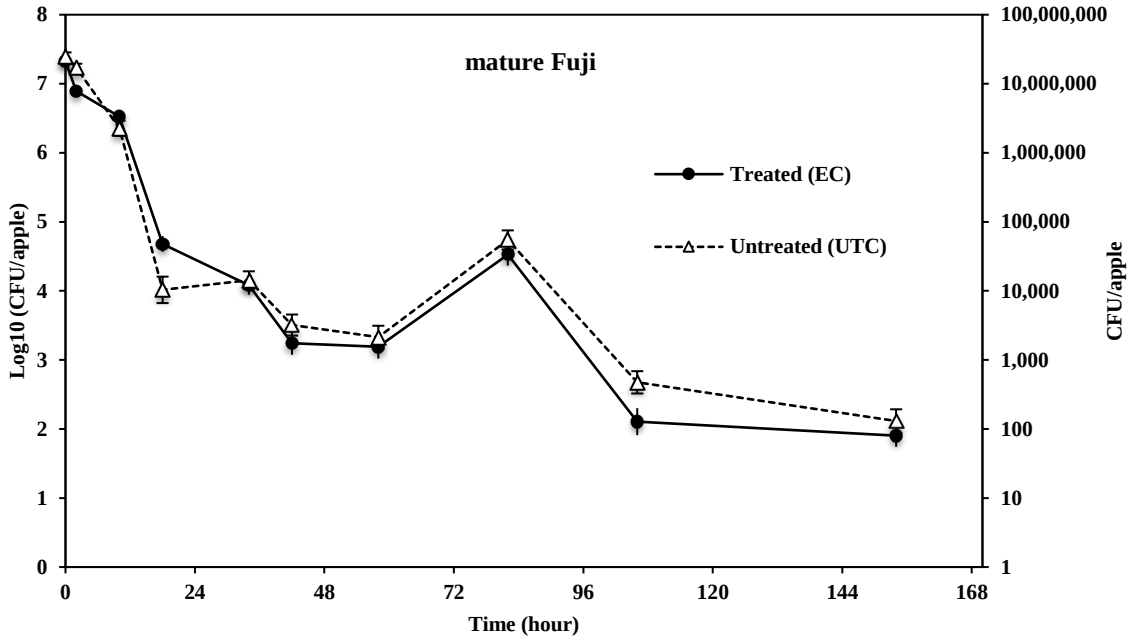


Figure 3. Generic *E. coli* levels on inoculated Golden Delicious apples with (EC: treated) and without (UTC: untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA from 2014 to 2016. Values reported in $\log_{10}$ colony forming units/apple.



Averaged over two years of data for **Fuji** apples, at 10 hours, average reduction in generic *E. coli* was 1.1 log for untreated Fuji and for fruit treated with evaporative cooling at 10 hours, average reduction was 0.8 log (Figure 4). For both treated and untreated Fuji apples, average values increased between 58 and 82 hours in 2015. Fluctuations in generic *E. coli* survival were observed in both years of the study in both locations between 42 – 82 hours; further analysis is ongoing. The key reasons to include Fuji in the study was to capture the response of fruit in different locations and at different times of the year (aka cooler weather close to harvest). Although the use of overhead cooling would be less frequent near harvest for Fuji, some orchards utilize overhead irrigation practices which may be utilized near harvest.

Figure 4. Generic *E. coli* levels on inoculated Fuji apples with (EC: treated) and without (UTC: untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA from 2015 and 2016. Values reported in $\log_{10}$ colony forming units/apple.



It is important to note that the standard deviations associated with each mean is typically around 1 log (detailed discussion provided in year 2 continuing report). Statistical analysis will be performed to fully evaluate project results. All results should be considered preliminary; for example, it is likely that seasonal influences impacted differences between varieties, and the statistical analysis will be useful in assessing this observation.

Mature fruit, Prosser region, Roza: Averaged over two years of data for untreated **Fuji** apples, at 10 hours, average reduction in generic *E. coli* was 1.1 log and for treated fruit with evaporative cooling at 10 hours, average reduction was 1.7 and 1.8 log respectively for EC and mist (Figure 5).

In general, the reduction in generic *E. coli* was lower (slower rate) for Fuji in both Sunrise and Roza orchards than for the other varieties included in the study, possibly due to the different harvest season of this variety (Figure 6). For example, during the first 10 hours after inoculation a 1.1 log reduction versus 2.1-2.6 log was observed.

Figure 5. Generic *E. coli* levels on inoculated Fuji apples with (EC: treated), without (UTC: untreated) and mist overhead evaporative cooling water application from an open surface water source near Prosser, WA in 2015 and 2016. Values reported in $\log_{10}$ colony forming units/apple.

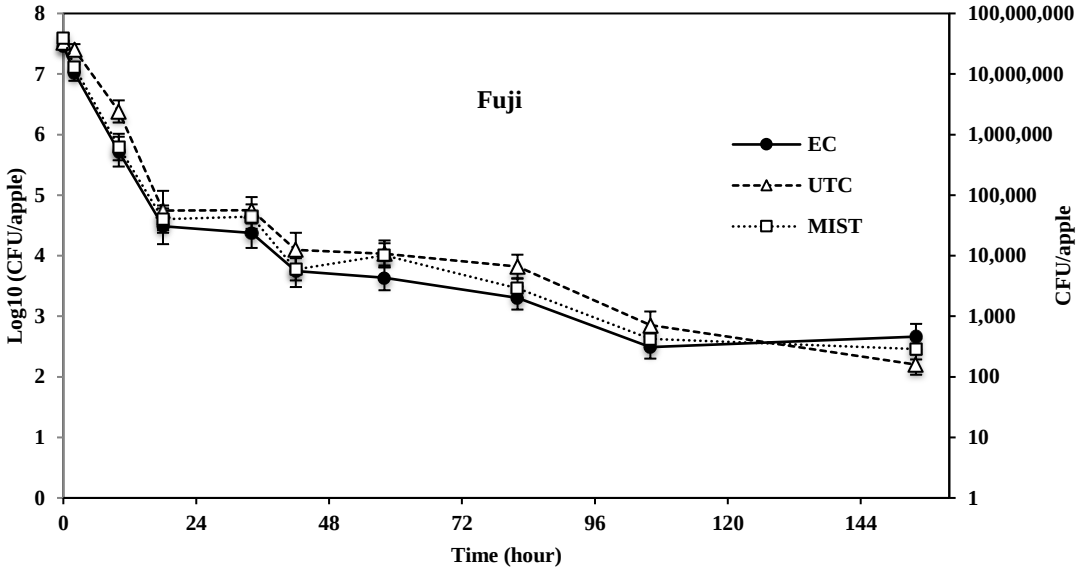
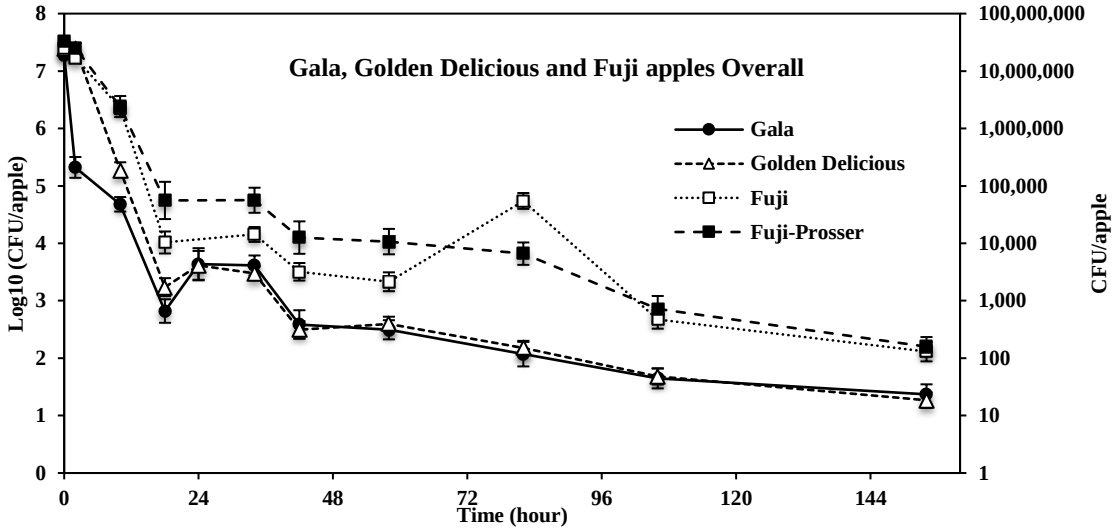


Figure 6. Generic *E. coli* levels on inoculated Gala, Golden Delicious and Fuji apples without (UTC: untreated) overhead evaporative cooling water from an open surface water source near Wenatchee and Prosser, WA in 2014 to 2016 (Fuji 2015-26 only). Values reported in $\log_{10}$ colony forming units/apple.



Immature fruit, Wenatchee region, Sunrise orchard (Gala, Golden Delicious): In 2015, a 2.7 and 3.8 log reduction of generic *E.coli* was observed during the first eight hours after inoculation in Golden Delicious and Gala treated immature apples, respectively (Table 2). Initial reduction rates were

greatest in the first eight hours after inoculation, and microbial reduction between treatments were similar. At most time points, generic *E. coli* levels were not influenced by EC (Table 2).

Table 2. Overall average of generic *E. coli* levels (log CFU/apple) on inoculated, immature Gala and Golden Delicious apples with EC practice (EC) or without (UC) in 2015. Values reported in $\log_{10}$ CFU/apple. Letters sharing the same subscript within a time point do not differ significantly at $P < 0.1$.

Variety	Treatment	Hours after inoculation								
		0	2	8	32	56	80	104	152	320
Gala	UC	7.25 a	5.35 a	3.53 a	2.73 a	NA	1.17 a	0.78 a	0.69 b	0.34 a
	EC	7.18 a	5.76 a	3.34 a	2.33 a	NA	1.14 a	1.03 a	1.23 a	0.28 a
Golden Delicious	UC	7.50 a	6.19 a	4.69 a	3.31 a	2.35 a	NA	1.83 a	0.70 a	0.43 a
	EC	7.41 a	5.90 a	4.66 a	2.50 b	2.21 a	NA	1.62 a	0.77 a	0.47 a

Canopy position: Type of canopy (traditional and modern) and fruit position within the canopy (high versus low) have the potential to impact microbial survival. In certain years of the study, both factors were examined. At Sunrise (a modern fruiting wall), two approaches were used to examine this factor: a) dividing the canopy into two (high vs. low) in 2014 and 2016 or b) three (high, low outside, low inside) distinct fruit locations in 2015. In all three years, canopy location appeared to have some influence on generic *E. coli* survival at specific time points, which warranted further investigation throughout the study. At the Roza orchard (traditional, free standing trees with dense canopy), in 2016, immature fruit located either in full sun or full shade were inoculated in two replications both in the field and over time. Generic *E. coli* levels were similar from inoculation up to 10 hours after inoculation (Figure 7); however, after 58 hours, generic *E. coli* levels were approximately one log higher for apples in full shade compared to apples in areas of full sun (4.2 log versus 3.5 log CFU/apple) (Figure 7). When performing studies on microbial survival on tree fruit, canopy location should continue to be evaluated to determine if and how canopy position influences survival of generic *E. coli*.

Inoculation level: In 2016, the impact of initial inoculation levels on survival of generic *E. coli* was performed using Fuji apples at the Roza location. The high inoculum level used for most experiments (approximately 10 million/31,000,000 CFU, 7.5 log) vs. a lower inoculum level (approximately 3,100 CFU, 3.5 log) were used in this study. Results are shown in Figure 8. Initial response differed between the high and low inoculum treatments between 2 and 10 hours post-inoculation. For the lower inoculum treatment, generic *E. coli* levels increased between 2-10 hours after inoculation (3.4 to 3.7 log CFU/apple), while for the higher inoculum treatment, generic *E. coli* levels decreased slightly (7.6 to 7.3 log CFU/apple). This trend generally differs from data from other experiments. For the remainder of the experiment, both fruit treated with high or with low inoculum levels showed a decrease in generic *E. coli* levels that was fairly consistent for both inoculum levels. For example, at 58 hours fruit from both treatments had achieved an average reduction in generic *E. coli* of 2.7-3.0 logs. For the low inoculum treatment, the majority of fruit reached the level of detection of the method by 42 hours. The use of a high inoculum level allows for quantitative analysis of microbial survival for longer periods of time after inoculation.

Figure 7. Generic *E. coli* levels on inoculated immature Fuji apples located in full sun (sun) or full shade (shade) within the canopy near Prosser, WA in 2016. Values reported in $\log_{10}$ colony forming units/apple.

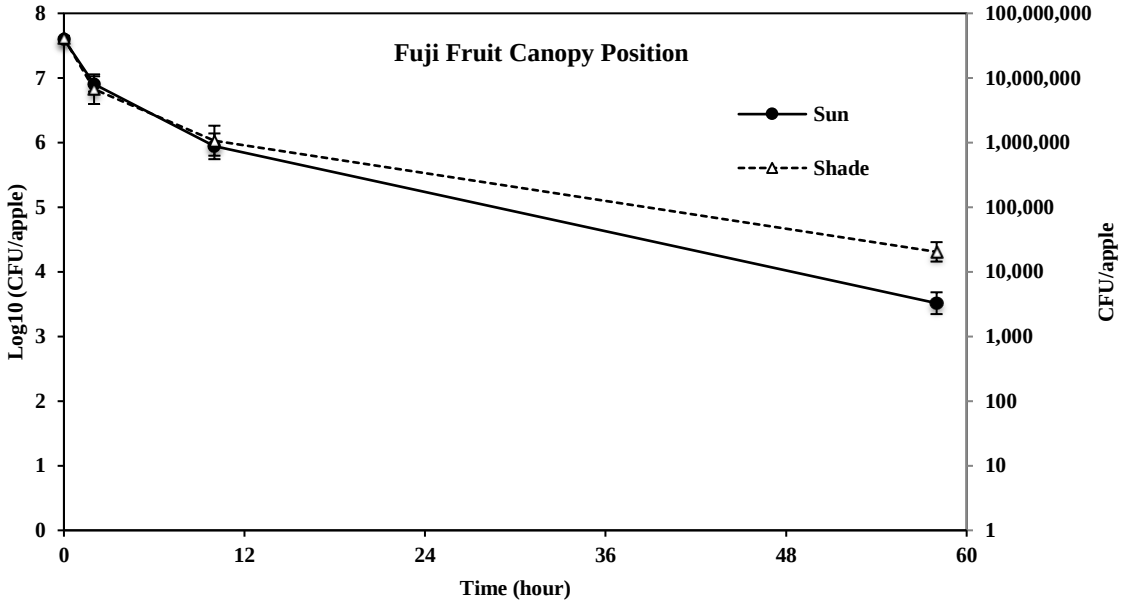
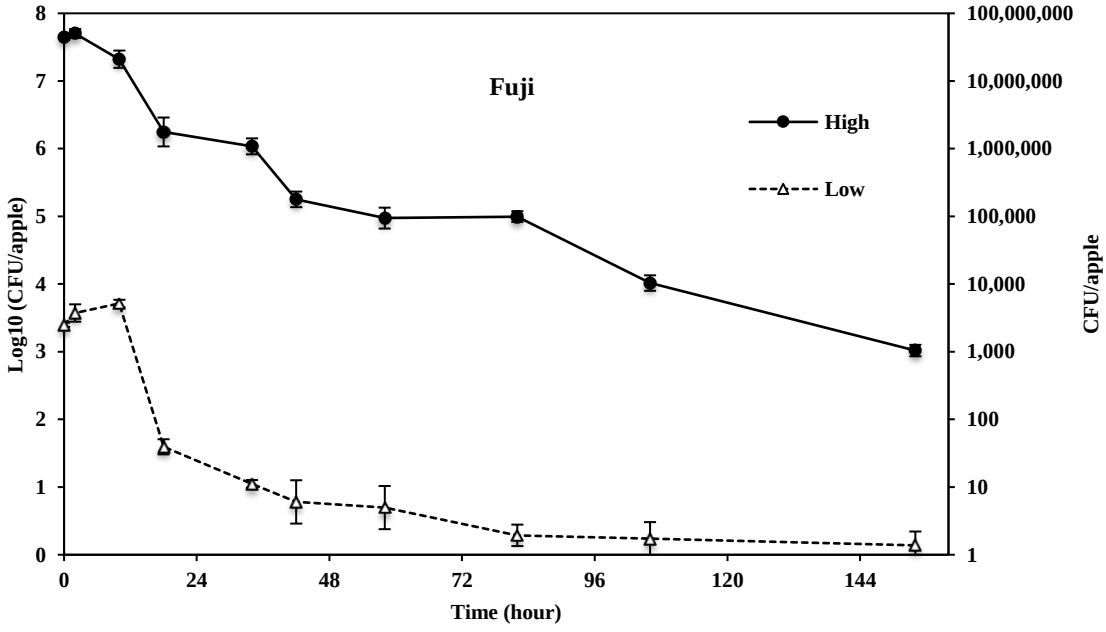


Figure 8. Generic *E. coli* levels on immature Fuji apples in an orchard near Prosser after high and low log inoculation, WA in 2016. Values reported in $\log_{10}$ colony forming units/apple.



EXECUTIVE SUMMARY

Overhead evaporative cooling (EC) using untreated surface water is frequently used in Washington to decrease sunburn in apples. While this technique prevents economic losses for farmers, its influence on food safety risk is uncertain as water is often applied near harvest. This study examined non-pathogenic surrogate survival in field studies, in order to understand potential risks associated with standard overhead cooling water application practices.

An important outcome of this study was the development of field inoculation methods for tree fruit, coupled with a specific staff training program to generate a highly consistent method for application of generic *E. coli* surrogates in an orchard setting. The development of this method and training program benefits long-term food safety research efforts involving field inoculation studies and strengthened the ability to draw conclusions from the study results.

This was the first long-term field study to examine survival of generic *E. coli* and impact of EC on multiple apple varieties. Collection of data over three years increased the strength of the data to draw conclusions by replicating over time and will allow for evaluation of weather conditions over several seasons. Mature Gala and Golden Delicious varieties (untreated and EC treated) were examined over three years in replicated field blocks; data to examine the impact of fruit location in the canopy and weather impacts were also collected. Time points differed slightly between years, but generally, fruit were sampled for up to one week after inoculation at the following time points: 0, 2, 10, 18, 34, 42, 58, 82, 106, 154 hours, and harvest times were selected to align with commercial practices, with picking starting at dawn. Generally, the greatest reduction rate of generic *E. coli* on apples occurred within the first 8-10 hours of inoculation, with additional reduction at a slower rate between 24 – 106 hours. Averaged over three years of data for untreated Gala and Golden Delicious, at 10 hours, average reduction in generic *E. coli* averaged 2.1-2.6 log CFU/apple for untreated and 2.8-2.9 log CFU/apple for EC treated apples. Additional EC water applications with overhead, evaporative cooling did not appear to impact average generic *E. coli* levels on apples within the first 96 hours (four days) after inoculation compared to the response on control apples that did not receive overhead cooling application; this observation represents an economic benefit to the apple industry, as the use of EC prevents economic losses due to sunburn and did not appear to negatively influence food safety risk within the first four days after inoculation. Additional statistical analysis will be performed to investigate the influence of EC, weather, fruit maturity, apple variety, orchard and canopy location to enhance study findings. All results should be considered preliminary; for example, it is likely that seasonal influences impacted differences between varieties, and the statistical analysis will be useful in assessing this observation.

By examining different varieties, generic *E. coli* response at different harvest time periods was determined. In general, the reduction in generic *E. coli* on Fuji apples during the first 10 hours after inoculation was slower (slower rate) than other varieties (Gala and Golden Delicious) examined in the study. These findings are important because there may be differences in generic *E. coli* survival on apples harvested later in the year, which is relevant for growers harvesting late season varieties, particularly if they utilize overhead irrigation practices.

A 2.7 and 3.8 log reduction of generic *E.coli* was observed during the first eight hours after inoculation in Golden Delicious and Gala treated immature apples, respectively. Microbial reduction between treatments were similar. Initial generic *E. coli* reduction was greatest in the first eight hours after inoculation, and at most time points, generic *E-coli* levels were not influenced by EC. Generally, average reduction of generic *E. coli* on immature fruit were nearly equivalent to or greater than those on mature fruit for both treated and untreated Gala and Golden Delicious.

Lastly, in all experiments, the reduction of generic *E. coli* varied dramatically among individual apples within the same variety at the same time point. Therefore, while average levels of generic *E. coli* generally appear to decline over time, certain harvested apples have the potential to carry high levels of generic *E. coli*.

FINAL PROJECT REPORT

Project Title: Effectiveness of foliar calcium applications in bitter pit management

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Cooperators: Stemilt Growers LLC, Oneonta StarrRanch Growers; Borton Fruits; CPC International, McDougall and Sons, Washington Fruit, Columbia Reach Orchards, Stormy Mountain Ranch, Allan Godwin; Luca Giordani WSU TFREC; Jim Mattheis, USDA-ARS

Total Project Request: **Year 1**: $69,052 **Year 2**: $63,158

Other funding sources: none

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	2015	2016
Salaries[1]	20,624	21,448
Benefits	3,686	3,835
Wages[2]	14,160	14,726
Benefits	1,682	1,749
Travel[3]	10,000	10,000
Goods and Services[4]	18,900	11,400
Total	69,052	63,158

Footnotes:

[1]Salaries for 50% Salary for Luca Giordani (Kalcsits) and 8.25% Research Associates (Sankaran and Khot).
[2]Wages for time slip summer wages for Luca Giordani and undergraduate assistant.
[3]For travel to field sites in Wenatchee, Quincy and Prescott including overnight travel from Pullman, WA.
[4]Goods and services include calcium isotope purchase, calcium isotope analysis, lab consumables and fruit purchase in addition to CT-imaging and FTIR instrument use service charges.

RECAP ORIGINAL OBJECTIVES

1. Examine the relationship between altitude (environment) and bitter pit development in Honeycrisp.
2. Evaluate the effectiveness of different frequencies of calcium applications and reduction in transpiration using ABA on bitter pit incidence.
3. Determine the optimum timing for foliar calcium applications using calcium isotope tracer application in the field.

SIGNIFICANT FINDINGS

1. Additional calcium sprays increase fruit calcium concentrations. For Honeycrisp apple, up to 50 lb/acre of Ca can be applied during the season using frequent, dilute sprays and being careful to avoid hot periods and leaf burn.
2. Environmental factors are not major drivers in bitter pit susceptibility. Horticultural management affects bitter pit the most.
3. When horticultural variables (training system, crop load, spray timing, etc.) are controlled, bitter pit incidence can still be highly variable.
4. Internal bitter pit incidence occurs before external symptoms and is detectable using CT imaging.
5. Within high-density apple trees, bitter pit incidence is greater in the lower half of the tree and this is related to differences in nutrient concentrations
6. Water status appears to be linked to fruit size and bitter pit incidence. More work is needed to see whether this can be used to control bitter pit incidence in Honeycrisp
7. From tracking the amount of calcium spray that can stick to fruit, we can model the potential % increases in calcium from frequent calcium sprays. Earlier in the season, when the surface area to fruit ratio is low and stomata are more active, calcium sprays are more effective. Later on in the season, the impact will be less but absorption should still be occurring. Using the isotope tracing, we will be able to confirm this.

RESULTS & DISCUSSION

1. **Examine the relationship between altitude (environment) and bitter pit development in Honeycrisp.**

Nine Honeycrisp orchards were selected for sampling in March 2015 that ranged in elevation from 405' to 1857'. All soils were sandy loam to loam soil with drip or microsprinkler irrigation and had a least weekly calcium applications. All orchards used upright training systems, with planting distances of approximately 3' x 12' and all were M-9 rootstock with the exception of one site that was on Bud-9. All orchards were between the 5th and 7th leaf. Fruit from nine trees were counted prior to thinning and then fruit was removed to targeted crop loads of 3, 5 or 7 fruit cm^{-2} TCSA (N = 3 trees). In 2015, there was a large range in growing environments with a range of approximately 850 growing degree-day differences between the coolest sites to the warmest sites (Table 1). Although there were approximately 400 less GDD in 2016 than 2015 (Table 2), the trends among sites were similar with the hottest sites in 2015 also the hottest sites in 2016. Soil temperature and air temperature were approximately 2°F less in 2016 than 2015. 16 fruit from each tree was labelled during thinning in June and the distance from the trunk and from the ground was measured for each fruit. Harvest dates were based upon commercial maturity and fruit was picked just prior to commercial harvest for each site. Harvest dates ranged from August 14-September 6th in 2015 and August 12 and September 4th in 2016. At harvest all fruit was picked from each tree for total weight and size distribution. The 16 tagged fruit was picked and placed into a tray for postharvest and post storage quality analysis. The locations of these fruit were linked to differences in fruit quality, nutrient distribution and bitter pit

incidence. Fruit quality data presented in this report represents 2015 data but 2016 data is available upon request and will be included in the final presentation.

Table 1. Site characteristics for each of nine Honeycrisp orchards in 2015. * = sensor failure

Site	Altitude (feet)	Rootstock	Mean Soil Temperature (°F)	Mean (°F) Air Temperature	GDD (50°F) (5/15/15-9/1/2015)
Burbank	405	Bud-9	72.4	71.66	2613
Royal City 1C	1162	M9	70.69	70.55	2389
Royal City 2W	1145	M9	69.89	71.00	2506
Quincy 1M	1369	M9	67.57	70.59	2555
Quincy 2S	1383	M9	*	*	2400
Quincy 3O	1354	M9	*	71.24	2409
Kittitas	1731	M9	65.84	67.1	2033
Chelan	1857	M9	63.82	64.6	1754
Tonasket	900	M9	69.11	68.2	2081

Table 2. Site characteristics for each of nine Honeycrisp orchards in 2016. * = sensor failure

Site	Altitude (feet)	Rootstock	Mean Soil Temperature (°F)	Mean (°F) Air Temperature	GDD (50°F) (5/15/16-9/1/2016)
Burbank	405	Bud-9	70.32	69.83	2253
Royal City 1C	1162	M9	69.31	68.66	2086
Royal City 2W	1145	M9	68.93	68.32	2015
Quincy 1M	1369	M9	66.46	67.09	1944
Quincy 2S	1383	M9	*	67.02	1877
Quincy 3O	1354	M9	67.89	67.51	1995
Kittitas	1731	M9	62.83	62.87	1531
Chelan	1857	M9	61.85	63.01	1556
Tonasket	900	M9	65.36	65.71	1810

Environmental and horticultural contributors to bitter pit development

Environmental conditions did not significantly affect bitter pit development. There were no notable trends in the incidence of bitter pit and the environmental conditions of each orchard. Cooler orchards were just as likely to get bitter pit as warmer orchards. In 2016, preliminary data indicates that, when crop load is tightly controlled, previous year's bitter pit incidence does not correlate with current year bitter pit incidence. But, more post storage analysis will confirm these observations. Even in an orchard where horticultural variables were highly controlled (training system, crop load, calcium sprays, etc.), bitter pit incidence can vary widely (Figure 1). In 2015, bitter pit incidence ranged from 4% to 38% in the 9 orchards. The second coolest site had the higher bitter pit incidence and one of the moderate sites had the least bitter pit. With many horticultural variables controlled and using similar systems, ages, and all Honeycrisp, the one commonality contributing to higher or lower bitter pit incidence was fruit size (Figure 2). Fruit size was significantly correlated with the plant water status. This was measured using a time-averaged indicator value ($\delta^{13}C$). Therefore, it appears that plant water status may be a significant factor affecting bitter pit susceptibility through changing fruit size within the orchard (Figure 3). Even with correct crop load and calcium sprays, excessive fruit size will lead to high bitter pit incidence.

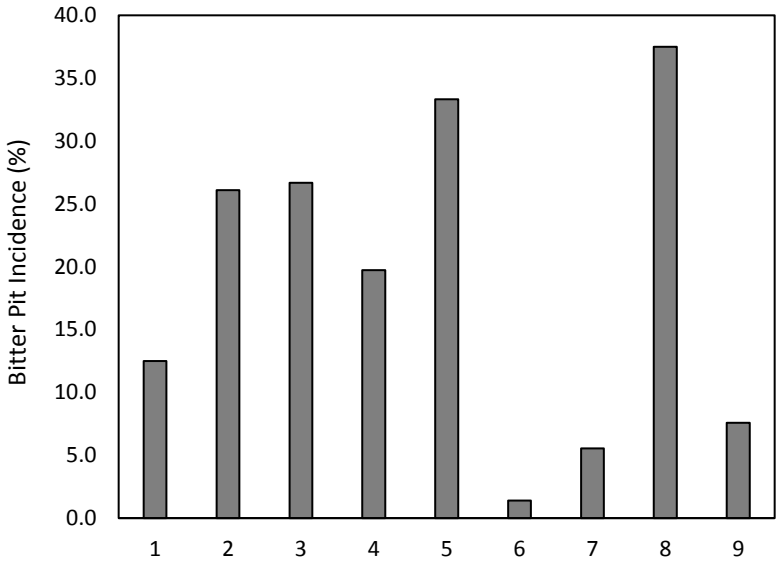


Figure 1. Bitter pit incidence among 9 environmentally diverse 'Honeycrisp' apple orchards in Washington State

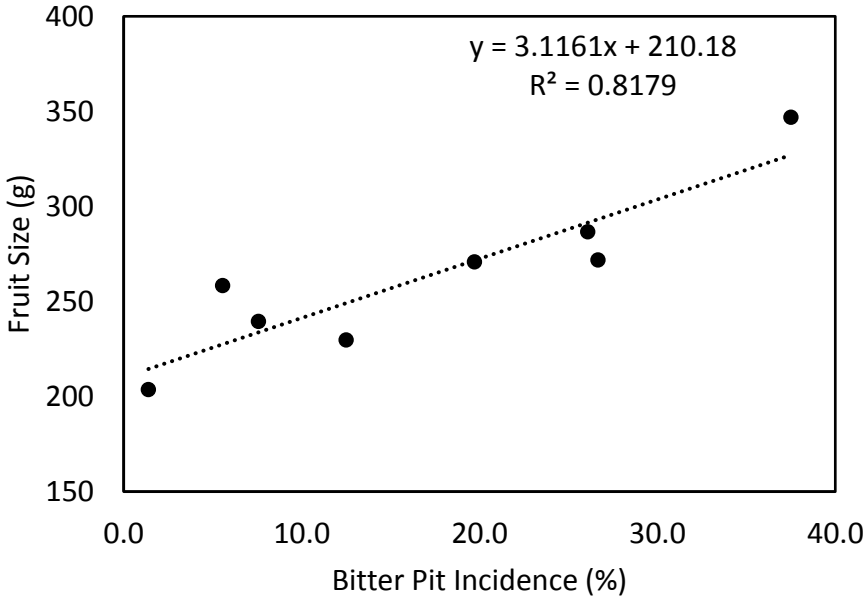


Figure 2. The relationship between average fruit size for nine environmentally diverse Honeycrisp orchards in Washington State and the bitter bit incidence observed after 4 months of cold storage

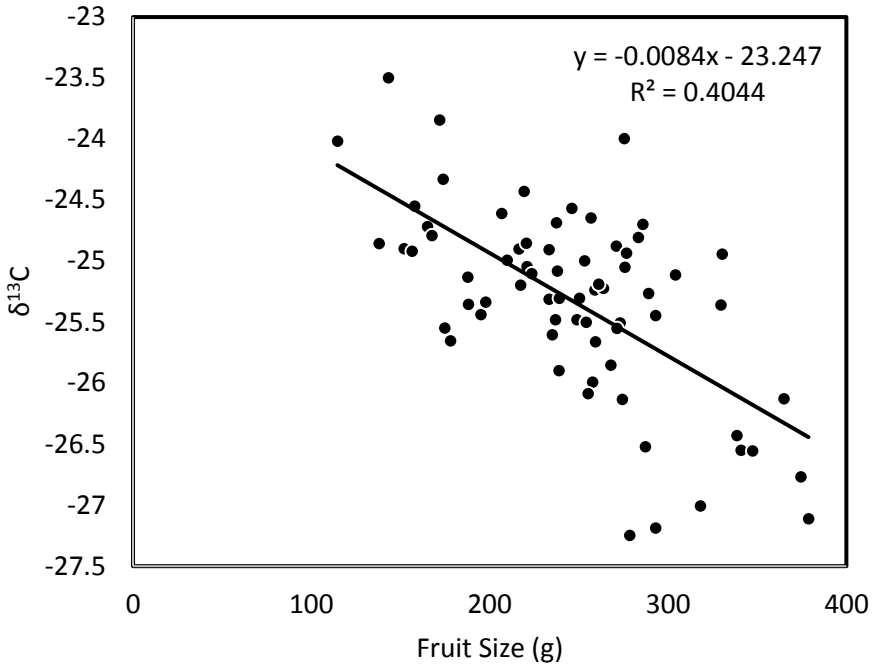


Figure 3. The relationship between $\delta_{13}C$ (a time-averaged measurement of plant water status) and fruit size for Honeycrisp apples harvested from nine environmentally diverse Honeycrisp orchards in Washington State. More negative values indicate less water stress than plants with less negative values.

Spatial variation within the canopy affecting bitter pit development and overall fruit quality

Fruit quality significantly ranged within the tree canopy, even for simple, high density systems. Dry matter, color development, soluble sugar content, titratable acidity and firmness were greater in the upper parts of the canopy compared to lower parts of the tree canopy. Nutrient distribution as also significantly affected by the position in the canopy. The potassium: calcium measured in June on fruitlets was significantly correlated with the potassium: calcium ratio measured on the same fruit at harvest using a portable x-ray fluorimeter. While the relationship was significant, there was still substantial variation between measurements and June measurements are not entirely predictable indicators of final ratios at harvest, particularly in situations where substantial thinning occurs after this date. At harvest, the potassium: calcium ratios were greater in the top of the tree than the lower half of the tree (Figure 4). However, there was still significant variation within the canopy. When all fruit at each height were pooled, fruit calcium concentration increased significantly as the relative height in the canopy increased and potassium concentrations decreased but not as rapidly (Figure 4). With these two opposing trends in potassium and calcium concentrations in the canopy, the potassium concentration was significantly greater in the lower half of the canopy than the upper and more exposed portion of the tree. These patterns in the potassium: calcium ratios directly correspond to the variation in the bitter pit incidence within the tree canopy. Bitter pit incidence was more than 30% for the lowest portion of the tree and decreased for apples higher in the tree to as low as 12%. It would be worthwhile to test whether segregation of fruit from lower or upper parts of the tree would result in better pack out % for fruit destined for long-term storage.

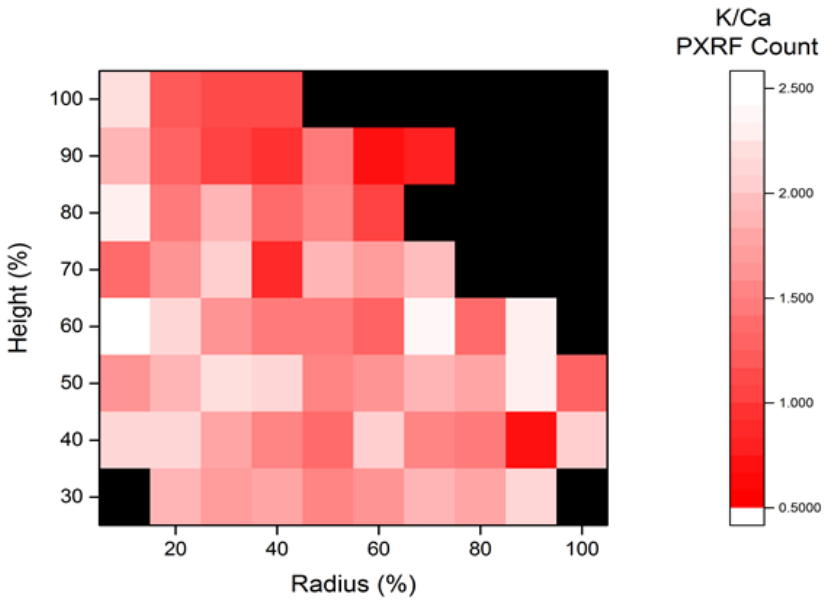


Figure 4. Potassium: calcium ratio measured at harvest using a non-destructive PXRF. Height represents the relative distance from the bottom of the tree where 100% is the top of the tree and the radius is the relative distance from the trunk of the tree.

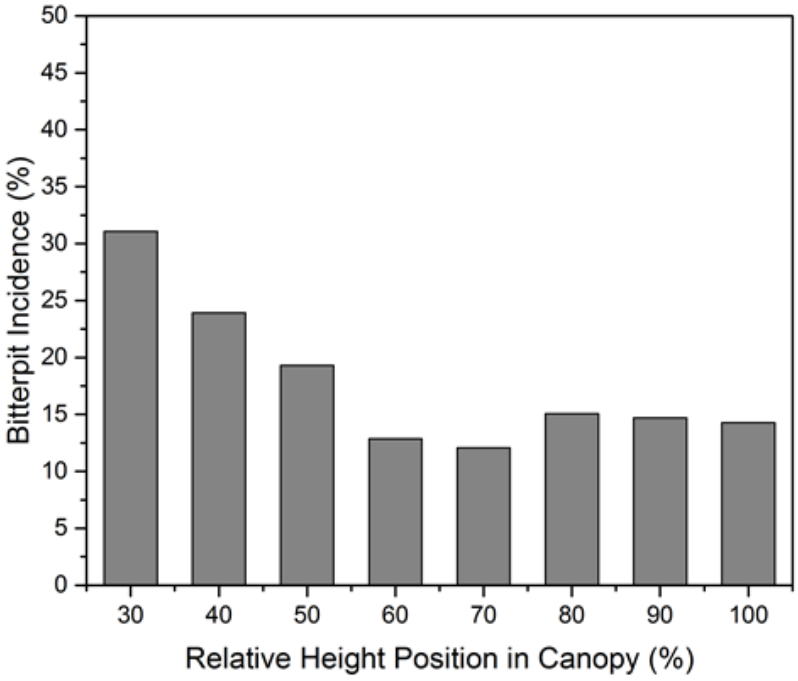


Figure 5. Bitter pit incidence (% of total harvested fruit) in Honeycrisp apples harvested from different heights in the tree canopy where 100% represents the top of the tree.

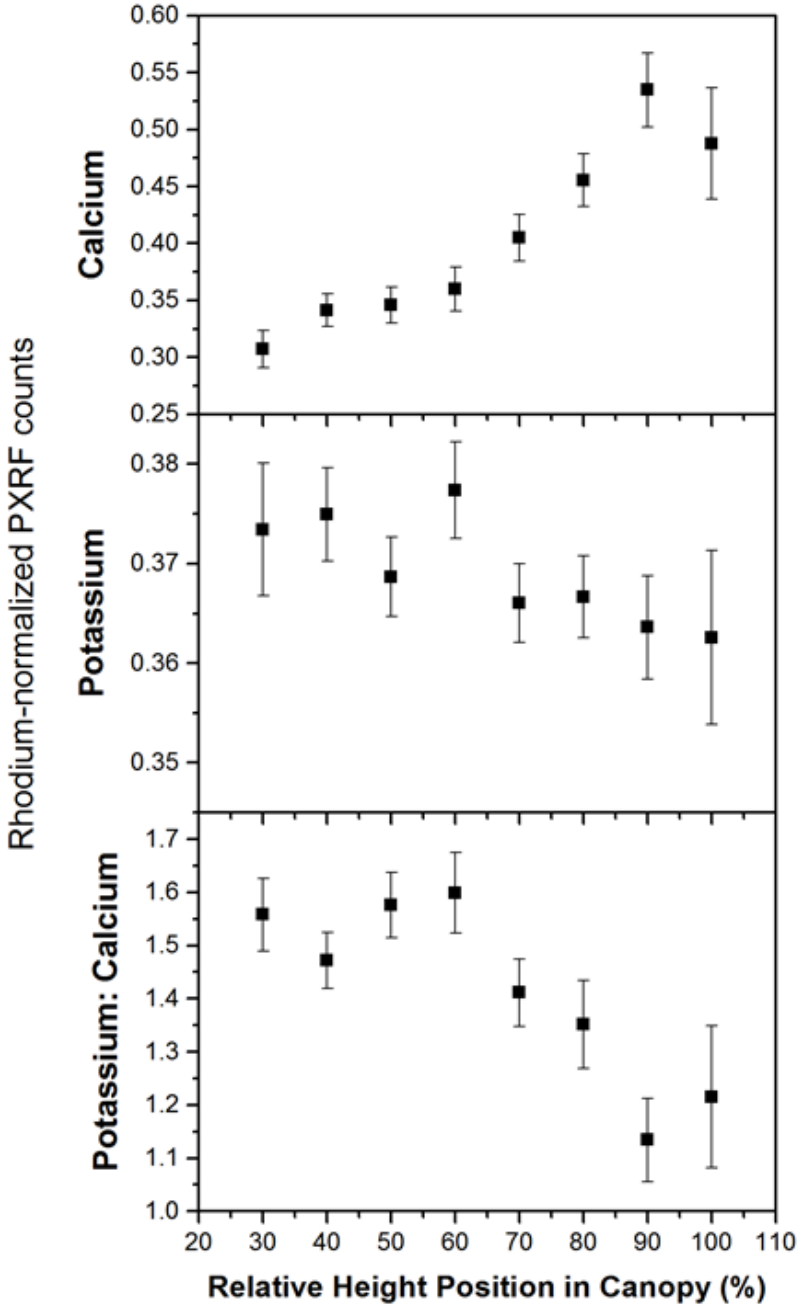


Figure 6. Calcium (top), potassium (middle) and potassium: calcium ratio (bottom) measured using PXRF on fruit from different heights in the canopy (100% represents the top of the tree).

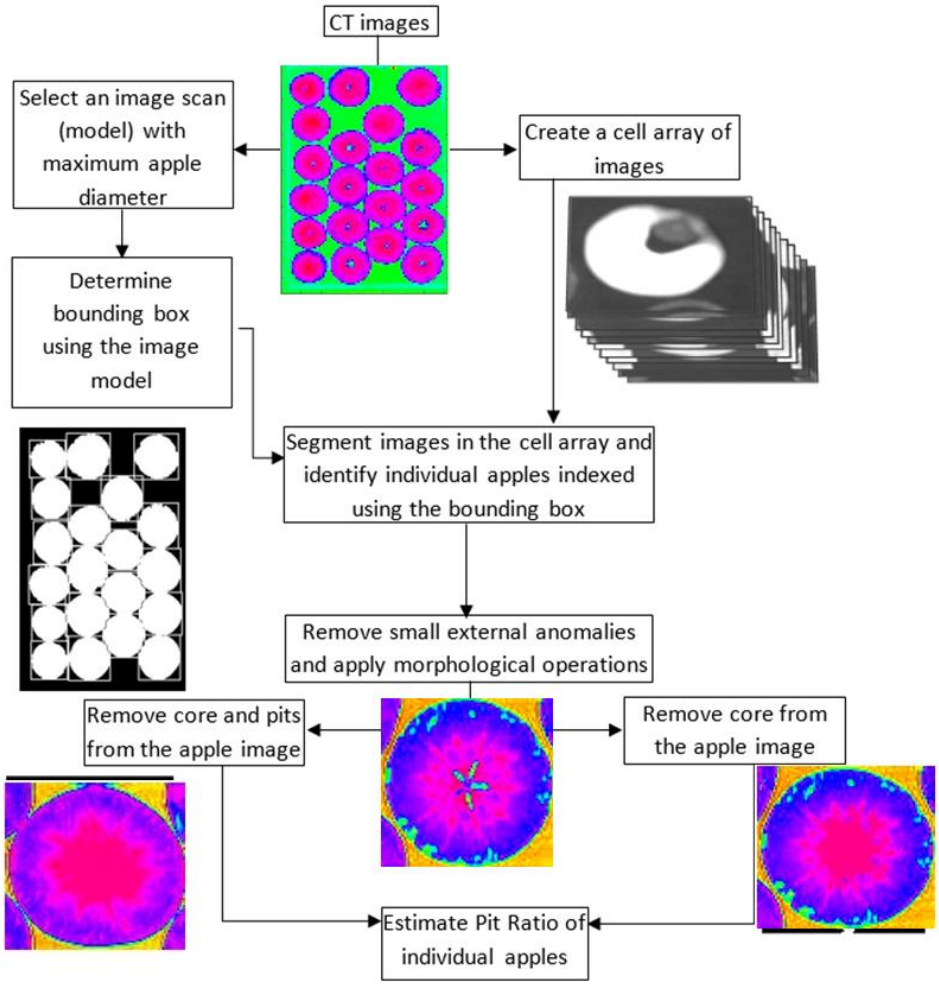


Figure 7. Image processing algorithm used to analyze CT images

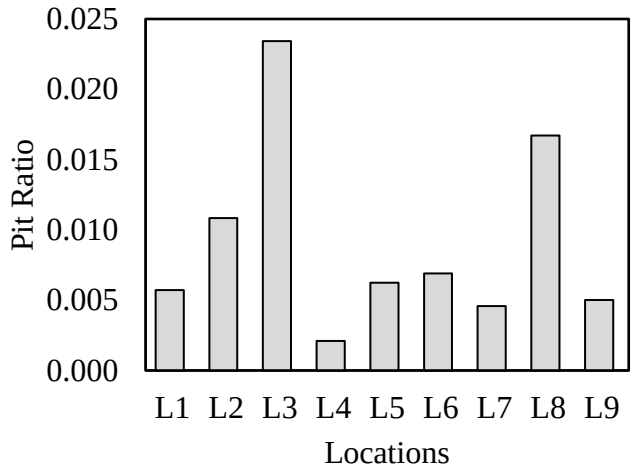


Figure 8. Pit ratio (pit area/total fruit area) of apples from nine environmentally diverse locations in Washington State

2. Evaluate the effectiveness of different frequencies of calcium applications and reduction in transpiration using ABA on bitter pit incidence.

A 9 year-old Honeycrisp orchard spaced at 6' x 14' was selected for this trial. In March 2015, sections of three rows each we marked off and treated with 2 lb/acre Ca either once per month, twice per month, once per week, or twice per week. This results in approximately 50 lb/acre applied in the twice per week treatment, 25 lb/acre in the once per week treatment, 12.5 lb/acre in the twice per month treatment, and 6 lb/acre in the once per month treatment. 10 trees per replicate were selected for sampling and hand-thinned to even crop loads. Fruit was harvested on August 26th, 2015. The average fruit size was 220 g. For this, there was higher calcium in the twice per week treatment compared to the once per week applications when measured with traditional lab analysis and PXRF analysis (Figure 8). However, the incidence of bitter pit after four months of regular atmosphere cold storage was less than 5% across all treatments and there were no differences in bitter pit among treatments, even for fruit that had 6 lb/acre of Ca applied throughout the season.

In 2016, for practicality, calcium treatments were limited to once per week or twice per week because once per week treatments can be tank-mixed with other sprays and do not amount to major additional costs for the industry. However, twice per week sprays require calcium-specific sprays and increase the risk of foliar burn. The additional spray per week amounted to a 10% increase in fruit calcium (Figure 8). However, again in 2016, the average fruit size was about 230 g and bitter pit incidence was exceptionally low at harvest. Fruit will be removed after four months of storage and bitter pit incidence scored but it is expected that the incidence will be low similar to 2015. When looking at the results from Objective 1, orchards that have exceptionally large fruit will likely benefit from additional calcium sprays.

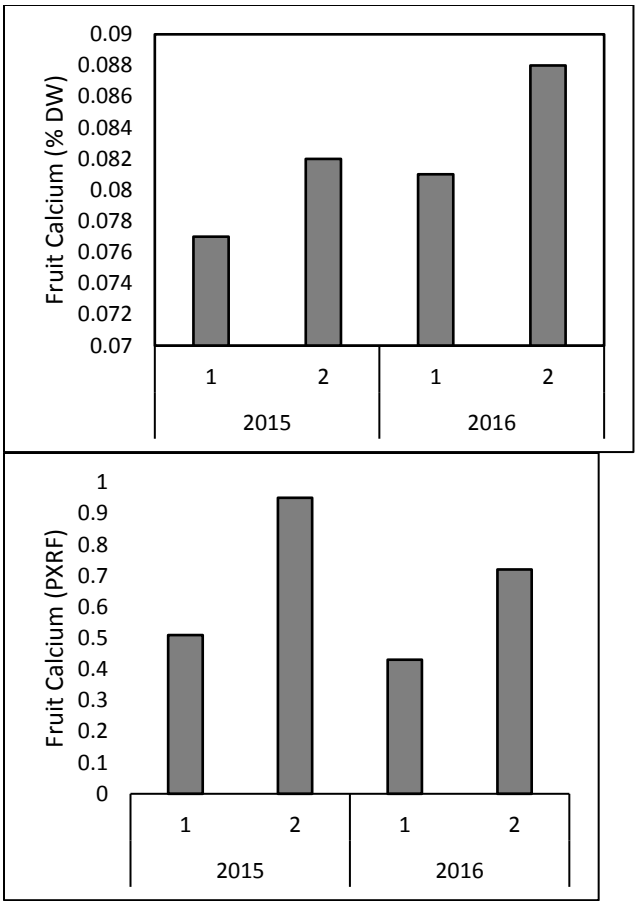
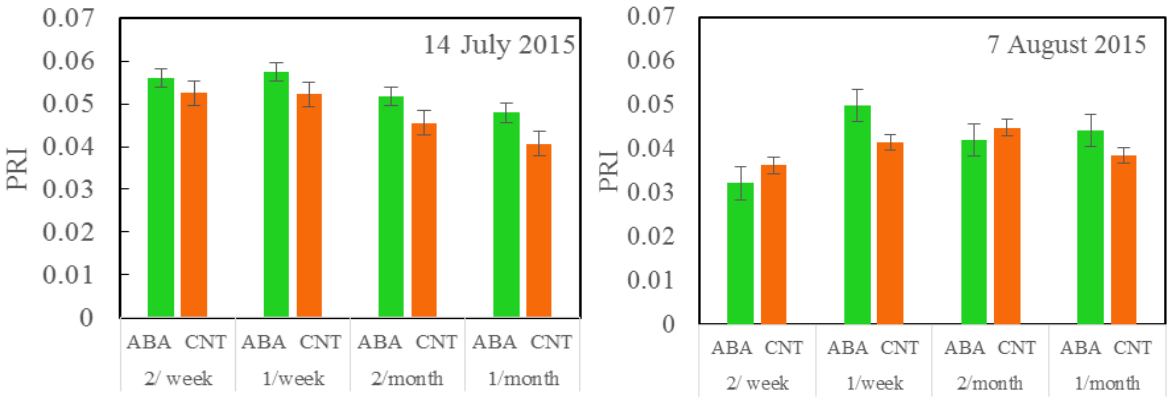


Figure 9. Fruit calcium content measured using traditional lab analysis (left) and PXRF (right) on fruit harvested from trees in 2015 and 2016 treated with 2 lb. /acre Ca once per week or twice per week.

In 2015, ABA was also applied to trees within all treatments three times in June, July and August to reduce plant water-use. Figure 10 shows the photochemical reflectance index after treatment in June and July, 2015. Vegetation indices were extracted from the visible-near infrared spectra. Amongst different vegetation indices, photochemical vegetation index (PRI) that is often used as an indicator of photosynthetic light-use efficiency, showed that ABA affected the physiology of the plant. During the mid-season (maximum canopy vigor and nutrient uptake), the PRI showed consistent differences between calcium treatment as well as effects of ABA. The ABA treated trees had higher PRI values in all four calcium treatments. However, in August, the effects are not as clear. This may be a result of leaf aging and lower responsiveness to the ABA. There were no differences in fruit nutrient concentrations or bitter pit incidence between the treated and untreated trees. There were also no significant differences in fruit quality between the treatments. This orchard had low vegetative vigor. However, this practice may be beneficial in orchards with more vegetative vigor and higher bitter pit risk. Future work trialing out ABA at more frequent doses could be helpful.

(a) Field Location (b) Proximal Sensing (c) NDVI False Color Image (d) Thermal Image



(e) Ground-based Photochemical Reflectance Index (PRI)

Figure 10. Ground and aerial-based sensing for changes in leaf physiological responses to ABA treatments.

3. Determine the optimum timing for foliar calcium applications using calcium isotope tracer application in the field.

For this objective, 15 trees were identified and thinned to even crop loads of 5 fruit/cm^2 TCSA. On five trees, 4 fruit per tree were sprayed with $^{44}CaCl_2$ every two weeks starting at 25 mm fruit size for a total of seven points during the season. At the end of the season, the fruit was harvested, separated into peel and flesh, and digested for isotope analysis (140 total fruit). This analysis will take place in January, 2016 and will be presented at grower meetings throughout WA state and in discussion with industry stakeholders. For the other 10 trees, fruit were subsampled weekly for 12 weeks and fruit weight, diameter and spray holding capacity was measured for each fruit. This was used to calculate theoretical gains and calcium application rates on fruit based on fruit size, adherence of spray to fruit and surface area. Calcium and potassium concentrations were also determined using traditional lab analysis weekly to look at changes in fruit calcium concentrations as fruitlets develop.

The surface area to volume ratio of fruit decreases as the season progresses and the fruit increases in diameter (Figure 11). The water holding capacity per unit of surface area also decreases as the season progresses as cuticle thickness increases and the fruit becomes more hydrophobic (Figure 11). These two factors support that early season applications of calcium are more important than later season applications simply because more calcium per unit volume is sticking to the fruit when it is less developed. The isotope data should provide more definitive data on what % of calcium applied is absorbed into peel and flesh, respectively. The theoretical increase in fruit calcium from calcium applications is all of it was absorbed would approach 60% depending on the frequency of application. However, fruit calcium only increased by 10% when the amount of calcium applied was doubled from once per week to twice per week (Figure 8). For this trial, we expect the isotope data to

show that between 15-20% of calcium applied is absorbed by the fruit. However, this will need to be confirmed with the final analysis that will be completed in February, 2017.

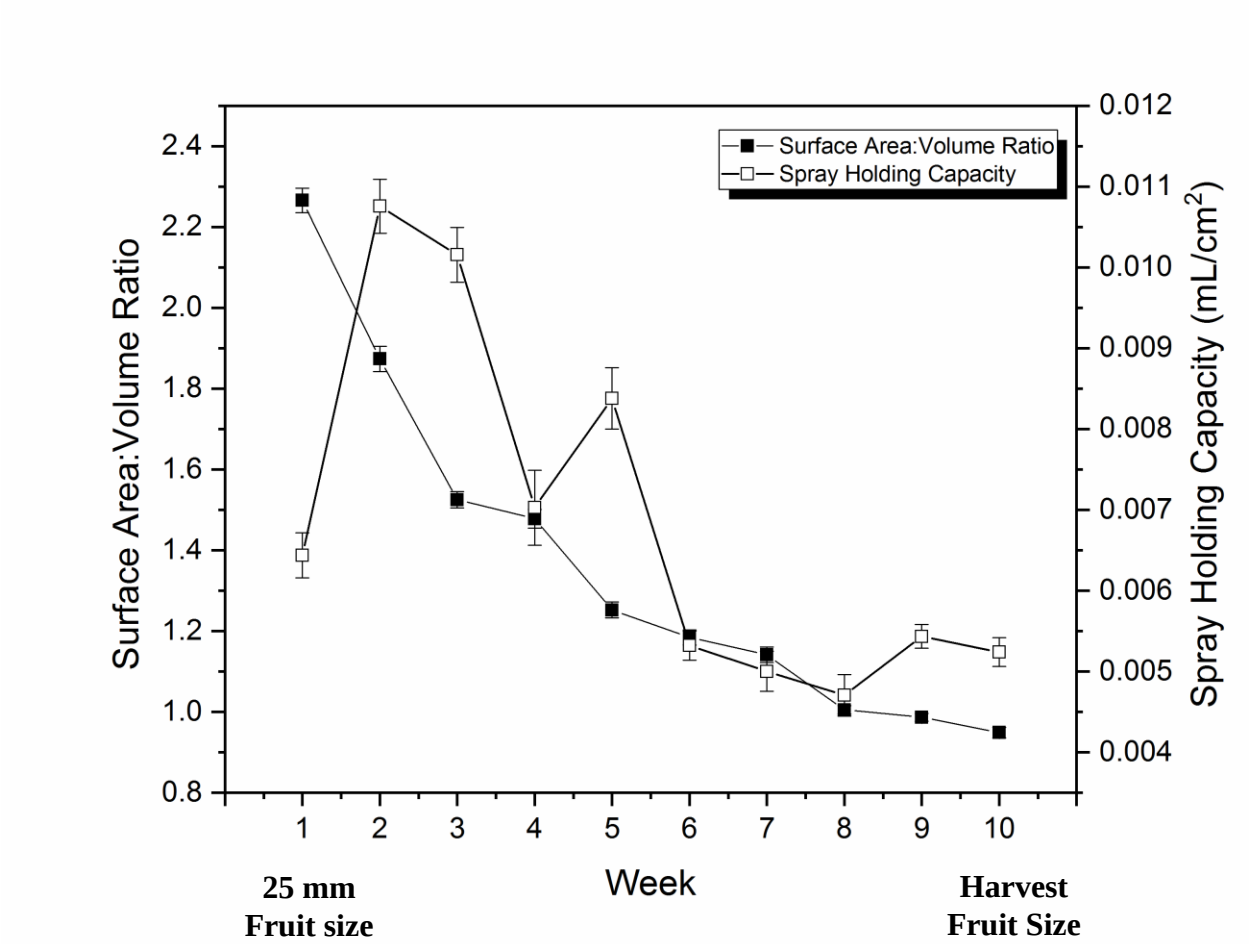


Figure 11. Surface area to volume ratio and spray holding capacity of apple fruit starting at 25 mm fruit (week 1) and ending at harvest (week 10)

EXECUTIVE SUMMARY

In 2015 and 2016, experiments were conducted to look at how environment impacts calcium uptake and distribution in Honeycrisp apple and how effective frequent calcium sprays are in improving fruit calcium levels and the optimum timing for calcium spray applications for Honeycrisp apple. Although there is still some final data to collect in early 2017, we have identified some critical factors contributing to bitter pit development in Honeycrisp apple.

Frequent, low rate calcium sprays are the most useful in improving fruit calcium levels. In situations when fruit calcium concentrations are low, calcium sprays have been shown to increase calcium levels by small amounts. However, even with frequent calcium sprays (even once per week), bitter pit incidence can still be very high (up to 50%). Calcium sprays show only minor increases in fruit calcium content compared to sound horticultural management (crop load, vigor control, irrigation and nutrient management). As fruit matures and the wax layer on the fruit becomes thicker, the amount of spray per unit volume of fruit becomes significantly smaller suggesting that early season application of calcium is more effective. However, because calcium needs to be applied frequently at low rates, late season applications could also be effective. With tank-mixing, applications of once per week are practical and the additional cost is minimal. Isotope data, when it is available, will identify the optimum timing or calcium application and will be shared at grower meetings or through individual grower discussions.

Abscisic acid (ABA) reduced plant transpiration and induced stomatal closure. However, this did not result in increased calcium in the fruit in this trial. However, this orchard was a low vigor situation and high fruit calcium concentrations. In a high vigor orchard, the result may be different and might be worth trialing under Washington State conditions. Frequent ABA applications would likely be needed to have an appreciable effect on calcium distribution within the plant.

While environment can impact the timing of fruit maturity and lead to better color development because of later maturity in cooler regions, there was no relationship between the environment and bitter pit susceptibility. Warm, low altitude environments had incidences of low bitter pit and cooler, high elevation sites had instances of high bitter pit rates. External bitter pit rates were related with the occurrence of internal bitter pit in independent subsamples of apples from the same trees. The main contributor to bitter pit development appeared to be fruit size. Larger fruit size was linearly correlated with bitter pit incidence where bitter pit incidence was under 20% for fruit that was 250 g or smaller (between 72 and 80 box size). Fruit size was also correlated with the time-averaged water status of the tree. Therefore, more work is needed to identify a link between water status and bitter pit in Honeycrisp to develop ways to use this as a tool to manage fruit size and bitter pit incidence in Honeycrisp.

Spatial variation within the tree was also assessed. While variation within the canopy has been identified for lower density orchards, within canopy variation in nutrient balance, bitter pit incidence and fruit quality has been less studied for high density orchards that are assumed to have more uniform canopies. Bitter pit incidence in the lower half of the tree was almost double that of the upper half of the tree. These differences in bitter pit incidence were related to the potassium and calcium concentrations in the fruit from different locations on the tree. This has potential to be adaptable for fruit segregation for harvest and storage management to maximize pack outs for long-term storage and make more informed decisions on handling of fruit in the field.

FINAL PROJECT REPORT

Project Title: Effects on physiology of apple under photoselective anti-hail nets

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Cooperators: McDougall & Sons, Inc., Sara Serra (WSU TFREC), Giverson Mupambi (WSU TFREC).

Total Project Request: **Year 1**: $84,102 **Year 2**: $85,713

Other funding sources

Agency Name: Washington State Department of Agriculture SCBG K1771
Amount awarded: $248,000
Notes: This project started in October, 2015 and will extend the project through 2017 and provide another year of data to the tree fruit industry that won't require support from the WTFRC.

Agency Name: Wilson Irrigation
Amt. awarded: Approximately $15,000
Notes: In-kind contribution of 6,500 sq. ft. of shadehouse structures for netting experiments

WTFRC Collaborative expenses:

Item	2015	2016
Wages and benefits[1]	8,000	8,000
Salaries and benefits[2]	5,000	5,000
Supplies	500	500
Travel	500	500
Total	14,000	14,000

Footnotes:
[1]Time slip wages for building shadehouses and harvesting fruit for quality analysis.
[2]Salaries for Tory Schmidt and Felipe Castillo.

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	2015	2016
Salaries[1]	16,000	16,640
Benefits[2]	4,882	5,077
Wages[1]	16,320	16,972
Benefits[2]	3,100	3,224
Travel[3]	4,000	4000
Goods and Services[4]	25,800	25,800
Total	70,102	71,713

Footnotes:
[1]Salaries for 33% research intern (Kalcsits) and time slip wages (Layne and Musacchi).
[2] Benefits at 30.5% and 19% for research intern and time slip wages, respectively.
3Frequent travel to orchard site (Quincy) where trials are being conducted.
[4]Goods and services include in-orchard temperature/humidity dataloggers, soil moisture and temperature monitors, isotope analysis, WSU TFREC fees for soil, leaf and fruit mineral nutrient analyses.

RECAP ORIGINAL OBJECTIVES

1. Determine characteristics of three net colors on light spectrum and their effects on the light quality and quantity of incoming radiation throughout the day.
2. Quantify the impact of nets on orchard microclimate, photosynthesis, vegetative growth and tree stress.
3. Evaluate fruit and leaf nutritional balance and fruit quality under different light conditions.

Working in a Honeycrisp orchard on Bud-9 that was planted in 2013, environmental sensors were installed, the physiological and growth status of the trees were measured, and fruit quality of the trees were assessed under red, blue and pearl netting compared to an uncovered control. A parallel experiment was set up at the WSU tree fruit research and extension center in Wenatchee using the same treatments to monitor the physiological changes in greater detail than would be possible when working in a commercial orchard.

SIGNIFICANT FINDINGS

- Photoselective netting reduced light intensity differently depending on color. All 20% netting reduced photosynthetic active radiation (PAR) by between 25% (red) and 21% (pearl). When using a new netting product, it is recommended that light intensity is verified using sample material before purchasing.
- Netting strongly reduced wind (by more than 50%) but did not affect air temperature in the tree canopy.
- Netting altered the spectra of incoming light and created a better light environment for plant and fruit growth. This was particularly true for the pearl netting that provides more scattered light than the other colors.
- Absorptive surfaces (fruit, leaves, soil) were more impacted by netting than the air under the canopy. Soil, leaf and fruit temperatures were all lower by using 20% netting (Figure 1, 2; Kalcsits et al. 2017).
- Netting reduced light and heat stress on the tree and improved leaf-level photosynthesis and light-use efficiency (Table 4).
- Netting increased flower induction and fruit set after a ‘heavy crop’ year in pearl and red netting.
- Canopy growth was greater under netting than for the uncovered control.
- Netting increased fruit size and had no major negative effects on fruit quality. There were small reductions in color development with the blue and red netting in 2015 (Table 5).
- Netting strongly reduced sunburn compared to an uncovered control and provided a similar level of sunburn control to evaporative cooling in 2016 (Figure 3)

RESULTS & DISCUSSION

Objective 1. Determine how photoselective anti-hail nets modify the microenvironment to mitigate stress-inducing conditions in WA State's growing environment

Environmental Monitoring

In June, 2015, at three separate locations each in the four experimental treatments, mini weather stations were positioned that included an EM50G datalogger (Decagon Devices, Inc., Pullman, WA) that recorded data every 10 minutes and transmitted data by cellular signal to a cloud-based server. Sensors at each station included a VP-4 humidity and temperature sensor (Decagon Devices, Inc., Pullman, WA), a Davis cup anemometer, a photosynthetically active radiation (PAR) sensor (Decagon Devices, Inc., Pullman, WA). These were used to measure, air temperature, relative humidity, wind speed and PAR in each treatment. For measurements that contain more variability, such as in-canopy air temperature and humidity and soil moisture and temperature, four replicate VP-4 sensors for measuring in-canopy air temperature and humidity were placed near the trunk at 1.6 m from the ground in each color of netting. Four 5TM soil moisture and temperature capacitance sensors (Decagon Devices, Inc., Pullman, WA) were installed at dispersed distances within each treatment. The capacitance sensors were installed at depths of 20 and 40 cm to measure volumetric water content (cm^3 cm^{-3}). Sensor locations were chosen to limit interferences from tree and post shadowing of irrigation microsprinklers and were equidistant from trees within the row. Data was downloaded monthly using DataTrac software (Decagon Devices Inc., Pullman, WA) from the online database to ensure that sensors were functioning correctly.

Results

Netting did not affect air temperature or relative humidity within the orchard canopy, but reduced wind speed by 40% compared to the uncovered control. Netting reduced soil temperature and increased soil moisture at 20 and 40 cm depths throughout the study period compared to the uncovered control. Amongst different colors of netting tested in this study, pearl and blue netting significantly reduced soil temperature compared to red netting. Netting also reduced photosynthetically active radiation (PAR) by approximately 20% and strongly reduced fruit surface temperature during hot periods. During full sunlight, differences in maximum fruit surface temperature between the uncovered control and the protective netting were 2.6 to 4.3°C under full sun conditions and reduced the incidence and severity of sunburn measured at harvest.

Table 1. Light intensity and wind speed in 2015 and 2016 in a commercial 'Honeycrisp' apple orchard under blue, pearl, and red netting compared to an uncovered control

	2015		2016	
	Light Intensity (umol m^{-2} s^{-1})	**Wind Speed (miles h^{-1})**	**Light Intensity (umol m^{-2} s^{-1})**	**Wind Speed (miles h^{-1})**
Control	1804 a	8.0 a	1782 a	10.3 a
Blue	1404 b	3.76 b	1407 b	4.7 b
Pearl	1459 b	3.96 b	1420 b	5.0 b
Red	1355 b	3.64 b	1354 b	4.7 b

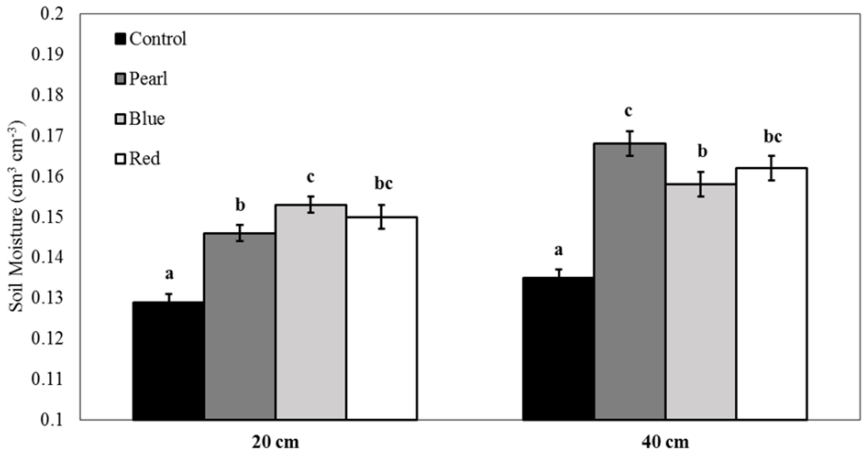

Figure 1. Mean volumetric soil moisture content (cm^3 cm^{-3}) at 20 and 40 cm depth under pearl, blue and red anti-hail netting compared to an uncovered control in a three year-old 'Honeycrisp' apple orchard in Quincy, WA (47.23° N, 119.85° W). Different letters denote significant differences between means determined using a one-way ANOVA (P<0.05).

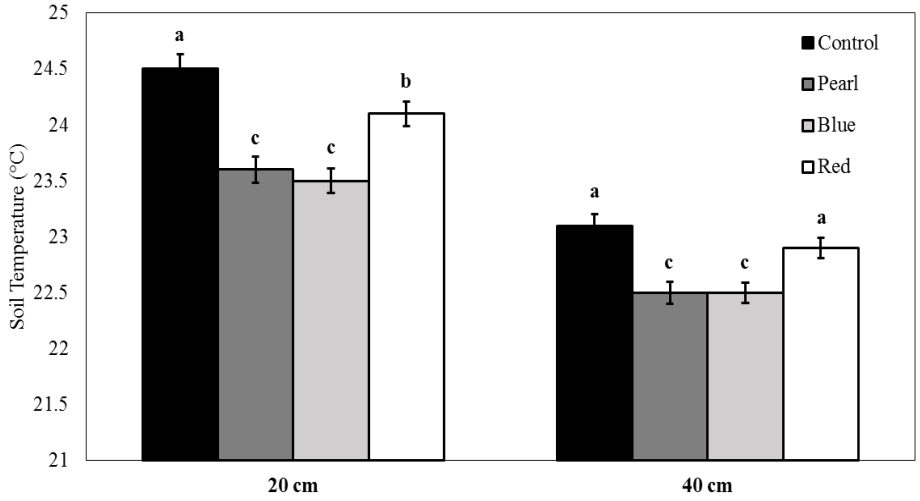

Figure 2. Mean soil temperature (°C) (±SE, N=4) at 20 and 40 cm depth under pearl, blue and red protective netting compared to an uncovered control in a three year-old 'Honeycrisp' apple orchard in Quincy, WA (47.23° N, 119.85° W). Different letters denote significant differences between means determined using a one-way ANOVA (P<0.05).

Canopy light interception (%)

The light interception of the canopies under three different netting treatments was significantly greater compared to the uncovered control in 2015 and 2016. Trees under colored nets developed more robust canopies than the uncovered control. This is supported by approximately 15% higher light interception for trees covered by nets than the uncovered control. Light interception was not significantly different among netting colors indicating similar canopy development between netting colors.

In 2015, the third year of growth, light interception was measured in trees with a crop (fruiting) and with all flowers removed (non-fruiting). In 2015, fruiting trees had a less developed canopy compared to the non-fruiting trees. However, in 2016, a targeted crop load was applied to all trees, fruiting or non-fruiting. In 2016, growth in the fruiting trees was greater because of low or no flowering and as such, there was no significant difference in light interception between fruiting and non-fruiting trees. The interaction between net color and fruiting treatment was significant ($p < 0.01$). The pearl net reported a higher light interception in the trees that bore fruit in 2015. This may be related to fruit set under the targeted crop load and higher vigor in 2016 when fruit load was low. Light interception increased from May to July, as the first-year tree growth increased, but were not statistically different.

Light transmittance (%) can be different for each colored net and is expressed as total light under each net / total light outside the net (open field) x 100. Transmittance describes the percentage of light coming through the nets relative to full sun and the shading percentage of each type of net is confirmed to range from 20-23% (as from manufacturer). Each colored net has a distinct transmittance curve calculated from the light spectra from 300 to 1000 nm. Variability during the season can be caused by how the sun hits the material or dust on the net. Blue net has a lower shading effect in the range from 400 to 550 nm (PAR range) than the red net. There was an increased in transmittance in in the infrared range (>780 nm) that is different from the other two net colors. The pearl net filtered the lowest wavelengths (UV-VIS) and shows a more uniform transmittance than the other colors. Red net has an increase in transmittance (%) immediately before 600 nm. In conclusion, transmittance measured in 2016 showed a similar trend with 2015 data and literature (Shahak and Gussakovsky, 2004). Spectra of scattered light under colored nets showed similar trends as reported in Shahak and Gussakovsky (2004) for scattered light on the total light under the different nets for all the wavelength.

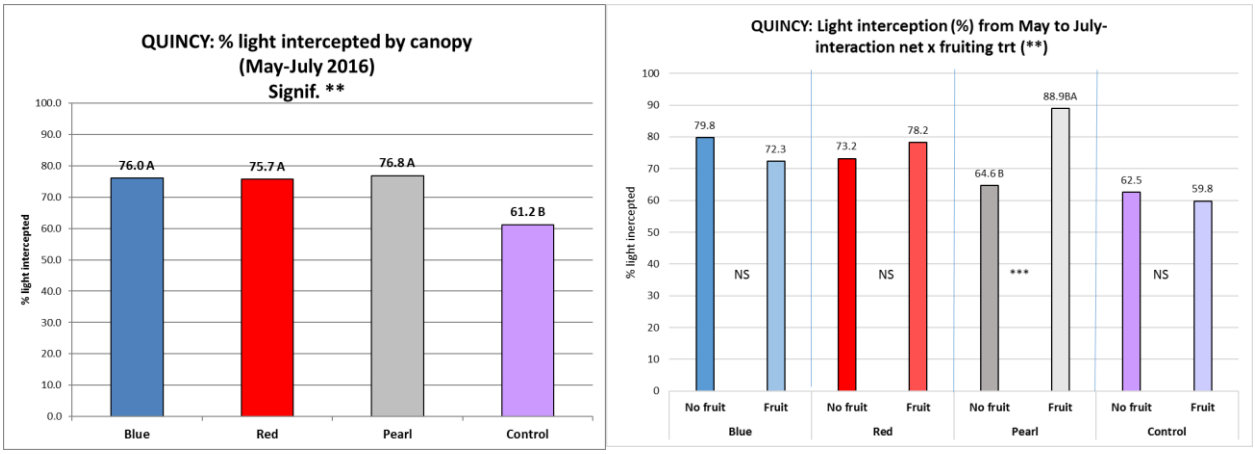

Figure 3: Light interception % from May to July in Quincy: comparison between colored nets and control (left) and within each color net between "fruiting" and "non-fruiting" (right).

Light spectra under the nets

At WSU TFREC and in the commercial orchard in Quincy, spectra of incoming total and diffuse (scattered) radiation were measured under the nets and in open field by using a spectroradiometer (Apogee Instrument, Inc., UT, USA) connected to a light cosine sensor via fiber cable. The open field readings were used as reference for the transmittance measurement. The entire data collection was performed orienting the detector perpendicular to the sun beams as reported in literature (Shahak and Gussakovsky, 2004, Kong et al., 2013). Transmittance of total light (%) for each colored net is expressed as total light under each net divided by the total light outside the net

(open field) x 100. Scattered light (%) was expressed as diffuse light under the net divided by the diffuse light in the open field, x 100. Light intensity parameters (PAR, UV, Blue, Red, Far Red) expressed as µmol m^{-2} s^{-1} were calculated in the same ranges as reported by Kong et al., 2013 (Table 2).

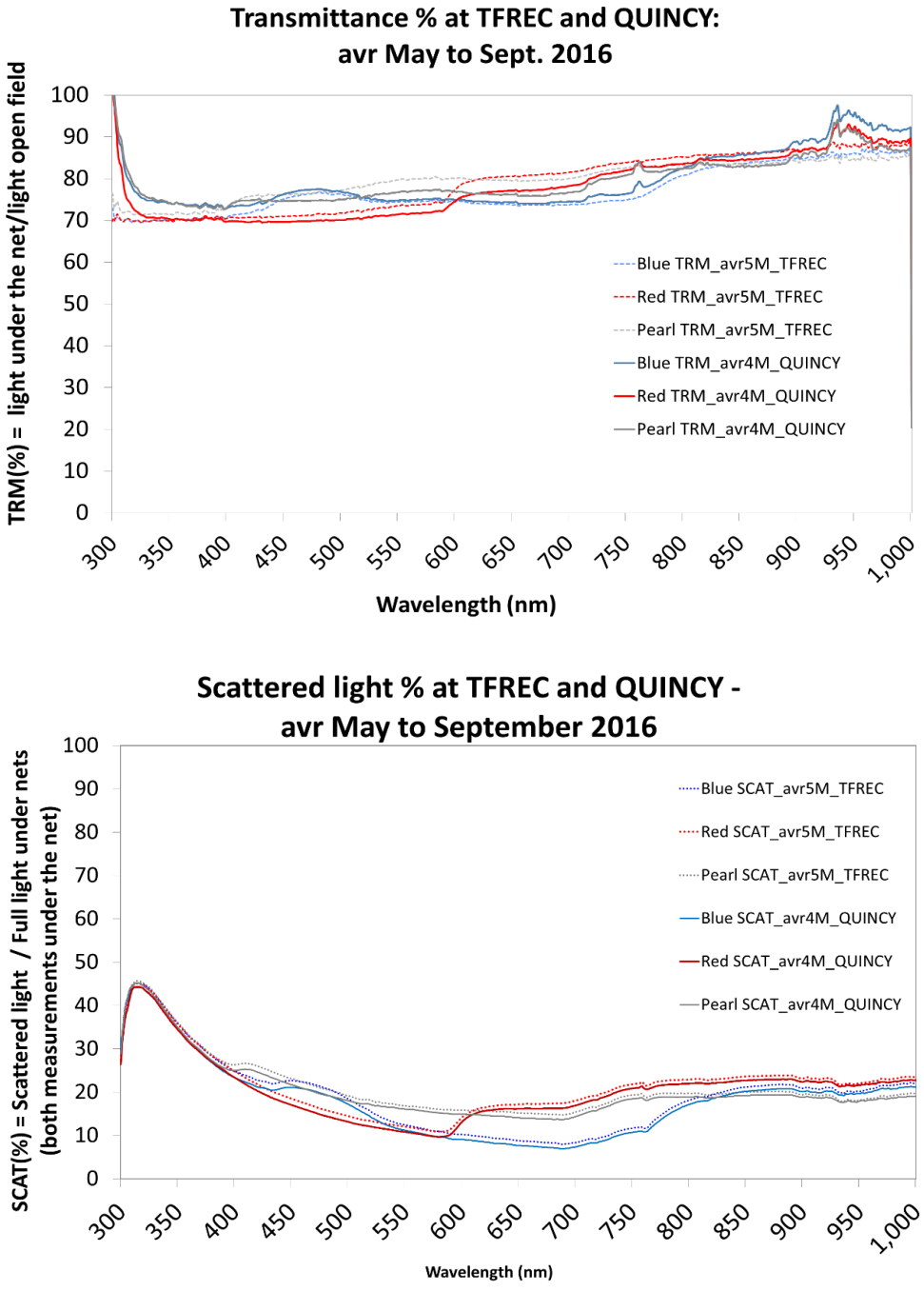

Figure 4: Transmittance spectra of total light under the nets (top) and percentage of scattered light on the total light under the nets (bottom) taken at both locations in 2016.

Table 2: Intensity and quality of total and scattered light under pearl, blue, and red netting compared to an uncovered control in Quincy and TFREC (Wenatchee) in 2016.

Quincy 2016			Light intensity (µmol $m^{-2}s^{-1}$)										Light quality (ratios)					
Light	Color Nets-ctrl	N	PAR: 400–700 nm		UV: 305–380 nm		Blue: 410–470 nm		Red: 640–680 nm		Far Red: 690–750 nm		Blue/Red		Red/Far Red		PAR/UV	
Scattered light (diffuse)	BLUE	48	159.5	B	20.4	B	42.5	A	14.5	C	23.0	C	2.93	A	0.63	B	7.89	C
	RED	48	183.4	B	19.6	B	32.8	B	36.1	A	59.5	A	0.91	D	0.60	C	9.47	B
	PEARL	48	213.0	A	21.0	B	44.7	A	27.5	B	46.5	B	1.63	C	0.59	C	10.21	A
	open field-NO NET	4	199.4	AB	31.5	A	51.7	A	20.2	C	26.6	C	2.55	B	0.76	A	6.44	D
	Significance		***		***		***		***		***		***		***		***	
Total full light (transmitted)	BLUE	48	1407.1	B	63.1	B	217.2	B	222.5	C	303.7	C	0.98	A	0.73	B	22.65	
	RED	47	1405.3	B	60.9	B	203.5	C	240.7	B	335.3	B	0.85	C	0.72	C	23.45	
	PEARL	48	1470.4	B	64.5	B	220.9	B	237.1	BC	329.7	B	0.93	B	0.72	C	23.09	
	open field-NO NET	4	1888.1	A	90.0	A	292.6	A	301.7	A	407.7	A	0.97	A	0.74	A	21.33	
	Significance		***		***		***		***		***		***		***		NS	

Significance: * p<0.05, ** p<0.01, *** p<0.001, NS= not significant. Significance was established with proc GLM in SAS, type III SS and Bonferroni as *post-hoc* test to discriminate means. Same letters means no difference between treatments.

Data were collected in a commercial orchard and reported as averages across the season from May to September 2016 (August measures were removed due to clouds). Open field-no net was measured only as reference (N=4). PAR: 400-700 nm, UV: 305-380 nm, Blue: 410-470 nm, Red: 640-680 nm, Far Red: 690-750 nm.

TFREC (Wen) 2016			Light intensity (µmol $m^{-2}s^{-1}$)										Light quality (ratios)					
Light	Color Nets-ctrl	N	PAR: 400–700 nm		UV: 305–380 nm		Blue: 410–470 nm		Red: 640–680 nm		Far Red: 690–750 nm		Blue/Red		Red/Far Red		PAR/UV	
Scattered light (diffuse)	BLUE	30	194.7	B	22.8	B	50.3	B	18.6	C	28.0	C	2.80	A	0.66	B	8.61	C
	RED	29	230.2	A	23.1	B	42.5	C	42.3	A	65.9	A	1.00	C	0.64	B	10.06	B
	PEARL	30	272.8	A	23.9	B	56.5	A	36.4	B	56.1	B	1.56	B	0.65	B	11.50	A
	open field-NO NET	3	222.0	AB	31.0	A	55.9	AB	23.4	C	32.4	C	2.50	A	0.73	A	7.27	C
	Significance		***		*		***		***		***		***		***		***	
Total full light (transmitted)	BLUE	30	1391.7	C	64.4	B	223.1	C	214.8	C	291.6	C	1.04	A	0.74	A	21.82	
	RED	29	1450.6	BC	65.3	B	217.0	C	243.9	B	335.8	B	0.89	C	0.73	B	22.50	
	PEARL	30	1521.1	B	67.2	AB	235.7	B	240.8	B	330.9	B	0.98	B	0.73	AB	22.90	
	open field-NO NET	3	1793.2	A	82.1	A	279.9	A	285.4	A	385.8	A	0.98	B	0.74	A	22.05	
	Significance		***		*		***		***		***		***		***		NS	

Significance: * p<0.05, ** p<0.01, *** p<0.001, NS= not significant. Significance was established with proc GLM in SAS, type III SS and Bonferroni as *post-hoc* test to discriminate means. Same letters means no difference between treatments.

Data were collected in an experimental shade house and reported as averages across the season from May to September 2016. Open field-no net was measured only as reference (N=3). PAR: 400-700 nm, UV: 305-380 nm, Blue: 410-470 nm, Red: 640-680 nm, Far Red: 690-750 nm.

In both locations, nets reduced the light intensity. Combined PAR, UV, Blue, Red, Far Red light were always significantly lower compared to the uncovered control (Table 2). PAR and UV across the season (May to September 2016) were similar for the three colors, while the intensity for Blue light was the lowest under the red net and Red and Far Red intensities were the lowest under the Blue net. The scattered light data in both locations showed a higher intensity in the PAR range for the Pearl net compared to the other colored nets and similar to the uncovered control as reported in literature (Kong et al., 2013). Blue net showed the lowest amount of scattered light intensity in the PAR range. Scattered light is the type of light that can reach the inner part of the canopy and modifying the physiology of the tree and fruit quality.

Blue/Red ratio was significantly ($p<0.001$) different among the four treatments: the highest under Blue net as total transmitted light and even more as scattered light, while the lowest values were registered under the Red net (as reported in literature, Shahak and Gussakovsky, 2004). Blue/Red ratios for Pearl and the uncovered control fell in between. Those trends in differences were more enhanced in the scattered light data than the total light. Red/Far Red ratio has a key role in phytochromes transition in their stages (activated P_{FR}/inactivated P_R) and therefore in how efficiently the light energy is captured for photosynthesis purposes (Batschauer, 1998). Red/Far Red ratio has the highest values in the uncovered control followed by Blue net. PAR/UV ratio of scattered light was significantly different among treatments showing the highest values under the Pearl net and the lowest in the open field. These results confirmed the beneficial effect of red and pearl nets for improving light quality in orchards.

Objective 2. Identify the impact of photoselective anti-hail netting on fruit quality and horticultural management

Shoot growth significantly increased under the netting compared to the uncovered control. In 2015, shoot growth was approximately 10% higher than the control and in 2016, when the netting was deployed earlier, growth was more than 25% greater under the netting compared to the uncovered control. Netting color did not affect shoot growth. Trunk cross sectional area was not significantly different among treatments (Table 3). However, bloom density was significantly greater for trees under pearl and red netting and the percentage of flowers set to fruit was greater for trees under netting than the uncovered control.

Net photosynthesis was greater under netting compared to the uncovered control (Table 4). This was likely driven by increases in stem water potential and increases in the light harvesting efficiency of the leaves. More work will be done in 2017 as part of the leveraged WSDA/USDA Speciality Crop Block Grant that will carry the project through 2017.

Table 3. Net photosynthesis of apple leaves at 120 days after full bloom in Honeycrisp apple under pearl, blue, and red netting compared to an uncovered control

Treatment	Net carbon assimilation rate ($\mu mol\ m^{-2}\ s^{-1}$)
Control	10.21 b[z]
Pearl	13.84 a
Red	15.11 a
Blue	15.44 a
Treatment Pr>F	0.0068

Table 4: The effect of nets and fruiting treatments in Quincy 2016 on averaged trunk cross-sectional area, weight of pruned material, return blossom, fruit set, and crop load of Honeycrisp and statistical results comparing treatments.

Treatment	Winter 2016 TCSA (cm^2)		Weight of wood pruned in winter 2016 (lb/tree)		Blossom cluster density (no. cluster/ cm^2 TCSA)		% Flower set to fruit	
Net								
Control	5.20		0.41		6.20	**b**	10.06	**b**
Pearl	5.07		0.37		11.71	**a**	15.85	**ab**
Blue	4.80		0.30		6.25	**b**	15.82	**ab**
Red	4.83		0.34		10.47	**a**	17.91	**a**
Significance	***NS***		***NS***		***		*	
Fruiting trt								
Non fruiting	5.31	**a**	0.43	**a**	12.75	**a**	10.12	**b**
Fruiting	4.63	**b**	0.28	**b**	4.56	**b**	19.71	**a**
Significance	***				***		***	
Significance net*fruit	***NS***		***NS***		***NS***		***NS***	

p<0.05, *; p<0.01, **; p<0.001, ***; NS, not significant for Type III sums of squares model significance.
Arithmetic means are presented; *post hoc* tests were done with LSMEANS option and the Bonferroni adjustment provided letter.

Objective 3. Evaluate fruit and leaf nutritional balance and fruit quality under different light conditions.

Fruit size was significantly greater under netting compared to the uncovered control in both 2015 and 2016. Netting reduced sunburn to a comparable level compared to evaporative cooling that was installed in 2016 (Figure 5). Blue netting had less sunburn than red or pearl. However, that appeared to come at a cost of slightly reduced color development (Table 6). To see the subtle differences between colors, another year of data is needed.

Table 5. Fruit size in 2015 and 2016 harvested from trees with even crop loads under pearl, blue, and red netting compared to an uncovered control.

	2015	2016
Control	231 *a*	366 *a*
Pearl	274 *c*	394 *b*
Blue	252 *b*	388 *b*
Red	260 *bc*	386 *b*

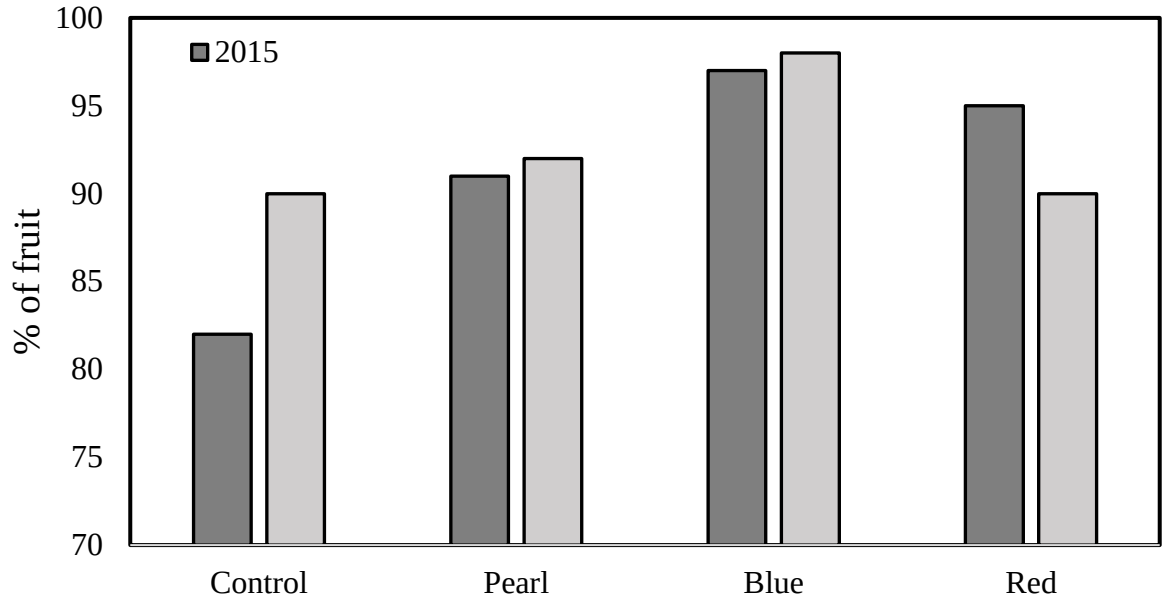

Figure 5. The proportion of harvested fruit (%) belonging to clean, Y1 and Y2 sunburn classes for fruit harvested from under pearl, blue and red protective netting compared to an uncovered control. 2015 had no evaporative cooling in the control and 2016 had evaporative cooling for sunburn protection in the control.

Table 6. Soluble solids content and foreground color development on apple fruit harvested from trees under pearl blue, and red netting compared to an uncovered control

	SSC (°Brix)	Foreground Color
Control	15.0 *a*	2.63 *a*
Pearl	14.7 *ab*	2.47 *ab*
Blue	14.5 *b*	2.39 *b*
Red	14.5 *b*	2.42 *b*

Leveraged External Grants

(2015-2018) 'Physiological responses of apple under photoselective hail netting'. Washington State Department of Agriculture Specialty Crop Block Grant. ($248,608).

Publications

Kalcsits LA, Mupambi G, Serra A, Musacchi S, Layne DR, Schmidt T, Mendoza M, Asteggiano L, Jaralmasjed S, Sindhuja S, Khot LR, Zúñiga Espinoza C. 2017. Above and below-ground environmental changes associated with the use of photoselective protective netting to reduce sunburn in apple. *Pending minor revisions*

Kalcsits LA, Asteggiano L, Schmidt T, Serra A, Layne D, Mupambi G. Shade netting reduces sunburn damage and soil moisture depletion in 'Granny Smith' apples. *Submitted to Acta Horticulturae October 15th, 2016.*

Presentations

Kalcsits L, Wheeler C, Asteggiano L, Jarolmasjed S, Khot L, Layne D, Mendoza M, Musacchi S, Sankaran S, Schmidt T, Serra S, Zuniga C**. Horticultural Management and Environmental Manipulation to Limit the Effects of Water Stress.** Washington State Tree Fruit Association Horticultural Show. Yakima, WA. December 7, 2015.

Kalcsits L, Wheeler C, Asteggiano L, Jarolmasjed S, Khot L, Layne D, Mendoza M, Musacchi S, Sankaran S, Schmidt T, Serra S, Zuniga C. Managing Stress in Tree Fruit. Washington State Tree Fruit Association Horticultural Show. Yakima, WA. December 8, 2015.

Kalcsits L. Netting to reduce risk and stress in tree fruit. Wenatchee Apple Day. Wenatchee, WA. January 21, 2016.

Kalcsits L. Update on photoselective netting trials in Washington State. International Fruit Tree Association. Grand Rapids, Michigan, February 10, 2016.

Kalcsits LA. Update on photoselective netting trials in Washington State. Olympia Fruit Annual Meeting. February 16, 2016

Kalcsits L, Wheeler C, Asteggiano L, Jarolmasjed S, Khot L, Layne D, Mendoza M, Musacchi S, Sankaran S, Schmidt T, Serra S, Zuniga C. Light, Air, and Soil Environment Manipulation Using Photoselective Anti-Hail Netting in a High Latitude, Desert Environment. ISHS Symposium on orchard systems, rootstocks and environmental physiology. Bologna, Italy. August 29 - September 2, 2016.

Kalcsits L, Wheeler C, Asteggiano L‡, Jarolmasjed S, Khot L, Layne D, Mendoza M, Musacchi S, Sankaran S, Schmidt T, Serra S, Zuniga C**.** Overhead Netting to Modify Orchard Environments. Washington State Tree Fruit Association Horticultural Show. Yakima, WA. December 7, 2016. Invited Presentation

Media Coverage

http://www.goodfruit.com/orchards-under-cover/
http://www.wenatcheeworld.com/news/2016/aug/20/local-color-researchers-find-multi-hued-netting-could-benefit-apple-production/
http://fruitgrowersnews.com/article/project-evaluates-photoselective-netting/
http://www.capitalpress.com/Orchards/20160512/researcher-studies-tools-ranging-from-netting-to-x-ray-meters
http://www.goodfruit.com/if-netting-is-the-future-what-color/

Extension Bulletin

http://treefruit.wsu.edu/news/photoselective-anti-hail-netting/

Field Days

Netting Field Day - August 17, 2016

The project team hosted 4 additional tours to the industry netting experiment in 2016

EXECUTIVE SUMMARY

This project tested the effect of pearl, blue, and red netting on the orchard environment, physiology and fruit quality of Honeycrisp apples compared to an uncovered control. Environmental sensors recorded air temperature, relative humidity, wind speed, light intensity, soil temperature and soil moisture in each treatment through the 2015 and 2016 seasons. In both years, fruit sunburn data and fruit quality was recorded at harvest and after four months of regular atmosphere storage. Vegetative growth, flower induction and fruit set was also measured. Plant water status, photosynthesis and light-use efficiency was recorded throughout the growing season in 2016.

Netting reduces wind speed, even when the sides are not closed. The netting material (sold as a 22% reduction in light) used reduced light intensity by 20-25% depending on the color or netting. Netting did not reduce the air temperature of the netting. It altered the spectra and intensity of light reaching the plant canopy and the soil. These changes in light produced cooler plant canopies, fruit and soil. This produced higher soil moisture under the netting, more growth and reductions in sunburn. Depending on the color of netting, the spectra of light reaching the plant canopy is different. Pearl netting, appearing white but is semi-transparent, scattered the light under the canopy providing a more uniform light environment and less shadowing (Table 2). It also transmitted more photosynthetically active radiation relative to UV light compared to other colors or the uncovered control.

Apple trees cannot use the total light made available on almost all summer days in Washington State. This excess light can be a cost to the tree as it needs to use energy to deal with this excess light. In many cases, a reduction in 20-25% light would not cause photosynthetic limitations that would lead to reduction in carbon assimilation and lower plant productivity. Plant productivity increased through increased photosynthetic rates, particularly later in the day. The light harvesting efficiency of trees under netting was higher than trees in the uncovered control. These gains in productivity led to increased growth shown through a 25% increase in terminal shoot growth and a 15% increase in light interception in tree under netting compared to the uncovered control. Flower induction was also greater under netting compared to the uncovered control.

Netting reduces fruit surface temperature below the sunburn threshold on days when the sunburn risk is high. Fruit sunburn was lower under the blue netting than the pearl or red. However, all netting reduced the incidence of sunburn to the same or lower sunburn severity as when using evaporative cooling. Fruit size was approximately 10% larger under netting. While this could be a concern for a cultivar like Honeycrisp, this is an advantage for other cultivars. Furthermore, the fruit size increase is likely a result of increased soil moisture and a less stressful environment. When evaporative cooling was not used in 2015, fruit maturity was accelerated outside the netting. However, when evaporative cooling was used in 2016, there were no differences in fruit maturity at harvest between netting treatments and the uncovered control. Blue appears to slightly limit color development but one more year of data is needed to confirm this. Pearl and red netting did not negatively affect color development in these trials.

As with any new horticultural approach, there are positives and negatives. Reductions in sunburn, increased plant productivity, hail, and wind protection are major positive aspects of netting. Increased fruit size and increased shoot vigor can be either positive or negative depending on the cultivar. Horticultural management could limit some of these potential negative effects of netting and each operation will need to develop their own strategies to manage their orchards under netting. We report than netting is a viable alternative for evaporative cooling for sunburn protection and other environmental stresses.

CONTINUING PROJECT REPORT **YEAR:** 3 of 4
WTFRC Project Number: TR-13-101

Project Title: Mechanical pruning in apple, pear and sweet cherry

PI: Karen M. Lewis
Organization: Washington State University
Telephone: 509-754-2011 X 412
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City/State/Zip: Ephrata, WA. 98837

Co-PI (2): Matthew Whiting
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Co-PI (3): Stefano Musacchi
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Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA. 98801

Cooperators: Olsen Brothers, Keith Oliver, McDougall & Sons, Brent Milne, Sara Serra (WSU-TFREC)

Total Project Request: **Year 1:** 77,536 **Year 2:** 47,959 **Year 3:** 50,210
Percentage time per crop: Apple: 60% Pear: 10% Cherry: 30% Stone Fruit: 0%
Other funding sources: None
WTFRC Collaborative expenses:

Item	2013	2014	2015
Wages	3,000	3,000	3,000
Travel	1,000	1,000	1,000
Total	4,000	4,000	4,000

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

Item	2013	2014	2015
Salaries[1]	26,295	26,307	27,359
Benefits[2]	2,183	2,271	3,135
Wages	7,214	7,503	7,803
Benefits	844	878	913
Equipment[3]	25,000		
Supplies	5,000	2,000	2,000
Travel	7,000	5,000	5,000
Total	73,536	43,959	46,210

Footnotes: [1] Salary for student. [2]Medical costs include increase of 4% per year. [3] Purchase or lease of 1 sickle-bar pruner and 1 circular saw pruner and tractor attachments.

Note: Written report to be submitted to the technology committee. Budget above for informational purposes only.

FINAL PROJECT REPORT

Project Title: Refinement/integration of vacuum-based end effector for fruit picking

PI: Curt Salisbury, Ph.D.
Organization: Abundant Robotics, Inc.
Telephone: 650.859.5050
Email: curt@abundantrobotics.com
Address: 333 Ravenswood Ave
Address 2: EL194
City/State/Zip: Menlo Park, CA 94025

Co-PI (2): Dan Steere
Organization: Abundant Robotics, Inc.
Telephone: 650.868.8467
Email: dan@abundantrobotics.com
Address: 333 Ravenswood Ave
Address 2: EL194
City/State/Zip: Menlo Park, CA 94025

Cooperators: N/A

Total Project Request: Year 1: $300,000 (ROM)

Percentage time per crop: Apple: 50% Pear: 30% Cherry: 10% Stone Fruit: 10%

Other funding sources

Agency Name: SRI International
Amt. awarded: $425,000
Notes: Internal Research and Development funds to support this effort

WTFRC Collaborative expenses: $15,000

Budget 1
Organization Name: SRI/Abundant
Telephone: 650 868 8467

Contract Administrator: Dan Steere
Email address: dan@abundantrobotics.com

Item	2015
Salaries	$160,000
Benefits	
Wages	
Benefits	
Equipment	$120,000
Supplies	
Travel	$20,000
Miscellaneous	
Plot Fees	
Total	**$300,000**

ORIGINAL OBJECTIVES

The original objectives of the 2015 funded research were:

- **Refined the nozzle design**
    a. The first proposed refinement to the nozzle design was aimed at reducing stem pulls and spur pulls.
    b. The second refinement to the nozzle design focused on minimizing damage caused to the body of the apple by the nozzle.
- **Developed an ultra-compact decelerator**
    a. Because the flow rates needed to apply a pull force on an apple from a distance are high, the speed of the apple once it enters the nozzle is exceptionally high. We proposed to develop a mechanism to decelerate the apples without bruising them.
- **Integrated the End-Effector on a Commercial Robot Arm**
    a. We proposed to integrate our end-effector with a commercial robot arm to ensure that our end-effector design is compatible with a robot arm and to facilitate a demonstration of the manipulation subsystem.
- **Demonstrated Integrated Manipulation Solution**
    a. We proposed to take the integrated test platform into the fields during 2015 harvest to evaluate its performance and demonstrate the system to the commission and growers.

SIGNIFICANT FINDINGS

The significant findings from our 2015 activities were:

- **Refined the nozzle design**
    a. We commissioned a careful study of the effect of pulled stems on apple storage life.
    b. We moved the urethane-polycarbonate interface to a location that would not cut the apples.
- **Developed an ultra-compact decelerator**
    a. We identified memory foam as a material with preferred viscoelastic properties for decelerating apples without bruising them.
- **Integrated the End-Effector on a Commercial Robot Arm**
    a. We integrated our end-effector with a commercial robot arm and a 3D stereo sensor developed by Carnegie Melon University and showed that our end-effector design is compatible with a robot arm.
- **Demonstrated Integrated Manipulation Solution**
    a. We tested autonomous picking in 7 different orchards in 2015, demonstrated the system to growers and the commission, and gathered data on a subset of the autonomously picked apples.

RESULTS AND DISCUSSION

Refine the nozzle design

Stems

The first proposed refinement to the nozzle design was aimed at reducing stem pulls and spur pulls. Early in the performance phase, we discovered that a couple of small experiments had been conducted to determine the effect of stem pulls on apple decay in storage. The results suggested that there might be little to no difference in decay between apples with an intact stem and apples with pulled stems. Rather than invest resources in minimizing the machine-induced stem pulls, we instead took the preliminary step of commissioning a formal experiment across multiple varieties to determine the effect of pulled stems on fruit decay in storage.

A postharvest study is currently underway to evaluate the quality of stored apples that were harvested without stems. In the fall of 2015, five bins of Granny Smith, Jazz, and Pink Lady, and six bins of Fuji (three of first pick and three of second pick) were harvested with an approximate ratio of 50:50 stem-on to stem-off. Photos of the test groups are shown in Figure 1 below. The test bins were placed on trailers with 'normal' fruit, bound for storage facilities. Test bins were placed randomly on a trailer and multiple trailers were used for each variety. Test bins were drenched using Scholar fungicide. Drench cycle number was recorded. Currently, the apples are in CA storage and will be evaluated alongside 'normal' harvested bins of fruit. Each bin will be evaluated for stem bowl rot or other defects attributed to a stemless condition. Evaluation is expected to start in March after approximately six months of storage.

Figure 9 Photos of Fuji apples included in our experiment to determine the effect of pulled stems on the decay of apples in storage. Note apples with stems and apples without stems in the image on the right

Damage reduction

The second refinement to the nozzle design focused on minimizing damage caused to the body of the apple by the nozzle. The nozzle used during the 2014 field trials was a urethane extension on a polycarbonate tube. The transition between the urethane and the polycarbonate was abrupt and caused some cuts and indentations to the apple. We moved this transition to be just beneath the urethane orifice. This put the sharp edge transition in a place that the apple could not contact.

Develop an ultra-compact decelerator

We determined that the least expensive, most reliable, and most compact decelerator would be a monolithic piece of viscoelastic foam. The viscous property decelerates the apple without causing the apple to bounce back at a high rate of speed, and the elastic element enables the material to restore its shape before the next impact. It was important to find a material with the right balance between these two properties. We found that Memory foam had the right balance of viscous and elastic properties. Originally developed by NASA for improved seat cushions, this material does an excellent job of distributing contact forces across the surface of the apple, and quickly decelerating the apples without bruising them. Initial experiments were conducted by dropping apples from a 19 ft height.

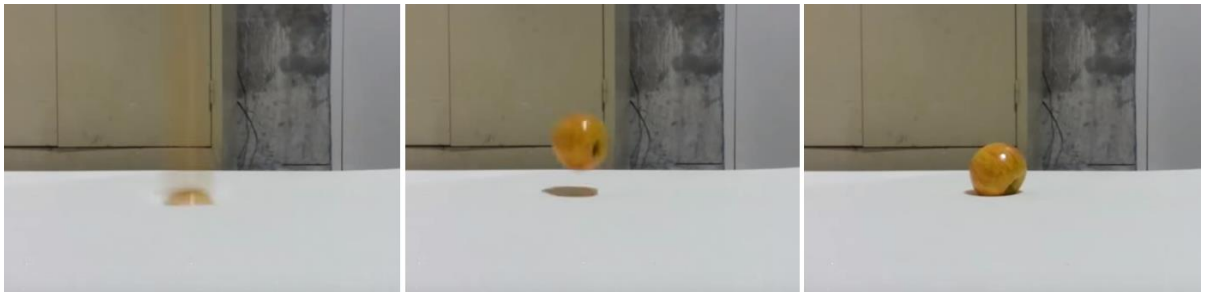

Figure 10 Drop testing of apples onto Memory foam. Left: Impact. Center: Rebound. Right: Resting Position.

Gravity accelerated the apples to a speed of 35 feet per second (approximately 24 miles per hour) upon impact and no bruising was observed across different apple varieties and sizes. Despite the drop height of 19 ft, the apples did not rebound more than approximately 3 inches (see Figure 2 above), but the memory foam would recover to near its original geometry within under a second. This is the optimum tradeoff between viscous and elastic behaviors. We then integrated the memory foam into our nozzle design and showed with lab testing that apples could be pulled into the vacuum nozzle, exit through check-valve doors and decelerate by colliding with the memory foam, all without bruising.

Integrate the End-Effector on a Commercial Robot Arm

We proposed to integrate our end-effector with a commercial robot arm to ensure that our end-effector design is compatible with a robot arm and to facilitate a demonstration of the manipulation subsystem. The integrated system is shown in Figure 3 below. The end-effector was mounted to the commercially available pick-and-place robot which was in turn mounted to the structure of a utility trailer. Also attached to the trailer structure was the vision system developed by Carnegie Melon University. We subcontracted a part of our grant funds to Carnegie Melon University to integrate their existing sensor with our picking platform in an effort to demonstrate fully autonomous apple picking.

Other significant hardware shown in the photo is the vacuum system, driven by the PTO of the tractor pulling the system. When an apple is not passing through the end-effector, the vacuum system draws 15 hp: 3 hp across the silencer, 6 horsepower across the pump, and 6 hp across the hoses and nozzle. The event of separating and apple from the tree and passing it through the end-effector has a duration of approximately 100 ms. At a picking rate of 1 per second, the average additional horsepower draw is approximately 2 hp.

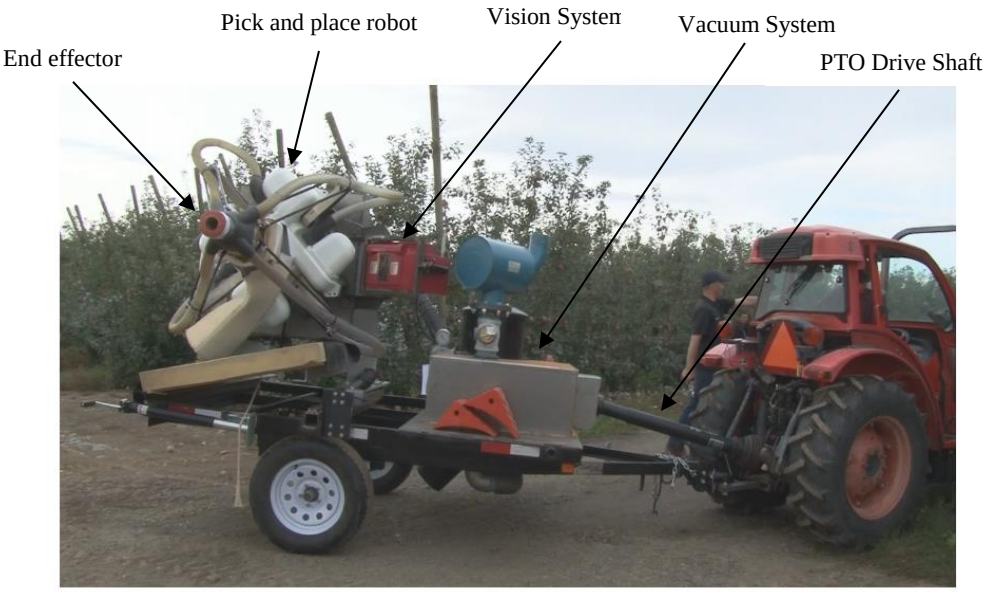

Figure 11 Photo of the demonstration and evaluation system

Demonstrate and Evaluate the Integrated Manipulation Solution

We brought the demonstration platform to Washington State for some initial testing September 8-11. Based upon those results, we returned the platform to SRI for some repairs and modifications. After the updates, the platform was sent again to Washington State for additional testing October 5-9. Our September testing was conducted at Yakima Valley Orchards' Glead ranch, Chiawana Orchards' Glead ranch and Doornink's Selah Ranch. Our October testing was conducted at Matson's Fruit Orchard in Wapato, McDougall and Sons' Gambler Ranch, Yakima Valley Orchards' Airport ranch and Flemming Farms in Quincy. We tested in multiple locations to enable us to evaluate our performance with a variety of different horticultural (pruning, training, etc) approaches. We also attempted to test in northern and southern geographies to enable as many growers to observe our evaluation and demonstration as possible. A map with the test locations is shown in Figure 4 below.

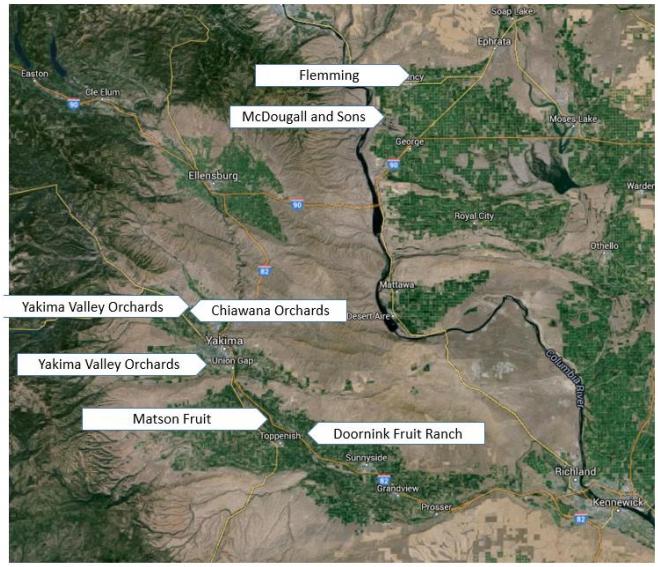

Figure 12 Map of Eastern Washington showing the 7 test locations

Our testing procedure was to park the system in an orchard row and press a key on the keyboard to initiate an autonomous picking sequence. Once initiated, the sensor would capture a photo of the trellis, recognize apples from leaves, determine the 3D coordinates of the recognized apples and then send those coordinates to the robot. The robot would then deploy the end effector to those coordinates and in the ideal case, the end effector would successfully pick the apples. A set of frames from one of our autonomous picking runs is shown in Figure 5 below. The image in the center shows the photo captured with the 3D sensor and includes circles drawn to indicate where the vision algorithm identified apples. The frames show the end effector moved to each of those locations. The system successfully picked all seven of the apples shown in the image in the center. This was one of hundreds of picking sequences we did throughout our field testing. We picked thousands of apples.

Figure 13 Frames of video capturing the pick events. The sensor output is shown in the center and the pick sequence is clockwise from the top left frame

As a part of this activity, the Washington Tree Fruit Research Commission collected 180 of the apples which we picked. The variety was Fuji. The results are shown in the table below.

SAMPLE SIZE		180	APPLES
BRUISING	DOWNGRADE	4	PERCENT
	CULL	2	PERCENT
PUNCTURE/CUT		12	PERCENT
TOTAL CULL		14	PERCENT

22% of the apples were found to have stem pulls, and none of the apples had spur pulls. The photos below show an example of the bruises and cuts in the experiment that were considered culls. An interesting finding was that the likely cause of nearly all of these culls was the apple rubbing across a branch during the picking event. If the canopy had long flexible branches, the branch would be sucked into the end-effector before the apple, and as the apple was subsequently sucked into the end effector it would slide along the branch causing either a bruise or a cut.

Figure 14 Left: Bruised Apple, Right: Cut Apple

CONCLUSIONS

Based upon our development and testing activities in 2015, we were able to demonstrate a vision system and end-effector solution that are capable of recognizing, localizing, and picking apples without bruising the apples. We demonstrated the ability of these systems to work together to support a picking rate of faster than 1 pick per second. We identified cuts as the principal cause of culls, and have a working hypothesis of the cause of these cuts. Specifically, it appears that the presence of long flexible branches near apples cause the end-effector to be prone to cutting the apples. We understand from growers that this issue can be reasonably addressed by pruning the long flexible branches from the tree.

EXECUTIVE SUMMARY

Refine the nozzle design

We took the preliminary step of commissioning a formal experiment across multiple varieties to determine the effect of pulled stems on fruit decay in storage. Currently, the apples are in CA storage and will be evaluated alongside 'normal' harvested bins of fruit. Each bin will be evaluated, beginning in March, for stem bowl rot or other defects attributed to a stemless condition. The second refinement to the nozzle design focused on minimizing damage caused to the body of the apple by the nozzle. The transition between the urethane and the polycarbonate was moved to be just beneath the urethane orifice. This put the sharp edge transition in a place that the apple could not contact.

Develop an ultra-compact decelerator

We determined that the least expensive, most reliable, and most compact decelerator would be a monolithic piece of viscoelastic foam. The viscous property decelerates the apple without causing the apple to bounce back at a high rate of speed, and the elastic element enables the material to restore its shape before the next impact We found that Memory foam had the right balance of viscous and elastic properties. We then integrated the memory foam into our nozzle design and showed with lab testing that apples could be pulled into the vacuum nozzle, exit through check-valve doors and decelerate by colliding with the memory foam, all without bruising.

Integrate the End-Effector on a Commercial Robot Arm

We integrated our end-effector with a commercial robot arm to ensure that our end-effector design is compatible with a robot arm and to facilitate a demonstration of the manipulation subsystem. We conducted some initial lab test to tune the integrated test platform.

Demonstrate and Evaluate the Integrated Manipulation Solution

We brought the demonstration platform to Washington State for demonstration and evaluation September 8-11 and October 5-9. The system successfully autonomously picked thousands of apples. As a part of this activity, the Washington Tree Fruit Research Commission collected 180 of the apples which we picked. The variety was Fuji. The results are shown in the table below.

SAMPLE SIZE		**180**	**APPLES**
BRUISING	**DOWNGRADE**	**4**	**PERCENT**
	CULL	**2**	**PERCENT**
PUNCTURE/CUT		**12**	**PERCENT**
TOTAL CULL		**14**	**PERCENT**

22% of the apples were found to have stem pulls, and none of the apples had spur pulls. An interesting finding was that the likely cause of nearly all of these culls was the apple rubbing across a branch during the picking event. If the canopy had long flexible branches, the branch would be sucked into the end-effector before the apple, and as the apple was subsequently sucked into the end effector it would slide along the branch causing either a bruise or a cut.

In conclusion, our development and testing activities in 2015 demonstrated a vision system and end-effector solution that are capable of recognizing, localizing, and picking apples without bruising the apples. We demonstrated the ability of these systems to work together to support a picking rate of faster than 1 pick per second. We identified cuts as the principal cause of culls, and have a working hypothesis of the cause of these cuts. Specifically, it appears that the presence of long flexible branches near apples cause the end-effector to be prone to cutting the apples. We understand from growers that this issue can be reasonably addressed by pruning the long flexible branches from the tree.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-15-103

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

PI:	Girish M. Ganjyal	**Co-PI**:	Meijun Zhu
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City/State/Zip:	Pullman, WA, 99164	**City/State/Zip**:	Pullman, WA, 99164

COOPERATORS: Van Doren Sales, Inc., Laura Grunenfelder – Northwest Horticultural Council, Stemilt Growers LLC., Double Diamond Fruit Co., Pace International LLC., US Syntec, Hansen Fruit Company, Symms Fruit Ranch, Washington Fruit & Produce Company and others packing houses.

BUDGET: **Year 1**: $73,951 **Year 2**: $74,798 **Year 3: $75,898**

OTHER FUNDING SOURCES: Part of the new faculty start-up funds of Dr. Girish M. Ganjyal. Support from co-operators for some of the materials and time on their packing lines.

Organization Name: WSU **Contract Administrator:** Katy Roberts/Ben Weller
Telephone: 509-335-2885/509-335-0052 **Email address:** arcgrants@wsu.edu/wellerb@wsu.edu

Item	2015	2016	2017
Salaries[1]	40,000	40,000	**42,000**
Benefits[1]	11,960	11,960	**12,558**
Wages[1]	3,750	6,000	**4,500**
Benefits	368	132	**441**
Equipment[3]	4,000	2,000	**0**
Supplies[2]	6,873	12,706	**11,399**
Travel[4]	7,000	2,000	**5,000**
Miscellaneous	0	0	**0**
Plot Fees	0	0	**0**
Total	$73,951	$74,798	**$75,898**

Footnotes:
[1] Salaries, Wages and Benefits for technical and student support
[2] Supplies and analysis fees, including for microbial testing
[3] Equipment related to biosafety level two microbial analysis
[4] Travel for industrial experiments

RECAP OF ORIGINAL OBJECTIVES

The objective of this proposal is to evaluate the potential of using hot air impingement drying to enhance the safety of the fresh packed apples. The specific objectives of the proposal are as detailed below:

1) Determine the impingement drying characteristics for broad range of wax coated apples and the impact on quality of the apples with in-plant testing.

2) Study the effectiveness of impingent drying in reducing the microbial levels in apples.

3) Develop scale-up strategies for commercial packing lines and complete the energy efficiency analysis.

SIGNIFICANT FINDINGS

In the year 2015 and 2016, significant progress was made on the testing of this technology on different varieties of the apples. As proposed in the original proposal, the first objective has been completed during the first year of the project and part of the 2nd objective has also been completed.

The following were the major findings from the 2 years of work so far:

Wax and Apple Quality

- From the first year of testing with a focus on ensuring the wax does not get deteriorated, it was found that the wax and apple quality retained well for up to 300^{o}F drying temperatures.
- The maximum temperature of drying is dependent on the apple variety.
- The "red delicious" variety was most sensitive to heat, among all the varieties tested.
- There were no significant negative impacts on the quality parameters (pH, soluble solids, acidity), for most of the varieties when dried at or below the optimum temperature.
- Temperatures above 300^{o}F were found to be negative for both types of waxes and for all the apples tested.
- This drying method can be used to reduce the current dryer footprint and increase the throughput through the dryer by reducing the drying times.

Microbial Reduction Testing

- The preliminary testing using high temperatures did not show any significant reduction in the total microflora. This testing was done with the natural microbial loads on the apples.
- The dryer was modified after taking to Pullman once so far, i) closed the ends by using rubber flaps to help retain the heat inside the chamber more, ii) decreased the clearance by an inch and iii) increased the air speed by 20% compared to before.
- After the modification, more testing was done with inoculation. This time we got a max reduction of 1 log. This shows that the above modifications helped.
- We will do one more modification to the dryer during early 2017 (under progress at this time in the machine shop). This will be to increase the air speed to the maximum practical level. Final series of testing will be done soon after this modification.

Overall, we had very encouraging results from the first year of the project with respect to the apple quality. The second years works showed that we can probably get about 1 log reduction. In the final year we will optimize the dryer and finish the microbial studies and also complete the energy savings and economic benefits analysis for the industry.

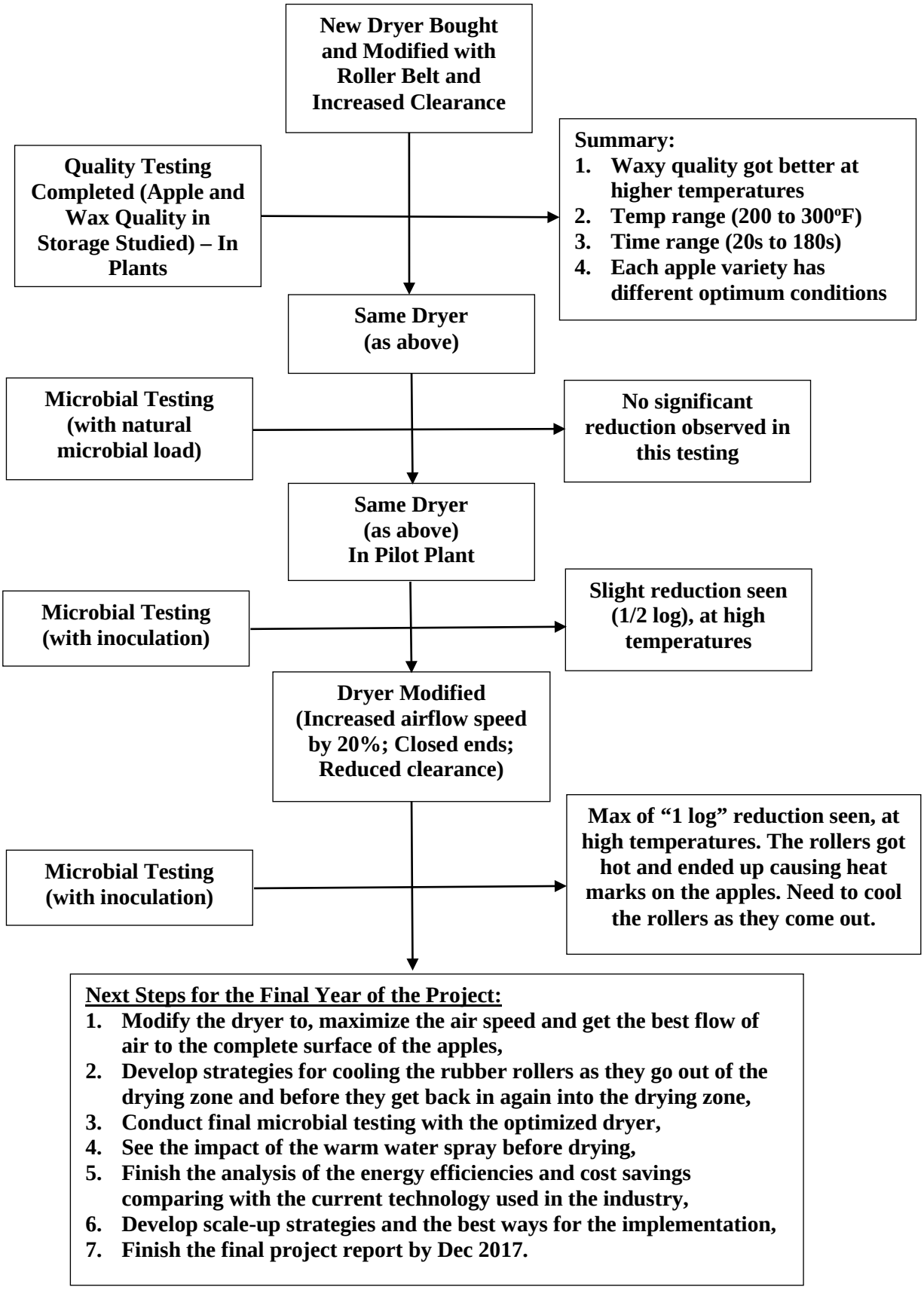

Fig. 1. Summary of the project progress

METHODS

Objective #1: Determine the impingement drying characteristics for broad range of wax coated apples and the impact on quality of the apples with in-plant testing

A detailed analysis of hot air impingement drying processes was conducted, with inputs from the co-operators of this research project. We worked with the packing houses to conduct in-plant testing with the new hot air impingement drying process, by comparing it side by side with the current drying processes.

The modified impingement dryer was moved into the packing houses and located close to the existing dryer in the respective plants. The fruit being run at the time was dried in the new dryer, side by side with the regular dryer. Fruit from both the dryers were tested for quality by using the standard procedure that is followed by the WTRFC.

Following hot air impingement drying characteristics were studied:

a. Temperature of the drying air (150 to 300°F)
b. Time of drying (15 to 90 seconds)

Following data and observations were recorded during the drying tests:

a. Surface and internal core temperature of the apples
b. Quality of the apples (glossiness before and after drying, color before and after the drying process, as well as assessments of wax quality, such as dripping, flaking, and cracking)

Once the apples are dried in the modified impingement dryer and the regular dryer in the plants, they were packed in regular packing cartons and stored in cold storage for the shelf life studies. Storage studies were conducted at WSU (Pullman Campus). During the shelf life study, all the standard quality parameters for the apples were measured. This included the total soluble solids, pH and titratable acidity. The protocols were set based on the initial review and discussions with the co-operators.

Impingement Oven

The original dryer we bought is the Hot Air Impingement Oven (PS628E, WOW^2, Middleby Marshall, Elgin, IL) as shown in Fig. 2. The oven was modified for drying of the range in apple size. The modified oven/dryer is shown in Fig. 3, during the testing process in a packing house.

Fig 2. The original oven

The oven has a maximum drying area of 16-inch x 16-inch. For the first year work, we had increased the clearance from the top of the belt to the top air holes, to facilitate the use of big sized apples. This ended up creating irregular flow or air, but did not matter for the wax conditioning during drying.

This is the modified oven used in the first year of the work. It worked out really well for quality testing. The quality of the wax and the quality of the apples was retained well. Although, every variety that was tested had different optimum temperature that it could withstand. Also, for the wax types, the maximum temperatures were different .

Fig 3. The modified oven/dryer in action

This shows the dryer modification in progress in the machine shop. The two plates on the right side, show the hole patterns for the air flow.

Fig. 4 Updates in progress to the dryer

This shows the dryer that was modified with the following:

i) The sides are now closed with rubber flaps, to help reducing the heat loss from the drying area.
ii) The clearance was decreased by an inch.
iii) The air flow speed was increased by 20%, compared to the initial set-up used for quality testing.

Fig 5. Slightly modified dryer (closed sides) and increased air flow speed by 20%

Objective #2: Study the effectiveness of impingent drying in reducing the microbial levels in apples.

The modified impingement dryer has been transported to Pullman (with the assistance of the WTFRC staff) and placed in the pilot plant. The microbial studies were conducted in the pilot plant by utilizing surrogate microorganisms (non-pathogenic *E. coli* and *Listeria* strains). Three strains were selected for both non-pathogenic *E. coli* and *Listeria* strains for the pilot-plant experiments.

Experimental procedure for microbial testing:

- Prepare 3-strain cocktail of *Listeria innocua* at ~ 10^6 CFU/ml
- Dip inoculate Fuji and Granny Smith apples for 10 min
- Dry the inoculated apples at room temperature for 24 h
- Set the dryer to target temperature
- Spray inoculated apples with sterile water and load onto the dryer
- Stop the conveyor when the apples move to the middle and time 1, 2, or 3 min with a timer
- Restart the conveyor to bring the apples out of the dryer
- Put each apple in one stomacher bag, rub, and plate immediately for enumeration

RESULTS & DISCUSSION

i) Impingement drying studies of different varieties of apples:

From the plant trials related to the testing for quality of the apples and the wax, we concluded that the best tempeartures for the red delicious apple variety is 250°F or lower. The "golden delicious" variety of apples are not negatively impacted by high temperatures (up to 300°F) of drying. Please refer to last years report for detailed data on this objective.

ii) Microbial testing:

Fig 6 and 7, show the data from testing, with the original dryer used in the quality testing.

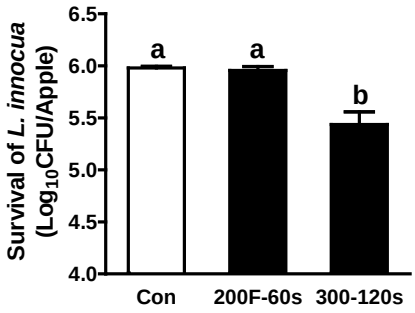

Fig. 6. Survival of *L. innocua* on Granny Smith apples (Trial 1)
Mean ± SEM, n=12, bars with different letters on the top are statistically different at $P < 0.05$.

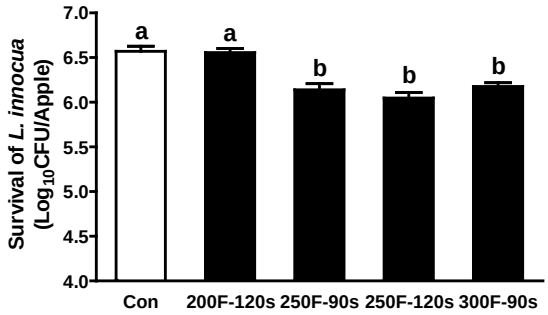

Fig. 7. Survival of *L. innocua* on Granny Smith apples (Trial 2)
Mean ± SEM, n=12, bars with different letters on the top are statistically different at $P < 0.05$.

Fig 8 and 9, show the data from testing, with the modified dryer with increased air speed, closed sides and reduced clearance.

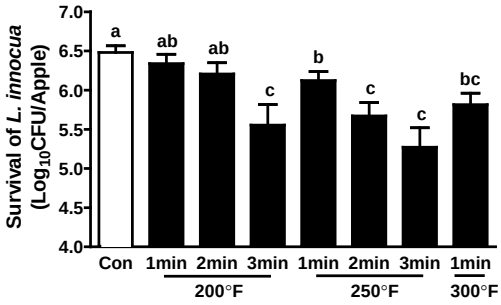

Fig. 8. Survival of *L. innocua* on Fuji Smith apples (Trial 2)
Mean ± SEM, n=12, bars with different letters on the top are statistically different at $P < 0.05$.

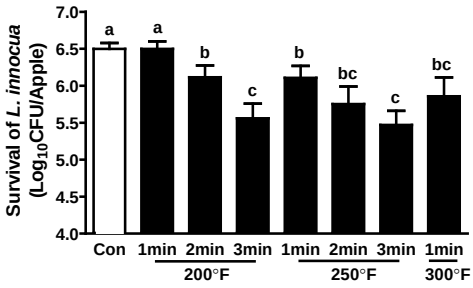

Fig. 9. Survival of *L. innocua* on Granny Smith apples (Trial 2)
Mean ± SEM, n=12, bars with different letters on the top are statistically different at $P < 0.05$.

Summary of the results and next steps:

- The results showed that the dryer modification went in the right direction.
- We saw, reduction with the increase in the air flow speed and with retaining of the hot air in the drying zone.
- This suggests that the hot air was not impinging uniformly on to the apples before. This helps us to go one more step forward to modify the dryer so that we can do the best we can to imping the hot air on to the apple surfaces uniformly including the calyx and the stem.
- In this drying procedure, the apples were wetted with cold water by spraying on them before drying. The reasoning was that the moisture will help with the process. But perhaps, we should have used warm water instead.
- So as the next steps, we would like to modify the dryer on final time to optimize it and conduct final microbial studies.
- Soon after this, we will conduct the analysis of the energy inputs and cost comparisons with the current drying technology used in the industry, by working closely with our cooperators.
- Following the final studies, we will submit the final report by the end of 2017.

CONCLUSIONS FROM THE STUDIES SO FAR

- This drying method can be used effectively to reduce the current dryer footprint and thus providing the opportunity to use additional food safety interventions on the packing line.
- Drying times can be reduced significantly by using higher drying air temperatures and thus increasing the production capatcity.
- We have seen a maximum of 1 log reduction so far at higher temperatures. But at the higher temepratures, we have to ensure that the rollers are cooled as they come out of the dryer and go back in, to avoid heat damage to the fruit surface because of the hot rollers.
- Overall, this drying technique can provide economic benefits to the packing houses.

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

WTFRC Project Number: AP-16-100A

Project Title: Ozone in apple storage: microbial safety and decay management

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Cooperators: Glade Brosi, Stemilt Growers LLC.; Matt Miles, Allan Brothers

Total Project Request: **Year 1:** $104,707 **Year 2: 108,515**

Other funding sources

Agency Name: Center for Produce Safety (CPS)

Amt. requested: $298,000 (Pending)

Notes: The project was submitted to CPS, which is proposed to complement interventional studies to control *Listeria monocytogenes* on fresh apple during cold storage.

WTFRC Collaborative expenses: $7,000 per year

Item	2016	2017
Salaries		
Benefits		
Wages	5,000	5,000
Benefits	2,000	2,000
Total	7,000	**7,000**

Footnotes:
Timeslip wages for 16 weeks.

Budget 1: Meijun Zhu

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

Item	2016	2017
Salaries[1]	$22,000	$22,880
Benefits	$10,193	$10,601
Wages[2]	$27,565	$28,667
Benefits	$5,623	$5,848
Supplies[3]	$25,326	$26,219
Travel[4]	$2,000	$2,300
Miscellaneous[5]	$5,000	$5,000
Total	$97,707	**$101,515**

Footnotes:

[1/] Technical support: four month salaries plus benefits are requested. For a research Intern (0.4 FTE) at 42.4% benefit rate.

[2/] PhD graduate student partial stipends and undergraduate assistant wages. Timeslip wages for 16 weeks.

[3/] Chrom *Listeria*, MOX plates, BLEB and selective reagents (Acriflavine hydrochloride, Nalidixic acid, Natamycin, Moxalactam), Chrom ECC, TSBYE and TSAYE, PCR reagents, other chemicals and medium for microbial culture and identification; Disposable consumable and supplies including dynabeads *Listeria*, filtration membrane, spreader, petri dishes, Whirl-PAK bags, PCR strips and 96 well plates, microtubes, pipette tips, serological pipettes, autoclave bags and others. Supplies include Petri dishes and artificial media for fungal growth.

[4/] Travel funds are requested to cover travel costs related to the project work such as trips to the packing facilities in central Washington for fruit collection and in plant testing. Travel to packing houses for trials and fruit sampling.

[5/] We have all instruments needed for proposed studies. Funds are requested to partially cover the Biosafety Level 2 facility and equipment maintaining fees and Biohazard disposal fees.

OBJECTIVES

1. Examine fate of *Listeria* spp. and natural microorganisms on apple fruit surfaces when stored in refrigerated air, controlled atmosphere in the presence or absence of continuous low doses of ozone.
2. Evaluate the efficacy of continuous low doses of ozone on postharvest decay
3. Examine the effect of ozone in the storage environment on final fruit quality.

PRELIMINARY DATA AND SIGNIFICANT FINDINGS

1. *L. monocytogenes* is a tough foodborne pathogen. A limited reduction of *L. monocytogenes* on apple surfaces occurred during 12 weeks of refrigerated storage either at 1°C/33°F, 4°C/39.2°F or 10°C/50°F. There was about ~1 Log reduction of *L. monocytogenes* on apple surfaces when they were stored at room temperature (22 °C / 71-72 °F).
2. We determined a~2-Log reduction of *L. innocua* (a *L. monocytogenes* surrogate) on apples after 6 weeks of storage under a commercial RA and CA storage environment at 33°F. Continuously low dose ozone gas application (70 ± 5 ppb) in CA storage generated an additional 1-2 Log reduction. Upon further storage for 12 additional weeks, a gradual reduction was observed.
3. Data indicates that cold storage with or without continuously low dose ozone gas application can be an additional hurdle for controlling *Listeria* on apple fruits.
4. A systematic/hurdle approach is needed to ensure apple microbial safety.

METHODS

We have established methods for proposed objective 1-3 studies as detailed in the following.

Objective 1: Examine fate of Listeria spp. and natural microorganisms on apple fruit surfaces when stored in refrigerated air, controlled atmosphere in the presence or absence of ozone.

a. Examine fate of *Listeria* spp. on apple fruit surfaces under different cold storage conditions

1. 3-strain *Listeria* inoculum preparation and established on apple surface

A 3-strain *L. monocytogenes or L. innocua* cocktail will be prepared via mixing equal numbers of each respective strain suspension. A 3-strain *L. monocytogenes* cocktail was used for examining the fate of *L. monocytogenes* on fresh apple under different temperatures in food microbiology lab (Biosafety level 2), while 3-strain *L. innocua* cocktail was used for studying the fate of *Listeria* on fresh apple during different cold storage with or without ozone in a commercial packing facility.

Apples were individually and separately inoculated to establish 1×10^{6} or $1\times10^{3\text{-}4}$ CFU/apple of 3-strain cocktail of *Listeria* strains through dipping inoculation, and held at room temperature for 24 h prior to different storages.

2. Storage treatments in BSL2 food microbiology lab

Apples established with high and low level of *L. monocytogenes* were randomly separated into four groups and subjected to different temperature storages (1 °C, 4 °C and 10 °C) for up to 12 weeks. Apples under different storage conditions were sampled at 0d, 1d, 4d, 7d, 14d, 28d, 56d and 84d of storage to analyze the survival of *L. monocytogenes* on fresh apples.

3. Cold storage treatments in a commercial packing facility

Apples established with 1×10^6 CFU/apple of *L. innocua* were randomly separated into three groups and subjected to three different storages: refrigerated air (RA, 33 °F), controlled atmosphere (CA, 33 °F, 4.0 % O_2, 0.9% CO_2), and CA with a low dose (70 ± 5 ppb) ozone (CA+O_3) for up to 6 months weeks. Apples under different storage conditions were sampled at 0wk, 1wk, 3wk, 6wk, 12wk, 18wk, and 24wk of storage to analyzer the survival of *L. innocua* on fresh apples.

4. Microbial analysis

At each sampling day, apples under the respective storage condition were sampled and transferred to sterile bags, rinsed with 10 ml of 0.1% buffered peptone water in Whirl-PAK bag, shaken and rubbed the apple surface for 2 min to release attached microorganism, then serial diluted. Appropriate dilutions will be plated on agar plates. Plates will be incubated at 35˚C (95˚F) for 24 ± 2h and enumerated manually.

b. To evaluate natural microbial reduction on apple fruit surfaces under different cold storage conditions

1. Cold storage treatments in a commercial packing facility

Non-waxed, non-inoculated Fuji apples were subjected to different storage conditions (RA, CA and CA+O_3) as described previously. Apples were sampled at 0wk, 6wk, 12wk, and 24wk of storage for total plate count and yeast and mold enumeration.

2. Survival microorganism analysis

At each sampling day, apple was sampled and transferred to a sterile bag, rinsed with 10 ml of 0.1% buffered peptone water in Whirl-PAK bag, shaken and rubbed the apple surface for 2 min to release attached microorganism, then serial diluted. The appropriate dilution was plated onto TSAYE plates for total plate count and potato dextrose agar (PDA) plates for yeasts and molds, respectively. Colonies will be counted manually after incubation at 35°C (95˚F) for 48h.

Objective 2. Evaluate the efficacy of continuous low doses of ozone on postharvest pathogens

a. Evaluate the efficacy of ozone on pre-wounded and inoculated apple fruits.

1. Apple surface disinfection and wounding

Freshly harvested organic apples of the selected varieties were surface-disinfected in 0.8% sodium hypochlorite, rinsed with tap water, and air-dried. Then, fruits were wounded using a sterile-3-mm diameter cork-borer at the stem-end zone of each fruit (2 wounds/fruit).

2. Fungal inoculation

Surface disinfected fruits were inoculated with 20 µl of a spore suspension of Penicillium expansum (blue mold), *Botrytis cinerea* (gray mold), *Neofabraea perennans* (bull's eye rot), *Sphaeropsis pyriputrescens* (Sphaeropsis rot), *Mucor piriformis* (Mucor rot*), Rhizopus stolonifer* (Rhizopus rot) or *Phacidiopycnis washingtonensis* (Phacidiopycnis rot). Each pathogen was inoculated at three different concentrations i.e. 10^3, 10^4, and 10^5 spores/ml. Four replicates of 20 fruit each was used for each pathogen/spore concentration combination.

3. Cold storages and apple analyses

Inoculated apples were subjected to different cold storage (CA, and CA+O_3) as described above. The incidence and severity of blue and gray, mucor, and Rhizopus molds, bull's eye rot, and Sphaeropsis and Phacidiopycnis rots (slow pathogens) were/will be determined after 2, 3 and 4 months, respectively.

b. Assess ozone efficacy on natural infections caused by major pathogens in combination with or without fungicides.

1. Interaction of ozone and postharvest fungicides on natural infections occurring in the orchard or in storage rooms

Four bins of apple fruits without drench or drenched with either TBZ, fludioxonil (Scholar) or pyrimethanil (Penbotec) were subjected to CA or CA plus ozone cold storage, respectively. Apples at are being analyzed for fungicide residue levels to determine whether ozone degrade fungicides when applied simultaneously.

2. In year 2, we plan to evaluate the efficacy of radical (wet) ozone versus gaseous ozone against the four aforementioned pathogens.

Non wounded fruits will be sprayed with spore suspensions of *Penicillium expansum, Botrytis cinerea, Neoabraea perennans* and *Phacidiopycnis washingtonensis* and will be treated with continuous gaseous ozone at 60 to 80 ppb or with 1 ppm radical ozone or 6 hours. Disease incidence and severity will be determined.

3. In year 2, the efficacy of gaseous ozone and wet radical ozone to sanitize storage rooms post-storage will be further evaluated.

The fungal populations in storage rooms will be monitored using the SAS Air-Sampler then ozone in its two forms will be applied to rooms at 500, 800, and 1,000 ppm for 6, 12, and 24 hours. The fungal populations in storage rooms will be monitored again after each treatment.

Objective 3: Examine the effect of ozone in the storage environment on final fruit quality.

At harvest and at the end of storage, flesh firmness, total soluble solids and titratable acidity of fruits are being analyzed per published methods (Both et al., 2017). Ozone burn will be assessed after storage and subsequent 1 week at room temperature.

PROGRESS/RESULTS AND DISCUSSION

Objective 1. (Ongoing)

1. Fate of *L. monocytogenes* established on fresh apples of selected varieties during storage at different temperatures

Currently, there is barely any information availbale on how easily *Listeria monocytogenes* survives on fresh apples under different storage conditions. Thus, we first did a short term storage study with *L. monocytogenes* established on fresh apples of selected varieties (Fuji, Granny Smith) under different storage temperatures. In this study, we choose the following storage temperatures per stated reasons.

- 1 °C (33 degrees Fahrenheit, a typical cold storage temperature).
- 4 °C (36 to 38 degrees F, a temperature commonly used for Honeycrisp long-term storage).
- 10 °C (50 degrees F, a temperature condition often used for Honeycrisp in preparation for storage).
- 22 °C (72 degrees F, mimic situation of consumer purchased apples which are put on their kitchen count before consumption, though unlikely in commercial scenario).

During two weeks of short-term storage, *L. monocyotogenes* level on organic Granny Smith apples stored at 1, 4, and 10 °C stayed stable, though there was ~0.3 Log CFU/apple after 1-day storage (Figure 1A). More *L. monocyotogenes* reduction was observed when organic Granny Smith apples were stored at 22°C; there was ~1.0 Log CFU/apple reduction after 14-day storage (Figure 1A). Similar survival patterns of *L. monocytogenes* on conventional Granny Smith apples (Figure 1B) and Fuji apples (Figure 1C) were observed during the 14-day storage.

We further examined fate of *L. monocytogenes* on fresh apples during 12-week cold storage. Very little die-off of *L. monocytogenes* was observed on fresh organic Granny Smith apples during the 12-week of cold storage, whether apples were inoculated with high level (Figure 2A) or low level (Figure 2B) of *L. monocytogenes*. There was no significant difference in survival of *L. monocytogenes* among the three storage temperatures (Figure 2).

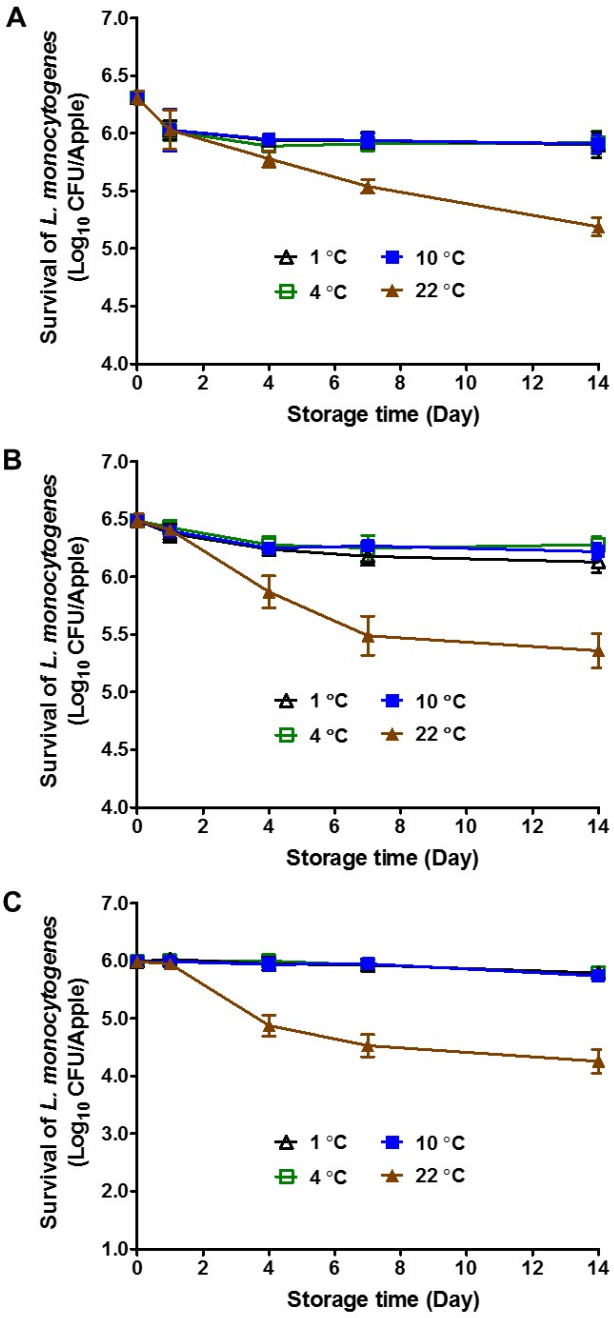

Figure 1. Fate of *Listeria monocytogenes* on fresh apples during short-term storage under different temperatures when inoculated at 1×10^6 CFU/apple. A. Organic Granny Smith; B. Conventional Granny Smith; C. Conventional Fuji. Mean ± SEM, n=12

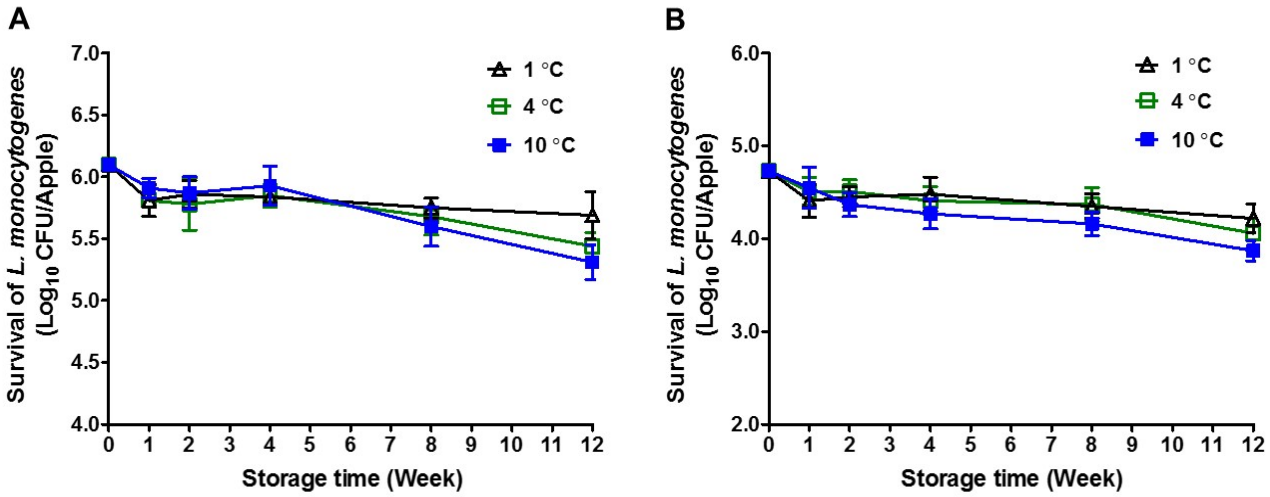

Figure 2. Survival of *Listeria monocytogenes* on fresh organic Granny Smith apples during 3-month cold storage. A. Apples were inoculated at 1×10^{6} CFU/apple; B. Apples were inoculated at $1\times10^{4\text{-}5}$ CFU/apple. Mean ± SEM, n=12

2. Fate of *L. innocua* established on fresh apple during under different cold storage at a commercial packing facility

We further conducted a cold storage experiment in a typical commercial apple facility using *L. innocua* inoculated apples. There was a ~2 log die-off after 6 weeks of storage. Ozone gas application further enhanced the effect, up to 4 logs after 6-week storage (Figure 3), but there was very small reduction beyond 6 week of storage. These data indicate that storage intervention alone can not completely eliminate *Listeria* from apple fruits. To control *Listeria*, a system's approach is critically needed. It is worthwhile to mention that data on *L. innocua* does not completely align with our data generated with the actual pathogen in the lab, and may over-estimate the actual die-off rates one could expect when dealing with pathogenic strains of *Listeria*.

Currently, we have another trial with Fuji apples under different storage conditions, which will be evaluated in the following months.

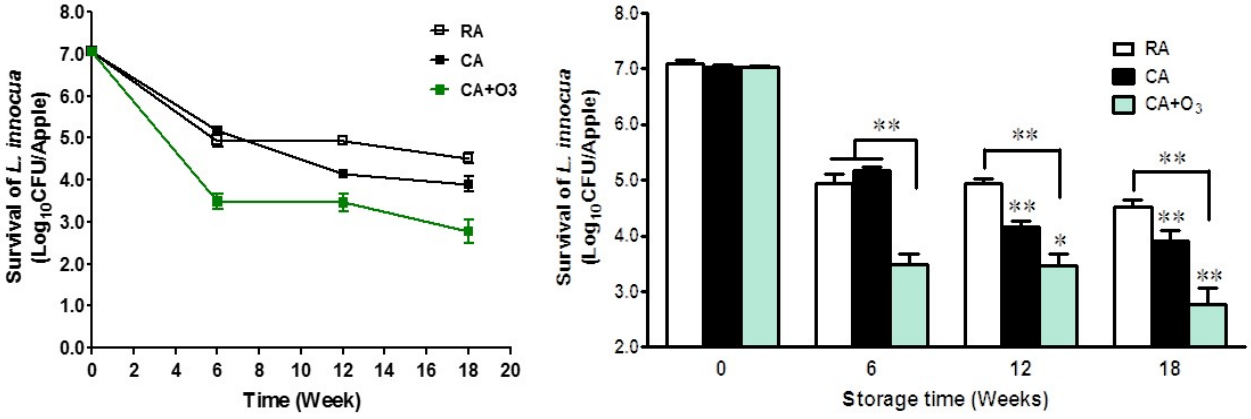

Figure 3. Survival of *Listeria innocua* established on Fuji apple surface under different cold storage at a commercial packing facility. Mean ± SEM, n = 40.

3. To evaluate natural microbial reduction on apple fruit surfaces under different cold storage conditions

Another set of Fuji apple fruits (non-waxed and non-inoculation) was subjected to different storage condition (RA, CA and CA with ozone) on October, 2016. We have analyzed total plate count (TPC) and yeast/mold (Y/M) count prior to and 6-week post respective storages. The TPC count was slightly decreased on apples under RA and CA storages, and more reduction in TPC count on apples under CA with ozone storage (data not shown) during 6-week storage. Similar results were found for yeast and mold count. This seems to indicate that cold storage does affect the surface microbial make up and affects their proliferation.

The same experiment using non-inoculated Fuji apples is still ongoing, which will be sampled for microbial analyzed at 12-week, 24-week of respective storages.

Objective 2. (Ongoing)

1. Evaluate the efficacy of ozone on pre-wounded and inoculated apple fruits

In October 2016, a trial was initiated to evaluate the efficacy of CA and CA plus a continuous low dose (60 to 80 ppb) gaseous ozone *against Penicillium expansum, Botrytis cinerea, Neoabraea perennans* and *Phacidiopycnis washingtonensis* inoculated to wounded fruit or to the surface of apple fruit from two cultivars Granny Smith and Fuji. Fruits are currently under storage, and will be evaluated in January 2017 for disease severity and incidence and/or the ability of ozone to kill spores of fungi on apple fruit surfaces.

2. Evaluate the interaction between ozone and postharvest fungicides

Another trial was conducted in October 2016 to evaluate the interaction between ozone and 3 postharvest fungicides: TBZ, pyrimethanil (Penbotec), and fludioxonil (Scholar). Apples were treated with these 3 fungicides and stored in CA and CA+Ozone and are being analyzed for fungicide residue levels to determine whether ozone can degrade the fungicides when applied simultaneously.

Objective 3. (Ongoing)

1. Examine the effect of ozone in the storage environment on final fruit quality

Fruits (non-waxed and non-inoculation) for this objective are currently under different storage conditions. We have analyzed maturity (flesh firmness, total soluble solids and titratable acidity) prior to respective storages. We will analyze maturity of fruits as well as ozone burn at the end of 24-week storage.

REFERENCE

Both, V., Thewes, F.R., Brackmann, A., de Oliveira Anese, R., de Freitas Ferreira, D., Wagner, R., 2017. Effects of dynamic controlled atmosphere by respiratory quotient on some quality parameters and volatile profile of 'Royal Gala' apple after long-term storage. Food Chem 215, 483-492.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP15-102A

Project Title: Apple scion breeding

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Cooperators: Bruce Barritt, Professor Emeritus, WSU; Amit Dhingra, Dorrie Main, Carolyn Ross, WSU Pullman; Tom Auvil, Ines Hanrahan, WTFRC; Roger Adams, Willow Drive Nursery, Ephrata; Craig Hardner, Australian Crop Genetic Services, Brisbane, Australia

Total Project Request: Year 1: $249,881 **Year 2:** $266,445 **Year 3: $260,362**

Other funding sources
Agency Name: WTFRC Apple Review
Amount awarded: $269,000 (2014-2016)
Notes: "After RosBREED: Developing and deploying new apple DNA tests" PI: Peace. Co-PIs: Hardner, Evans, Main. Synergistic project to develop and deploy DNA tests.

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. Synergistic project to identify sources of fire blight resistance.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $5.72M (2015-2017 with 2 more years likely)
Notes: "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars" PI: Iezzoni. Co-PIs: Peace, Evans et al. To further develop MAB for U.S. Rosaceae crops.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $2.7M (2014-2019)
Notes: "Genome Database for Rosaceae: Empowering Specialty Crop Research through Big-Data Driven Discovery and Application in Breeding" PI: Main. Co-PIs: Evans, Peace et al. Synergistic project for application of bioinformatics to tree fruit crops.

WTFRC Collaborative expenses:

Item	2015	2016	2017
Wages	21,500	11,700	14,700
Benefits	8,600	7,800	9,800
RCA Room Rental (x2)	8,100	8,100	8,100
Shipping	0	0	0
Supplies	1,000	1,000	1,000
Travel	3,500	3,500	3,500
Plot Fees	0	0	0
Total	42,700	32,100	**37,100**

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnson & Joni Cartwright
Telephone: 509 335 7667,509 663 8181 **Email address:** carriej@wsu.edu; joni.cartwright@wsu.edu

Item	2015	2016	2017
Salaries[1]	59,205	61,573	64,036
Benefits	20,697	21,525	22,386
Wages[2]	22,680	23,587	24,530
Benefits	4,309	4,482	4,661
Orchard establishment supplies	20,000	20,800	18,060
Genotyping supplies	17,000	18,500	20,000
Travel[3]	14,690	15,278	15,889
Miscellaneous (virus testing)	1,500	4,500	1,500
Plot Fees	8,800	8,800	8,000
Total	168,881	179,045	**179,062**

Footnotes:
[1]Salaries for Agricultural Research Technologist (Bonnie Schonberg @ 1.0 FTE) and for 3 months for genetic screening technician (Terence Rowland @ 0.25FTE)
[2]Wages for time-slip labor for orchard management and trait phenotyping
[3]In-state travel to research plots which are spread across the state.

Budget 2
Organization Name: Willow Drive **Contract Administrator:** Roger Adams
Telephone: 509 787 1555 **Email address:** roger@willowdrive.com

Item	2015	2016	2017
Seedling propagation	35,400	53,300	35,700
Phase 2 & 3 trees	2,900	2,000	8,500
Total	38,300	55,300	**44,200**

OBJECTIVES

1. Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.
2. Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

This project continues the existing WSU apple breeding program with the specific focus of producing new improved apple varieties for the Washington industry. This project addresses the highest priority of the WTFRC apple horticulture and post-harvest committee of 'Fruit quality pre and post-harvest'.

SIGNIFICANT FINDINGS

1. Fourteen new large families were made in 2016 with approximately 31,300 seeds produced in the WSU Apple Breeding Program (WABP).
2. Seedlings from approximately 13,000 seeds from 2015 crosses were grown in the greenhouse.
3. Approximately 9,000 seedlings were screened with DNA markers for fruit quality; just over 4,000 were culled leaving the remaining seedlings to be transplanted to Willow Drive nursery.
4. Seedlings at Willow Drive were propagated on M.9 rootstocks for future orchard evaluation. More than 3,000 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2017.
5. The final count of new Phase 1 trees planted in 2015 was approximately 4,600.
6. Promising selections already in Phase 2 trials (planted in 2007-2015) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
7. Nineteen new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2015.
8. Thirty five promising selections made in 2015 were propagated in 2016 for planting in 2018 Phase 2 trials at three diverse sites in Central Washington.
9. One new promising Phase 2 selection was propagated onto Geneva 41 rootstock for advancement to Phase 3 initially at the Quincy site only.
10. One new promising Phase 2 selection was top-worked onto trees at the Phase 3 Quincy site.
11. Fruit was harvested on three new selections advanced to Phase 3. None had bitter pit. Sunburn was slight. All are eating very well in RA storage in December with no issues.
12. Phase 3 Quincy 3rd and 4th leaf grafts of WA 38 had light crops of big fruit. Phase 3 Prosser's WA 38 grafts had one of the two leaders removed; the resulting vigor might have contributed to the very light crop. Bitter pit was not issue, although the very large fruit did accumulate some stem bowl splits.
13. A series of three WA 38 field days were held in WSU Roza and Sunrise.
14. Genetic identity was confirmed for all mother trees of WA 38 planted in the nursery mother tree blocks. All trees tested as true to type using several DNA markers.

METHODS

Objective 1: Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.

Objective 2: Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

The breeding program benefits from regular input from the Breeding Program Advisory Committee (BPAC) particularly in terms of the horticultural aspects of the elite selections in Phase 3. Regular orchard visits and an annual meeting provide several opportunities to get BPAC feedback on the quality of the selections and also the priorities and targets for the program itself.

Expected results are primarily new elite selections progressing into the Phase 3 trial and beyond. Decisions to release new varieties are dependent on the amount and quality of data available. Once a selection is identified for release, the program focuses on communicating results regularly through Field days, multiple opportunities to sample fruit, and reports usually in the Good Fruit Grower.

RESULTS & DISCUSSION

Objective 1: Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.

A Clicker survey to rate priority attributes was completed at the Apple Review dinner in January. Results from the non-researchers (growers, field-support and one supplier) who participated are presented in Table 1 and align well with the selection protocols of the program.

Crosses for this season were designed taking into account all the available DNA test information as well as phenotypic trait knowledge. DNA testing focused on the Ma-indel test and worked very efficiently to reduce population sizes. Only eight 96-sample plates of samples repeatedly failed and so full sets of seedlings from those plates were sent to the nursery for propagation without DNA information obtained.

Crosses were made with WA 38 to introgress fire blight resistance into the breeding program germplasm using the three *Malus sieversii* accessions selected from the Norelli project ('Fire blight resistance and fruit quality in new Washington cultivars', CP-15-100) as having the best fruit quality. Several hundred seeds were produced from each cross combination.

Harvest started and finished particularly early this season with the first selection harvested on July 25. Stored fruit from 2016 is still being evaluated.

Objective 2: Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

Evaluation of fruit from the 2015 season harvest was completed early in the year with a total of 304 seedling trees evaluated after storage. In addition, fruit from 105 Phase 1 'keeper' selections (selected in previous years for re-evaluation) was evaluated (497 samples in total), plus 51 Phase 2 selections and controls (961 samples) and 2 Phase 3 selections (18 samples).

Data at the end of the season was analyzed with 'Elite Advance' software, trait by trait, and top-ranking individuals were selected using a combination of this data and breeding team discussion.

New selections were made for Phase 2 and Phase 3. Thirty-five promising seedling selections were propagated in fall 2016 for inclusion in Phase 2 plantings in 2018. One advanced selection from Phase 2 was propagated in fall 2016 for inclusion in 2018 Phase 3 plantings. Three selections were grafted in groups of about 40 trees in the Phase 3 Prosser site. A few trees are still failing due to fire

blight susceptibility exacerbated by the 2013 hailstorm. In Quincy, 35 trees each of two selections were grafted to a different (new) elite selection, reducing their tree number to ~75 to 80. The new grafts did very well. The rootstock is G.41. More than 100 trees are scheduled to be planted in Quincy to start the Phase 3 process. The strategy of staggered start provides more fruit from one location sooner, allowing for more intense early storage and sensory work. Most selections discontinued in 2010-11 were due to storage or sensory problems.

Fruit was harvested on three new elite selections in Phase 3 from the Quincy site. None had bitter pit, however slight sunburn was observed. All are eating very well in RA storage in December with no apparent issues. One selection has a type 4 tip bearing growth habit, the other two are more similar to Gala/Cripps Pink type growth habit. Thinning requirement varied between the selections. In Quincy, two of the new selections were harvested on September 16, the third harvested August 31. All had tough/chewy peel in 2016. This block did encounter slight water stress in late August. The block has cooling.

Phase 3 Quincy 3rd and 4th leaf grafts of WA 38 had light crops of big fruit. Phase 3 Prosser's WA 38 grafts had one of the two leaders removed; the resulting vigor might have contributed to the light crop. Bitter pit was not an issue, however the very large fruit did accumulate some stem bowl splits.

Thanks to Dave Allan and Sarah Franco in Prosser, Scott Driscol and Dale Goldy for the Quincy trial and Ray Fuller for the Phase 2 planting in Chelan.

WABP Publicity

Numerous fruit samples of WA 38 and Phase 3 selections were distributed to the industry, allied industry and target audiences. A series of three double-site field days were organized to showcase WA 38 at different times in the growing season. http://treefruit.wsu.edu/videos/wa-38-cosmic-crisp-field-day/

'Market to Market' Iowa public TV attended and filmed a WA 38 field day (show not yet screened). WA 38 horticulture and commercialization was featured in a full afternoon session of the 2016 Washington Horticultural Association Show in December.

Talks, publications and posters

Jan 2016 – Korean nursery visit, TFREC, WA. (*Evans*): 'The interaction between the WABP and the U.S. Clean Plant Network.'

Aug 2016 – National Association of Plant Breeders poster, Raleigh, NC. (*Jamie Coggins, Evans Grad student*): 'Utilizing dry matter and Near-Infrared spectroscopy for selection in the WSU apple breeding program.'

Aug 2016 – WSU CSS 512 Field crop breeding students tour of the apple breeding program (*Evans*).

Oct 2016 – Hort 509/510 seminar, WSU. (*Evans*): 'The WSU apple breeding program'.

Oct 2016 – International Women's Association of Yakima, Yakima, WA. (*Evans*): 'From the U.K. to the U.S.: the science of breeding tasty new apples.'

Oct 2016 – Sarah Kostick and Jamie Coggins (Evans Grad students) hosted visiting High School students and presented the apple breeding program with a sensory evaluation activity.

Oct 2016 – ISHS 1st International Apple Symposium, Yangling, China. (*Peace*): 'Learning as we go: DNA-informed apple breeding at Washington State University.'

Oct 2016 – Henan Agricultural University invited seminar, Zhengzhou, China. (*Peace*): 'From QTLs to routine DNA-informed breeding: prospects, advances, & needs …and experiences in apple at Washington State University.'

Nov 2016 – Wageningen University & Research invited seminar, Wageningen, Netherlands. (*Peace*): 'From QTLs to routine DNA-informed breeding: prospects, advances, & needs …and experiences in apple at Washington State University.'

Nov 2016 – University of Maryland invited lecture, College Park, MD. (*Evans*): 'Development and application of DNA-informed breeding in the WSU apple breeding program.'

Nov 2016 – The breeding program hosted the Fruit evaluation class from Wenatchee Valley College.

Nov 2016 – 1st Tropical Genomes Conference keynote presentation, Cairns, Australia. (*Peace*): 'DNA-informed breeding successes in temperate rosaceous crops: What can tropical crops learn?'

December 2016 – Fruit of advanced selections was available for tasting at the Washington State Horticultural Association meeting in Wenatchee, WA. Dr Evans also presented talks entitled 'Introducing WA 38; A new standard of product excellence' and 'Tree fruit breeding and selection at WSU'.

Harshman J, Evans K, Hardner C. (2016) Cost and accuracy of advanced breeding trial designs in apple. *Horticulture Research* DOI 10.1038/hortres.2016.8

Hardner C, Evans KM, Brien C, Bliss F, Peace C. (2016) Genetic architecture of apple fruit quality traits following storage and implications for genetic improvement. *Tree Genetics & Genomes* 12:20. DOI 10.1007/s11295-016-0977-z

Ru S, Hardner C, Carter PA, Evans K, Main D, Peace C. (2016) Modelling of genetic gain for single traits from marker-assisted seedling selection in clonally propagated crops. *Horticulture Research* 3:16015. DOI 10.1038/hortres.2016.15

Table 1. Clicker survey grower/field-support/suppliers* results for ranking breeding program priority attributes (January 2016).

Rank	Attribute
1	susceptibility to bitter pit
2	crispness
3	consistency of flavor after storage
4	yield
5	susceptibility to sunburn
6	ease of harvest
7	firmness
8	resistance to powdery mildew
9	precocity
10	juiciness
11	resistance to fire blight
12	bearing habit
13	acidity
14	greater or less than 20 % red color
15	susceptibility to superficial scald
16	resistance to post-harvest disease
17	early vs. late harvest timing
18	fruit size below or above 88
19	sweetness
20	thickness
21	susceptibility to watercore
22	non-browning flesh
23	self-thinning
24	yellow vs. green background color
25	low vs. high incidence of russet

*11 growers, 2 field-support personnel and 1 supplier responded

CONTINUING PROJECT REPORT **YEAR:** 3 of 3 (no-cost ext.)
WTFRC Project Number: AP14-103A

Protect title: WA 38 rootstocks and training systems

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Cooperators: Sara Serra (WSU-TFREC)

Total Project Funding: **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:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 **Email:** arcgrants@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 (by Musacchi S.).*

- The highest trunk cross-sectional area (TCSA) and annual trunk growth were reported for spindle trees in both Sunrise and Rosa orchard locations (like in 2014-15)**.** In Sunrise, G41 had significantly higher TCSA and annual trunk growth than Nic29 (like in 2014-15) and opposite trend in Roza.
- Hand pruning the V systems in both locations took significantly longer to prune in hours per acre than the spindle system in both locations.
- Spindle had higher yield per tree (like in 2015) in both locations. Yield per acre was higher in V system in Roza only. No statistical difference between rootstocks in Roza for yield like in 2015, while G41 produced bigger fruit than Nic29 in Sunrise.
- In both orchards, approx. 58% of fruit graded were considered fancy (WFCY).

❖ *Objective 2: Identify growth and productivity characteristics on bi-axis (by Musacchi S.-Lewis K.).*

- The mechanical winter pruning of bi-axis in Roza showed a 93% reduction in time of pruning with 1h:26min/A versus 21h:47min/A for the hand pruned.
- The lowest yield per acre was observed for bi-axis Nic29-hand (34 bins/A), while all the other combinations are higher and similar (Bi-axis-Nic29-Mech 48 bins/A).

❖ *Objective 3: Conduct an economic analysis of WA 38 production (by Gallardo K.).*

- Total pruning cost in both Roza and Sunrise was the lowest with V, G41 and bending in Roza; and spindle, M9-NIC29 and bending in Sunrise.
- Commercial 'Fuji' pruning cost ranged \$0.12-\$0.17 per tree. The total pruning cost for the WA38 in Roza ranged from \$0.10 to \$0.17 per tree and in Sunrise \$0.05 to \$0.16 per tree, across all treatments.
- In both Roza and Sunrise, the highest gross margin is for WA38 spindle, M9-NIC 29 and click when the different prices of different fruit sizes are taken into account. On the other hand, when the different prices depending on fruit grades are considered, the highest gross margin is WA 38 V, M9-NIC29 and click in Roza; and WA38 spindle, M9-NIC29 and click in Sunrise.

METHODS

Two thousand trees of the new WSU scion WA 38 (to be released as Cosmic Crisp in 2017) propagated on M9-Nic29 and G41 rootstocks were planted in June 2013 in two locations at the WSU Sunrise (Wenatchee) and WSU Roza (Prosser) orchards to compare vegetative and productive performances. In both sites, two main training systems are compared: spindle (3 ft x 10 ft =1499 trees/A) and V system (1.5 ft x 10 ft= 2997 trees/A). Another trial with WA 38 bi-axis trees (1 year younger, 3 ft x 10 ft =1499 trees/A) on the same rootstocks has been set up to assess the possibility of mechanized thinning, pruning and harvest. 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 most horizontal limbs, "clicking" on 1yr old wood and trying to develop more flower buds close to the stem to avoid the "blind wood" issue that characterizes the "type IV habitus" apple varieties.

For *Objective 1,* winter pruning was done by hand in both locations in February 2016. No hand summer pruning was done in 2016. Blooming started at the beginning of April. Post-bloom thinning was not performed in any of the treatment groups at either location because WA38 spontaneously set 1-2 fruit per cluster. Fruit quality samples from harvest 2015 were sorted in homogenous batches accordingly to I_{AD} index at T0 and T1.

For *Objective 2* we are focusing in this report only on biaxis trees in Roza. In this trial we are comparing winter (February 2016) + summer (June 2016) mechanical pruning (with commercial sickle bar machine) versus normal hand pruning in winter. Harvest occurred September 12-13th and fruit were graded and sized as in Objective 1. As of the submission of this report, biaxis grown in Sunrise didn't have any mechanization treatment in 2016.

For *Objective 3 (by Gallardo K.),* we followed two approaches. First, we calculated the mean pruning time across three treatments: training system (spindle and angled), rootstock (M9-NIC29 and G41), and pruning technique (bending and click), then evaluated if the differences of pruning time between treatments are statistically significant. Pruning was done during the winter and expressed as hour per tree. To estimate the cost, we considered a wage of $12.00 per hour. Second, we compared costs and returns for the WA38 experimental trial with commercially produced 'Fuji' apples. We considered the management for WA38 is somewhat similar to 'Fuji'. This enables to have an approximate idea of what would likely be the WA38 costs at a commercial scale. In this report, we present results for the 4th year of establishment for both 'Fuji' and WA38. The returns for WA38 were estimated using two types of pricing: (a) 2-year average price of 'Honeycrisp' for different fruit sizes; and (b) 2-year average price of 'Honeycrisp' for different fruit grades (i.e., WAXF and WFCY). The 2-year averages were estimated from price data for 2014-15 and 2015-16 marketing seasons. To enable comparisons, the pruning costs per acre for each WA38 treatment were estimated given the density of trees in commercial 'Fuji' orchards — 1,089 trees per acre under a spindle system, and 1,452 trees per acre under an angled system. In addition, we included a royalty fee per box for WA38. Since prices considered were higher than $50 per 40-lb box, the royalty fee was at $3 per 40-lb box.

RESULTS AND DISCUSSION

Objective 1: Identify growth and productivity characteristics on spindle and V systems (by Musacchi S.).

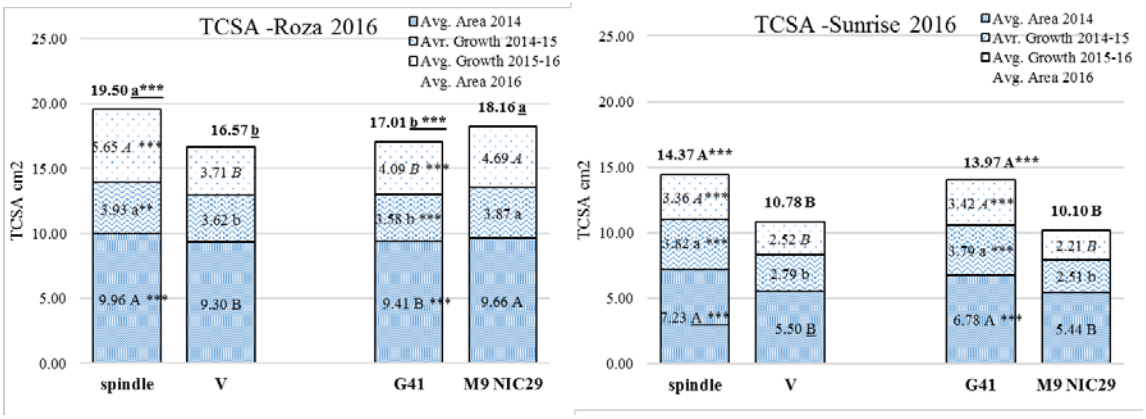

Figure 1: Trunk cross sectional areas in Roza and Sunrise from 2014 to 2016, included annual growth.
Significance: p<0.05, *; p<0.01, **; p<0.001, *; ns, not significant for Type III sums of squares model significance. Student-Newman-Keuls post hoc test to assign letter groups to arithmetic means where model was significant.**

Vegetative parameters

The highest trunk cross-sectional area (TCSA) and annual trunk growth were reported for trees trained to spindle in both locations (like in 2014-15, Fig. 1). In Sunrise, G41 rootstock had significantly higher TCSA and annual trunk growth than Nic29 (like in 2014-15) as well as the tallest trees. In Roza, Nic29 had higher TCSA and annual growth than G41; this difference was statistically significant for spindle, but not for the V system (like in 2015). TCSA was not significantly different in the comparison between "bending" pruning technique and "click" technique in Roza, while in Sunrise "bending" technique had higher TCSA than the "click", though only significant in V. As combinations, spindle-G41-bending was the most vigorous for TCSA 2015, 2016 and annual growth in Sunrise, while both combinations of spindle-Nic29 in were the most vigorous in TCSA in Roza. V-Nic29- click was the least vigorous combination in both orchards. The average number of rootsuckers per tree in Sunrise was higher in Nic29 than G41 (like in 2014-15).

Winter Pruning

The V system hand pruning in both locations took significantly longer than the spindle system. (26 h/A vs 20 h/A in Roza, Table 1). The hours dedicated to winter pruning per acre were slightly lower than in 2015 only in Roza. The material removed during winter hand pruning 2016 from spindle was significantly more than V (almost double) in Roza as in 2015, while no significance was observed for Sunrise. In Sunrise, trees on G41 rootstock required more pruning time than Nic29, while no differences in time were reported in Roza (data confirmed in 3 years). G41 reported a higher amount of wood cut during winter pruning by hand in comparison to Nic29 (0.4 kg/tree vs 0.2 kg/tree) as reported in 2015 (Table 1). The interaction of training system x rootstock in Sunrise revealed that within V system, G41 requires double the time in comparison to Nic29, while in spindle they were different but less far apart (data not shown). The "click" pruning technique took significantly more time than "bending" during winter for Sunrise orchard only (Table 1). In Sunrise, the most time-consuming combination to hand prune in 2016 was V-G41-click (as in Roza), the fastest to prune was spindle-NIC29-bending in Sunrise and Spindle-G41-bending in Roza (data not shown).

Table 1. Winter pruning labor hours at Roza and Sunrise orchards and material removed from cut (wood and fruit), 2016. Labor time is presented as hours per acre.

2016	ROZA				SUNRISE			
	winter PRUNING (hours:min:sec/Acre)#		cut material (wood) in winter (kg/tree)		winter PRUNING (hours:min:sec/Acre)#		cut material (wood) in winter (kg/tree)	
Trainin systems								
spindle	19:56:10	B	0.59	A	14:41:46	B	0.32	
V	26:08:31	A	0.28	B	25:01:47	A	0.26	
significance	***		***		***		NS	
Rootstocks								
G41	22:06:22		0.40		25:31:14	A	0.39	A
M9 NIC29	23:44:19		0.46		14:12:19	B	0.19	B
significance	NS		NS		***		***	
Pruning treatment								
BENDING	21:38:21		0.46		17:19:28	B	0.30	
CLICK	24:26:20		0.41		22:24:06	A	0.28	
significance	NS		NS		***		NS	
significance training system* rootstock	NS		NS		***		NS	
significance training system* treatment	NS		NS		***		NS	
significance rootstock* treatment	NS		NS		NS		NS	
significance training * rootstock* trt	NS		NS		NS		NS	

calculations done referring to 1 person pruning

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

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

Productive data

Spindle trees had more fruit per tree and higher yield per tree (like in 2015) in both locations (Table 2). Yield per acre was higher in V system than spindle in Roza only (41 Mton/A vs 29 Mton/A). There was no statistical difference between rootstock behaviors in Roza in term of productions like in 2015, while in Sunrise G41 produced bigger fruit than Nic29 (328g vs 307g, respectively). "Click" technique induced higher production than "bending" in terms of kg per tree but also Mtons/A in both orchards (Table 2), but no significant difference in average fruit weight. The combinations that resulted in the most production in terms of bins/A in Roza were V-Nic29-click and V-G41-bending, while the least productive were both combinations of spindle-bending (Nic29 and G41). The combinations that resulted in the most production in Sunrise was V-Nic29-click and spindle-Nic29-click while the least productive were both combination of V-Nic29 –bending (data not shown). In Roza 6323 fruit were graded for sorting defects and judged on the basis of a hypothetical future commercial standard. The percentage of cull on all the block was on average 31% of all harvested fruit, while 59% classified as fancy (WFCY) and only 10% as extra-fancy (WXF). This distribution was similar to Sunrise with slightly more cull probably due to the fact that in Roza the percentage of limb rub/bruises reached up to 30% with more vigorous trees. In Roza, the green spot incidence was 19%, and limb rub/bruises was 31%, while sunburn was 9%, similar to Sunrise. Russet was 11%, again similar to Sunrise, while insect damage was higher in Roza (9% versus 4% in Sunrise). Among the 8 combinations under comparison, the one that reported highest percentage of cull was spindle-Nic29-bending (32%), likely due to the high incidence of limb rub/bruises (40%) and green spot (20%). Spindle in general reported very low percentage of extra fancy fruit (approx. 3%) while in this condition V registered up to 19% of extra fancy on average. The combination showing the highest percentage of extra-fancy fruit was V-G41-bending (33%). The average of fruit affected by poor color is 13.5%, but both combinations of V-bending showed higher values (not shown).

Table 2: WA38 fruit yield at Roza and Sunrise harvest September 2016.

Training system	Rootstock	Pruning trt	total number fruit/ tree 2016		kg fruit/tree 2016		Average fruit weight (g) 2016		yield Mton/Acre 2015		yield Mton/Acre 2016		bins[#]/Acre 2016	
ROZA														
Spindle			72	A	19.16	A	269	B	16.07	B	28.73	B	71.97	B
V			49	B	13.64	B	281	A	28.43	A	40.87	A	102.40	A
		Sign.	***		***		*		***		***		***	
	G41		62		17.10		277		22.19		36.09		90.41	
	M9-Nic29		58		15.66		273		22.30		34.18		85.62	
	Sign.		NS		NS		NS		NS		NS		NS	
		bending	55	B	14.86	B	275		23.16		31.64	B	79.26	B
		click	66	A	17.84	A	275		21.34		38.67	A	96.88	A
		Sign.	**		***		NS		NS		**		**	
Training x rootstock			NS		NS		NS		NS		NS		NS	
Training x pruning trt			NS		NS		*		NS		NS		NS	
Rootstock x pruning trt			*		*		NS		NS		*		*	
Training x rootstock x pruning trt			NS		NS		*		NS		NS		NS	
SUNRISE														
Spindle			42	A	13.77	A	336	A	14.10		20.65		51.72	
V			23	B	6.78	B	299	B	15.20		20.33		50.94	
		Sign.	***		***		***		NS		NS		NS	
	G41		29		9.61		328	A	13.00		19.19		48.08	
	M9-Nic29		36		10.95		307	B	16.50		21.78		54.58	
	Sign.		NS		NS		*		***		NS		NS	
		bending	23	B	7.81	B	324		15.00		14.86	B	37.23	B
		click	42	A	12.75	A	311		14.40		26.12	A	65.43	A
		Sign.	***		***		NS		NS		***		***	
Training x rootstock			NS		NS		NS		NS		NS		NS	
Training x pruning trt			NS		NS		NS		NS		NS (5.9%)		NS (5.9%)	
Rootstock x pruning trt			NS		NS		NS		NS		NS		NS	
Training x rootstock x pruning trt			NS		NS		NS		*		NS		NS	

1 bin = 880 lb (by Tom Auvil)

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

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

In Sunrise (2690 fruit graded) the "green spot" disorder had an incidence of 31.6% on the graded fruit at harvest followed by limb rub and bruises (17%), while sunburn hit only 8% of fruit graded. Ten percent of the fruit presented a little bit of russet (10%) and 27% of fruit had a "leaf shade" breaking the red color coverage. Other defects were not present at noticeably high frequencies. The percentage of cull was on average 28.8% of all harvested fruit, while 58.2% classified as fancy (WFCY) and

13.0% as extra-fancy (WXF). Fruit with minor and acceptable defects not impacting the flesh were considered fancy while perfect fruit in terms of color in addition to acceptable defects were classified as extra-fancy.
Among the 8 combinations under comparison, the one that reported highest percentage of cull was spindle-G41-bending (45%) probably due to the high incidence of green spot (51.1%), but at the same time this combination showed the highest percentage of extra-fancy fruit (19%) followed by spindle-Nic29-bending (18%) and spindle-Nic29-click (17%). Both combinations of G41 in V reported the lowest percentage of extra-fancy fruit (5% approx.), while both combinations of Nic9 in V had double the amount of extra-fancy fruit (approx. 11%). The average of fruit affected by poor color is below 9%, but both combination of G41 in V showed higher values (data not shown).

Fruit quality harvest 2015 T0 (=1 month after harvest) and T1 (=6 months of cold air storage)
Spindle subsample was larger (by weight) than V system both at T0 and T1 (both Sunrise and Roza). However, V system fruits in Sunrise experienced higher percentage weight loss during cold air storage than spindle, while in Roza was opposite. Spindle fruits showed higher percentage of overcolor than V system fruits at T0, but not a significant difference in red intensity (both Sunrise and Roza).
Fruit harvested from spindle showed lower I_{AD} values than V system fruits both at T0 and T1 but only in Sunrise. Additionally, Sunrise spindle fruit I_{AD} values fell more dramatically during cold storage than V system fruits, while no differences were observed for Roza. Spindle fruits in Sunrise were more firm and had higher soluble solids content (SSC) than V fruits both at T0 and T1, while in Roza had showed similar behavior only at T1. No differences in starch, dry matter (DM), pH and titratable acidity were found between training systems at T0 and T1 in both locations. Not many significant differences emerged in general between rootstocks, in particular at T0. Nic29 fruits had higher soluble solids content (SSC) than G41 fruits at T0 (only in Sunrise). G41 fruits were more firm than Nic29 fruits at T1 (mainly in Roza), while not significant at T0. After 6 months of cold storage, "click" fruits had lower I_{AD} values than "bending" ones. "Click" pruning technique yielded fruit with more intense red color than "bending" at T0 (only in Sunrise), had higher soluble solids content (SSC) and higher dry matter % than "bending" fruits both at T0 (both locations) and T1 (only Sunrise). DM% was successfully predicted as a significant level by Felix F750 produce quality meter after 6 months cold storage in Sunrise, though not significantly in Roza. Together, spindle-Nic29-Click fruits had the highest soluble solids content at T0 over all other combinations and among the highest in T1 in Sunrise.

Objective 2: Identify growth and productivity characteristics on bi-axis (by Musacchi S., Lewis K.).
The mechanical winter pruning in Roza in bi-axis plots showed a reduction in pruning time of 93% with 1h:26min/A versus 21h:47min/A for the hand pruned.
The amount of wood removed mechanically in winter was only 45% of the total removed by hand. The percentage of wood removed is higher than in "previously clicked-spindle". This confirms fruiting wall as the best pruning method to use in conjunction with mechanical pruning (data not shown).
Combinations of mechanical plus hand fine pruning could be a good compromise.
In the comparison among 4 combinations, G41 by hand was the most time consuming and significantly different from Nic29 (5 hours/A less), while both mechanical combinations were similar.

Mechanical pruning in biaxis reported a higher number of fruit per tree but no significant difference was found in the production per tree, while the hand pruning showed higher average fruit weight than the mechanical ones, only due to bi-axis-Nic29 (ns in G41) that reported higher yield per tree and per Acre than hand treatment. The lowest yield per Acre was registered by bi-axis Nic29-hand, but the best in terms of average fruit weight. The highest percentages of cull were reported for both the combinations of bi-axis mechanical with up to 49%, but bi-axis-Nic29-Mech reported also the highest % of extra fancy fruit (12%). Defects tendencies don't show any particular problem that could be caused by mechanization. In the mechanized combinations the leaf shade seems highly reduced in comparison to the hand pruned combinations.

Objective 3: Conduct an economic analysis of WA 38 production (by Gallardo K.).

Table 3. Estimated pruning time (hr/tree) for WA38 during the fourth year of production (2016) given the interactions of various training systems, rootstocks and pruning techniques, and in comparison with the baseline ('Fuji').

Location	Treatments	Winter Pruning[1] (hour/tree)		Labor cost ($/tree)
Roza	Spindle, G41, Bending	0.0109	A[2]	$0.1306
	Spindle, G41, Click	0.0134	A	$0.1609
	Spindle, M9-NIC29, Bending	0.0142	A	$0.1702
	Spindle, M9-NIC29, Click	0.0141	A	$0.1698
	Angled, G41, Bending	0.0079	A	$0.0952
	Angled, G41, Click	0.0094	A	$0.1130
	Angled, M9-NIC29, Bending	0.0085	A	$0.1023
	Angled, M9-NIC29, Click	0.0094	A	$0.1125
Sunrise	Spindle, G41, Bending	0.0118	A	$0.1410
	Spindle, G41, Click	0.0120	B	$0.1438
	Spindle, M9-NIC29, Bending	0.0077	A	$0.0925
	Spindle, M9-NIC29, Click	0.0078	A	$0.0933
	Angled, G41, Bending	0.0092	A	$0.1102
	Angled, G41, Click	0.0130	B	$0.1561
	Angled, M9-NIC29, Bending	0.0042	A	$0.0504
	Angled, M9-NIC29, Click	0.0070	B	$0.0842
Baseline	'Fuji', Spindle, M9	0.0138		$0.1656
	'Fuji', Angled, M9	0.0103		$0.1236

[1]Winter pruning in March. Estimated time takes into account the number of persons involved (winter pruning – 2 people in Roza; 1 person in Sunrise). Labor rate is $12/hour.
[2]Different letters indicate numbers between treatments are statistically significant different at 10% level.

Overall, total pruning cost was the lowest with V, G41 and bending in Roza; and spindle, M9-NIC29 and bending in Sunrise. The total pruning cost was highest with spindle, M9-NIC29 and bending in Roza; and V, G41 and click in Sunrise. Results from the field trial were compared to pruning time and costs for commercial 'Fuji'. Pruning one acre of 'Fuji' trees in the fourth year takes on average 15 hours under either spindle or angled trellis system. Considering the density of trees for 'Fuji'-spindle (1,089 trees per acre) and 'Fuji'-angled (1,452 trees per acre), commercial pruning time ranges 0.01-0.014 hour per tree, and the cost ranges $0.12-$0.17 per tree. The total pruning cost for the WA38 ranges from $0.10 to $0.17 per tree in Roza, and $0.05 to $0.16 per tree in Sunrise (see table 3).

For the estimated yield, production costs and returns, of the different WA38 treatments, in the fourth year, the per-acre gross yield and net yield of commercial 'Fuji' are 35 bins (in Roza) and 28 bins (in Sunrise). Yields in Roza were higher than the fourth year 'Fuji' yields across all treatments. In Sunrise, WA38 yields were higher than 'Fuji' under the spindle system with G41 and click, and spindle system with M9-NIC29 and click; and lower than 'Fuji' under all treatments of the angled system. See table 2 (Roza) and table 3 (Sunrise). When considering the different prices depending on the fruit sizes of WA38, the gross margin is highest under the spindle system, M9-NIC29 and click in both Roza and Sunrise. On the other hand, when the different prices according to fruit grade are considered, the highest gross margins are given by the angled system, M9-NIC29 and click in Roza, and the spindle system with M9-NIC29 and click in Sunrise.

CONTINUING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project Number: AP-16-101

Project Title: Reducing scald after long-term CA storage

PI:	David Rudell	**Co-PI**:	James Mattheis
Organization:	USDA-ARS, TFRL	**Organization**:	USDA-ARS, TFRL
Telephone:	509 664 2280 (ext. 245)	**Telephone**:	509 664 2280 (ext. 249)
Email:	David.Rudell@ars.usda.gov	**Email**:	James.Mattheis@ars.usda.gov

Budget: **Year 1**: $30,690 **Year 2**: **$63,095** **Year 3**: $72,508
Collaborators: Loren Honaas, Girish Ganjyal, and Ines Hanrahan

WTFRC Collaborative expenses:

Item	2016	2017	2018
Salaries			
Benefits			
RCA Room Rental	$6,300	$6,300	$6,300
Total	$6,300	**$6,300**	$6,300

Footnotes: Costs for 1 RCA room

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

Item	2016	2017	2018
Salaries	$18,338	$39,004	$41,344
Benefits	$6,052	$12,871	$13,644
Miscellaneous *		$11,220	$11,220
Total	$24,390	**$75,695**	$66,208

Footnotes: One-third of instrument service contract

OBJECTIVES:

1. Identify rapid, stress provoking, at-harvest treatments that reduce scald levels during a prolonged supply chain.

2. Validate changes in peel chemistry as indicators of efficacy for stress-based scald treatments.

3. Determine how at-harvest treatments that provoke stress impact other fruit quality factors.

4. Determine if post-storage reduction of ethylene action is a feasible post-storage scald control technique.

Goals and activities for the next year:
Expand temperature study to include more conditioning temperatures and time points with or without stress treatment including intermittent warming. Follow up studies optimizing stress treatment as well as conditioning/stress treatment combinations will be another focus. A final focus for the new season will be investigating the impacts of reducing ethylene and optimizing the storage environment for scald reduction during a long supply chain following long-term effective CA storage. Focus will be on crop-protectant restrictive storage regimes.

SIGNIFICANT FINDINGS:

1. Physical stress most effectively controlling superficial scald- must occur prior to 1 week of cold storage.
2. Stress responses that lead to clearing may require warmer temperatures prior to cold storage.

METHODS:
Equipment and Cooperative Summary: Stress treatments (excluding impingement drier) as well as fruit quality, tissue sampling, processing and analysis of SRABs using analytical instrumentation (gas and liquid chromatography-mass spectrometry) will be performed at ARS-TFRL, Wenatchee. Treatment using the impingement drier was performed at BSYSE, WSU-Pullman in collaboration with Drs. Ganjyal and Hanrahan. Pressure treatment was performed by Dr. Honaas and staff at the ARS-Wenatchee location shop. Storage experiments will be performed both at ARS-Wenatchee and in Stemilt RCA storages. New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

Year 1 (includes activities outlined for Objectives 1, 2, and 3)

Temperature conditioning
Granny Smith apples will be conditioned at 68 °F for 0, 3, 5, and 7 days prior to storage in 0.7 or 1 % O_2 (0.5% CO_2) for 6 months. We expect to perform the initial experiment beginning in Year 1. Treatments in subsequent years will focus on testing fruit from multiple orchards and refining treatment duration to those that have the least impact on overall quality and still significantly reduce scald incidence during the simulated 3 month post-storage supply chain. Eventually, by year 3, we expect to focus on the best combination(s) of stress treatment and temperature treatment.

Granny Smith apples were conditioned at 68 °F for 2 and 7 days prior to storage in air or 1 % O_2 (0.5% CO_2) for up to 6 months. (All of the stress treatments below included fruit that went immediately into storage and a group that were left at 68 °F for 2 days). Another treatment included fruit that were immediately placed in 33 °F for 1 week, then 2 days at 68 °F, then back to 33 °F air or

1 % O_2 (0.5% CO_2) for up to 6 months. Fruit stored in CA will undergo scald and quality evaluation during and following a 3 month supply chain simulation at 33 °F.

Stress amendment treatment
Preliminary experiment: To indicate the time required for scald-free zones to form around a bruise, the impact of physical stress on scald was assessed using a standardized impact force to equally bruise Granny Smith apples at different time points beginning and then 2, 4, and 8 weeks after putting fruit in cold air storage at 33 °F. To bruise fruit, a 5.6 g ball-bearing was dropped directly onto two positions on the unexposed side of the fruit in a glass tube to assure an equidistant (38 cm) drop and therefore equal force. The initial indentation was 0.58 cm. Scald incidence and severity as well as diameter of scald free area around each bruise was evaluated monthly until 6 months.

A variety of other stress *treatments were applied at harvest with the idea of improving the effectiveness and/or reducing the duration of effective temperature acclimation of Granny Smith apples. Following treatment, apples will be placed immediately in 33 °F air or CA (0.6% O_2: 0.5% CO_2) storage or left at 68 °F for 2 d before being placed into CA. Subsequent years will focus on optimizing those treatments with the best supply chain quality outcomes. Fruit from the dry heat or pressure treatment were not stored in CA.

> *Treatments in Year 1 for the first experiment included hot water drenching, nitric oxide fumigation, H_2O_2 solution drenching, and superoxide solution drenching. For hot water treatment, fruit will be submerged in 118 °F water for 3 min. For nitric oxide fumigation, apples will be treated for 4 h with an initial concentration of 100 ppm ·NO will be injected into a treatment chamber containing apples in air at 68 °F. For H_2O_2 treatment, apples will be submerged for 60 min in a solution containing 3% H_2O_2. For superoxide treatment, apples will be submerged in a solution containing 100 ppm superoxide-- $O_2^{\cdot -}$ (generated using KO_2) for 60 min. Additional experiments used O3, dry heat treatment, and high atmospheric pressure. For O_3 treatments, apples were exposed to 1, 10, or 50 ppm O_3 for 60 min as well as 10 ppm for 0.5, 2, and 4 h in addition to 1 h. For high pressure treatment, a small pressure vessel was designed and compiled to apply up to 60 psi to batches of 10 fruit for 1 min. An impingement drier in Pullman was used for the heat treatment.

Quality and scald incidence assessment
Quality assessment (Firmness, TA, soluble solids) will be performed at harvest, following treatment, and upon removal from storage throughout the simulated supply chain period (held at 37 °F): at harvest, 3 months, 6 months, and 9 months for the temperature conditioning and O_3 experiments. Incidence of superficial scald and other defects will be identified and quantified with all quality assessments as well as repeatedly on all samples that are not destroyed.

Tissue sampling for metabolic profiling and SRAB monitoring
Peel has been and will be sampled following each stress treatment and/or temperature conditioning combination and then following 14, 30, 60, 90, 120, and 180 days after cold storage inception. Metabolic profiling will be performed on these samples with the idea of continuing to associate risk, as impacted by stress, with changes in peel chemistry. We will use untargeted metabolic profiling to better understand how peel chemistry changes with respect to different stresses imposed to find chemistries that indicate a treatment is effective at controlling scald as well as common changes associated with other postharvest disorders.

Existing scald risk assessment biomarkers (SRABs) are continually monitored by extracting wax and estimating levels using a spectrophotometer as outlined as an outcome of our previous project. These will be monitored for every treatment at least monthly for up to 4 months. This process will further test the existing risk assessment under these conditions.

RESULTS AND DISCUSSION:

Physical damage assessment

Physical injuries to the fruit surface including bruises, limb rubs, sunburn, as well as disorders that appear prior to or soon after harvest such as bitter pit can develop regions of the peel within and/or immediately around the pre-existing injury that do not develop scald symptoms (Fig. 1). These regions are typically attributed to some, yet undefined, form of innate immunity triggered by the prior injury that renders the peel insensitive to the chilling stress during the first month of cold storage that leads to superficial scald development months later. While it can be assumed that the stress initiating the immunity occurs prior to the chilling stress that leads to scald, which occurs cumulatively between cold storage imposition and 1-2 months in air storage, the actual best time when a stress most effectively leads to immunity is unknown. This set of experiments assessed whether the injury must occur prior to storage and how long into harvest would injury form a scald-clear zone. Injury was assessed at 4 and then 6 months. These results (Fig. 2) and other similar tests (not shown) indicated the immunity is initiated only at harvest and less so 1 week following cold storage initiation. Evidence indicates that physical stress (limb rubs, finger bruises, and the other aforementioned injuries) must occur prior to or very near the beginning of cold storage and that the resulting immunity is most likely an active metabolic process that may best arise during warmer temperatures.

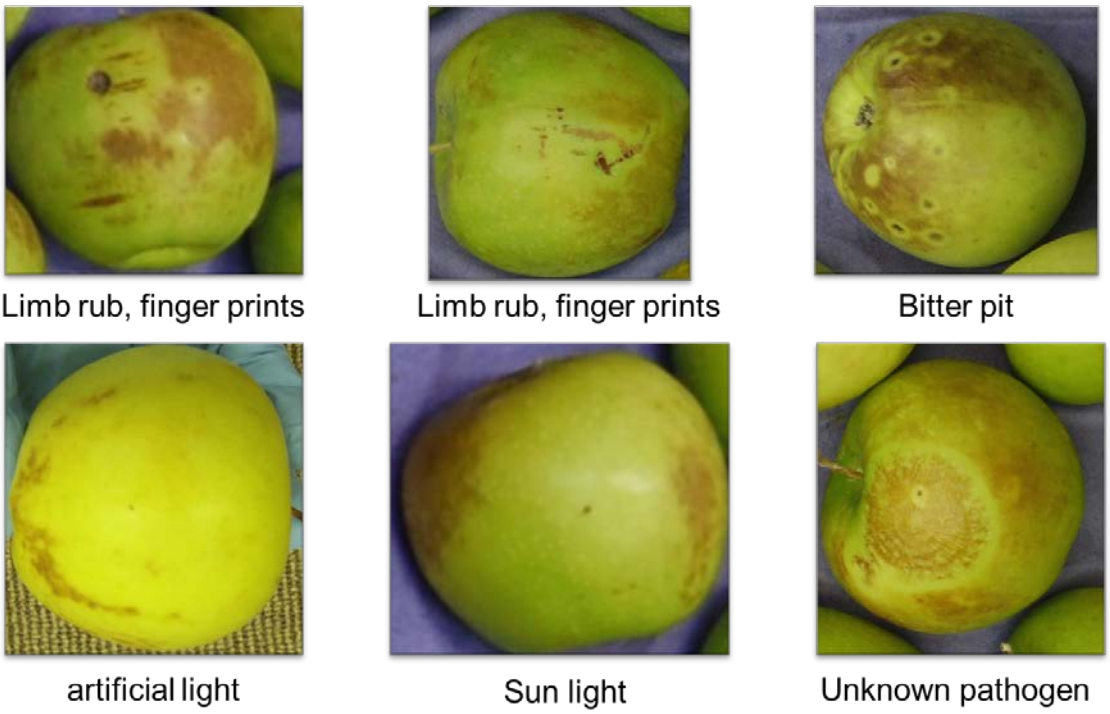

Fig. 1. Examples of some pre-storage injuries that can inhibit superficial scald symptoms from developing.

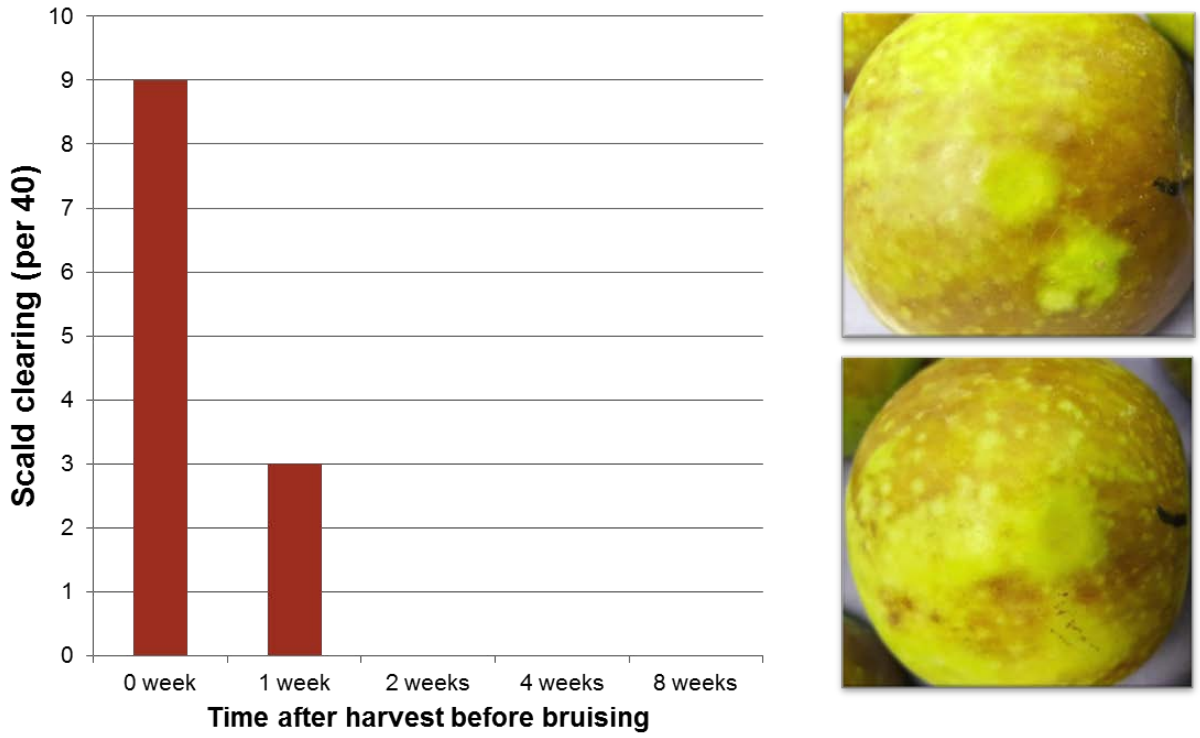

Fig. 2. Incidence of "scald-free" region (right) after 6 months 33 ºF air storage in and around peel intentionally bruised at 0, 1, 2, 4, and 8 weeks following cold storage inception.

Other stress response treatments

Our findings from the preliminary experiment indicate that some form of stress similar to bruising, wounding, sunburn, or bitter pit at some time prior to cold storage could provide some degree of scald protection. Of course, any stress employed as a control measure could not visibly injure the fruit and would optimally have no impact on internal quality. Consequently, for the current season, we have focused on screening a number of known chemical and physical stressors that might meet these criteria. These focused on gaseous treatments expected to provoke oxidative stress (O_3, $\cdot NO_2/\cdot NO$, H_2O_2, and KO_2) as well as non-bruising physical stressors at this key time point prior to storage. In each case, fruit were either placed immediately into 33 °F or left for 2 days at 68 °F to indicate if any process leading to scald immunity musters more effectively at warmer temperatures.

While these experiments started with the new season and scald symptoms have not yet appeared by the time of this report, already some evidence indicates that stress response processes were mustered during this 2 d adaptation period prior to cold storage. O_3 was applied for 1 h to Granny Smith at 3 different rates prior to cold air and CA storage. As of 3 months storage, the 50 ppm treatment developed typical O_3 damage (a lenticel blotch) but only on fruit that was placed in air storage immediately (Fig. 3). This may be indicative of a stress response process that transpired within the first few days following O_3 treatment but was impeded by chilling temperatures, resulting in cell death around the lenticels. As of 3 months, neither the 2 d acclimation period nor the O_3 treatment influenced fruit firmness (not shown).

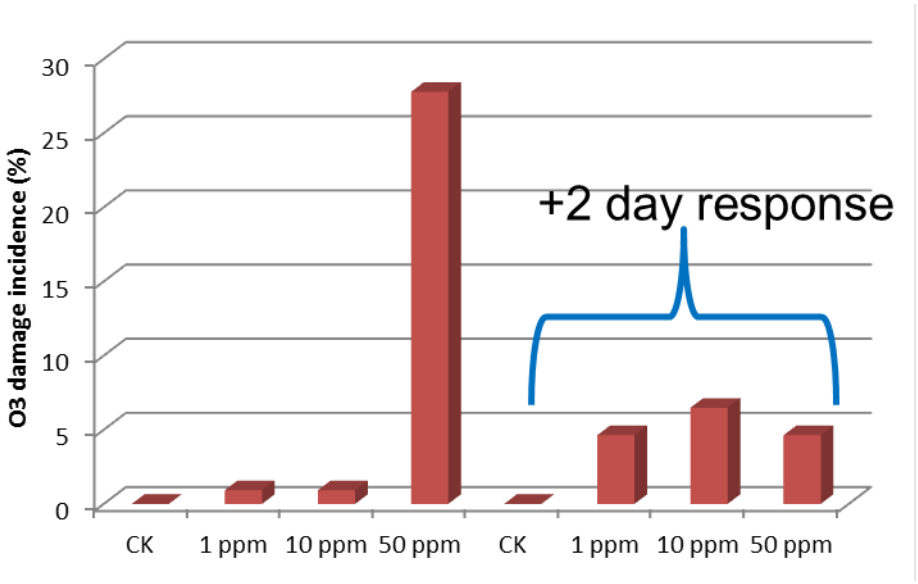

Fig. 3. O_3 injury incidence at 3 months 33 ºF air storage for fruit immediately placed in storage (left) or held for 2 d at 68 ºF following O_3 treatment prior to air storage (right). O_3 damage was not observed in the same fruit stored in CA by the time of this report.

Other evidence, including from physical injury and also past temperature acclimation studies aimed at ameliorating superficial scald, indicate any response to a beneficial stressor is most effective if the fruit is allowed to remain at a warmer temperature following treatment. Intermittent warming treatment (chilling apples for 1 week or less, then warming, then placing into long term storage) is particularly effective, as demonstrated by numerous studies, for possibly just this reason.

Next year, we plan to begin to optimize timing, including stress response timing for any stressor that shows promise. We will focus on optimizing temperature acclimation regimes as proposed with the focus on the supply chain following long-term CA storage.

CONTINUING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project Number: AP-16-102

Project Title: Risk assessment for delayed sunburn and sunscald

PI: David Rudell
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Co-PI: Carolina Torres
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Telephone: +56 9 6847 0541
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Budget: **Year 1**: $67,427 **Year 2**: **$70,865** **Year 3**: $72,595

Collaborators: Omar Hernandez

Other funding sources

Agency Name: CONICYT, Chile (proposed)
Amt. awarded: $88,700 (total over 3 years)
Notes: Funds for supplies and materials, travel, and analytical services.

WTFRC Collaborative Expenses: None

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

Item	2016	2017	2018
Salaries	$40,757	$43,342	$44,620
Benefits	$13,450	$14,303	$14,755
Wages			
Benefits			
Equipment			
Supplies			
Travel	$2,000	$2,000	$2,000
Miscellaneous*	$11,220	$11,220	$11,220
Plot Fees			
Total	$67,427	**$70,865**	$72,595

Footnotes: One-third instrument service contract

Objectives:

1. Identify changes in apple peel chemistry associated with response to light prior to and during cold storage.

2. Determine if changes in peel chemistry are specifically indicative of delayed sunscald and other sun-related postharvest peel disorder risk prior to symptom development.

3. Develop protocols to establish tissue viability before and during cold storage.

Goals and activities for the next year:
Refine studies in the orchard by more carefully analyzing light interception and even delimiting light from regions of the peel to more accurately assess which chemistries are most indicative of aberrant light exposure potentially leading to postharvest issues. Begin to analyze the capacity of peel exposed to high light to produce energy during the first part of storage.

SIGNIFICANT FINDINGS:

3. Peel appearance (symptoms) change in different ways during storage depending upon cultivar.
4. Aroma chemistry changes differentially depending upon pre-harvest sun exposure indicating continued oxidative stress on the exposed side of the fruit even following cold storage.

Methods: (see outline in Table 1)

Equipment and Cooperative Summary: Fruit quality, tissue sampling, processing and peel chemistry analysis using analytical instrumentation (gas and liquid chromatography-mass spectrometry) will be performed at ARS-TFRL, Wenatchee. UV-vis reflectance spectral deconvolution and modelling is being performed by Dr. Torres. Both storage experiments will be performed at ARS-Wenatchee . New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

2016-2017 (primarily Objective 1)
A summary progress on activities is included in Table 1.

The influence of pre-harvest light environment alone and during the transition to cold storage on peel metabolism is different among apple cultivars. We are analyzing apple peel chemicals to include our full metabolic analysis but with special focus on lipids, oils, and waxes and other oil-soluble metabolites that may be most impacted by light and temperature together. To begin to address this, we are analyzing peel chemistry of 4 apple cultivars that are differentially impacted by the combination of high light and chilling. We harvested approximately 360 apples each of 'Granny Smith' (9/20/16), Gala (8/5/2016), 'September Fuji' (8/23/2016), and 'Honeycrisp' (8/22/16) at around commercial harvest. Orchards were selected for smaller trees with high sun exposure. Fruit that was obviously exposed in clear contrast with back side with respect to exposure was picked and brought back to the laboratory for subsequent same-day sorting, maturity assessment, sampling, and storage. Exposed sides of the fruit were marked on the stem end upon picking. Once back in the lab, apples were further sorted to obtain the best front to back contrasts and cull any fruit that were not exposed enough on the exposed side or too exposed on the backside. Fruit with sunburn above the

median level representing that orchard were retained in their own category. Starch index and internal ethylene were assessed at harvest. Fruit were stored in air at 33 ºF.

At 0, 2, 4, and 8 weeks (and then monthly until 6 months) sun damage incidence was monitored on Gala, Fuji, and Honeycrisp and categorized on Granny Smith by rating the exposed side 0-4 (Table 1). At 0, 2, 4, and 8 weeks (and, finally, at 6 months), color was also monitored on both sides using a Minolta colorimeter as well as peel sampled. One goal is to carefully characterize visual differences in any delayed symptoms among cultivars and changes are photographically recorded on the front and back of the fruit for later analysis. Peel samples taken from those time points have already been analyzed for a subset of metabolites with the expectation that the full analysis of over 800 metabolites will be completed by spring. Data analysis of metabolite data has focused on determining differential changes between the exposed and unexposed sides of the fruit upon chilling stress, possibly linking specific changes in peel chemistry with specific delayed conditions.

Table 1. Sun damage severity rating table for Granny Smith. A rating of 4 constitutes delayed sunscald.

Rating	Description
0	Green (no damage)
1	Sun (yellow)
2	Blush
3	Darkening red
4	brown

Sunscald prediction model and UV-vis reflectance characterization

A model to predict delayed sunscald of Granny Smith based on the degree of sun exposure at harvest has been developed for Chilean apple producers by the Torres laboratory. To test this model and, possibly, work towards adaptation to our climate, 90 fruit from 5 different Granny Smith lots were sampled, the degree of sun exposure of each fruit cataloged and, then, these values entered into the mathematical model which, in turn, generated a % sunscald prediction. Fruit were then placed into 33 ºF air storage. The final sunscald evaluation will be following 4 months of storage. Results will be compared to predictions.

Ten other Granny Smith lots were picked from bins for analysis using a UV-Vis reflectance spectrometer at harvest. This work was performed by a visiting collaborator from the Torres Laboratory. Again, fruit were immediately placed in 33 ºF cold storage and final sunscald ratings will be at 4 months of storage. The UV-Vis spectrum from 108 fruit from each lot will be added to data from Chile to determine if this technique is effective, and, if so, which wavelengths can be best monitored to assess sunscald risk.

RESULTS AND DISCUSSION:

Evaluation of the sun damage incidence did not change for the first 3 months of storage of Gala, Fuji, Honeycrisp, or Granny Smith (Fig.1). In Fuji and Honeycrisp there were changes of symptom appearance (Fig. 2), although these changes were more towards a “muddy” background to even greening resulting from anthocyanin (red color) loss much like “stain”. Sunscald severity (0-4) of Granny Smith demonstrates the transition of blushed or sunburned sides of the fruit to more severe sun damage, including delayed sunscald, over the first 3 months of storage (Fig. 3). These changes and the timing of the changes in every one of these cultivars reference those from multiple previous

studies. Delayed sunscald is typically the progressive darkening or browning of the exposed side and is not thought to be related to cold stress but, rather, the continuation of the effects of solar irradiation well into storage. However, stain is a combination of irradiation and chilling stress, as it can be reduced using cold acclimation techniques at the start of cold storage. Honeycrisp and Fuji can develop stain in the Washington climate and Gala can in the Maule, Chile climate. Granny Smith develops delayed sunscald in both climates.

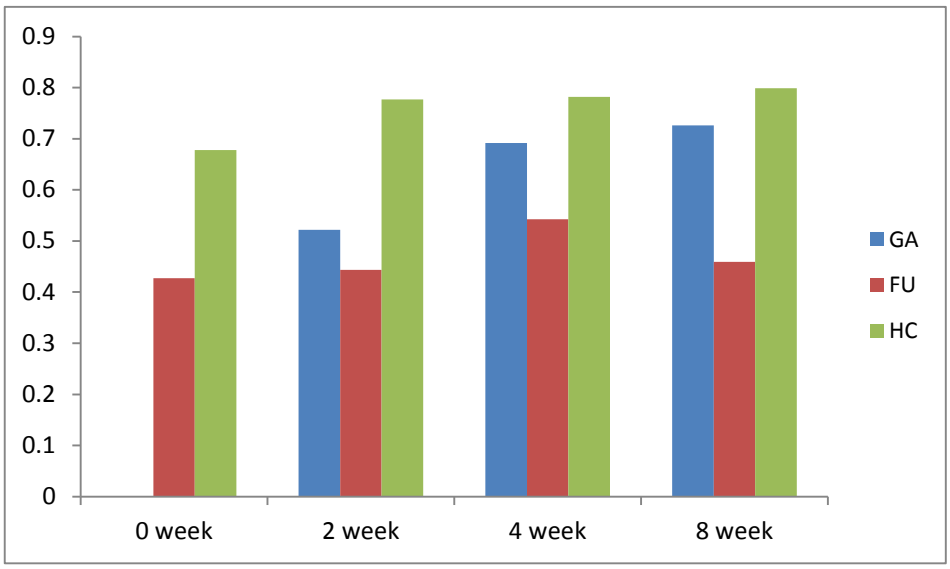

Figure 1. Incidence of sun damage at harvest and during the first 8 weeks of storage. Sun damage incidence remained largely unchanged for Gala, Fuji, and Honeycrisp, instead changing in appearance where existing symptoms and/or surrounding areas acquired a muddy appearance.

Fig. 2. Sun damage appearance changing on a Fuji apple. Photos are in chronological order from left to right: 0, 2, 4, 8, and 16 weeks. Typical of the Fuji sun injury during storage, symptoms typically develop a muddy color around the periphery, sometimes forming defined borders to form what is called “stain”. Honeycrisp and Gala can also get this disorder in certain climates.

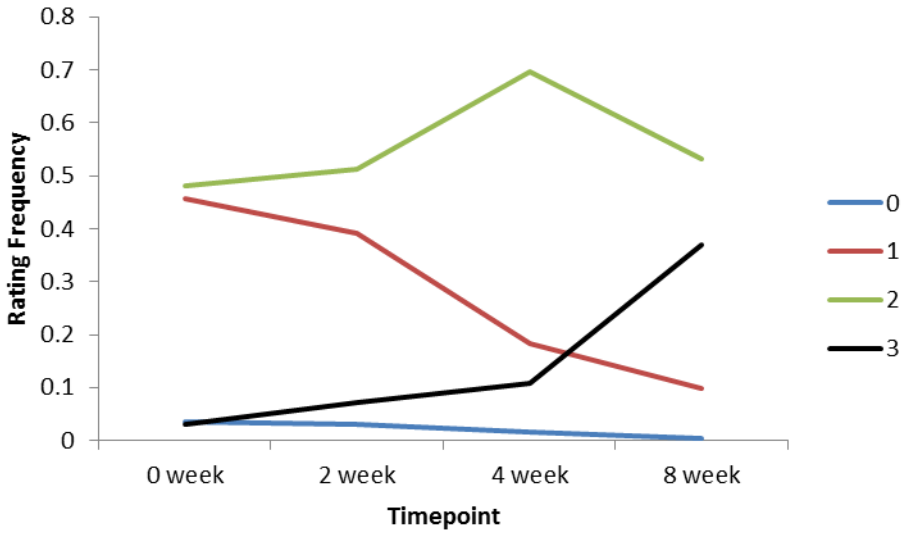

Fig. 3. Sunscald severity (0-4) (Table 1) on Granny Smith as rated during the first 8 weeks of 33 ºF air storage. Sunscald severity had already worsened between 2 and 4 weeks as can be noted by the decrease in fruit in category 1 and increases in categories 2 and 3. Typical Granny Smith sunscald symptoms (category 4) were only beginning by the time of this report and were not included in this figure.

To investigate any difference in chemical composition during the first 6 months of storage caused by the combination of cold stress and sun exposure, peel from either side of each cultivar was sampled during storage as outlined above. At the time of this report, 1 of 3 analyses, an analysis of volatiles, including natural chemicals composing apple aroma, was completed on 0-2 month samples from every cultivar. As expected, the volatile profile among cultivars was substantially different (not shown), so exposed and unexposed peel of individual cultivars over time had to be compared first to determine any differences. Using a statistical analysis that finds the main influence of experimental factors (treatments or differences in appearance we expect or employ in our tests), we were able to determine where peel was different between the front and back even though only one analysis of natural peel chemicals has not been completed.. Gala, Fuji, and Honeycrisp peel volatile profile were all different depending upon pre-harvest sun exposure while Granny Smith, with the exception of a few compounds, was not different (Fig. 4). Unlike the other cultivars, the Honeycrisp profile was similar between sides at harvest but differentiated during the first part of storage. Compounds associated with differences during cold storage included those that are associated with stress during high light stress, suggesting oxidative events responsible for the genesis of these compounds continue after the fruit have been removed from the orchard. It is expected that many more chemistries characterizing the transition into cold storage will be discovered once our entire analysis is completed. We also expect a further differentiation of severe sunburn reaction to chilling stress upon our 6 month evaluation.

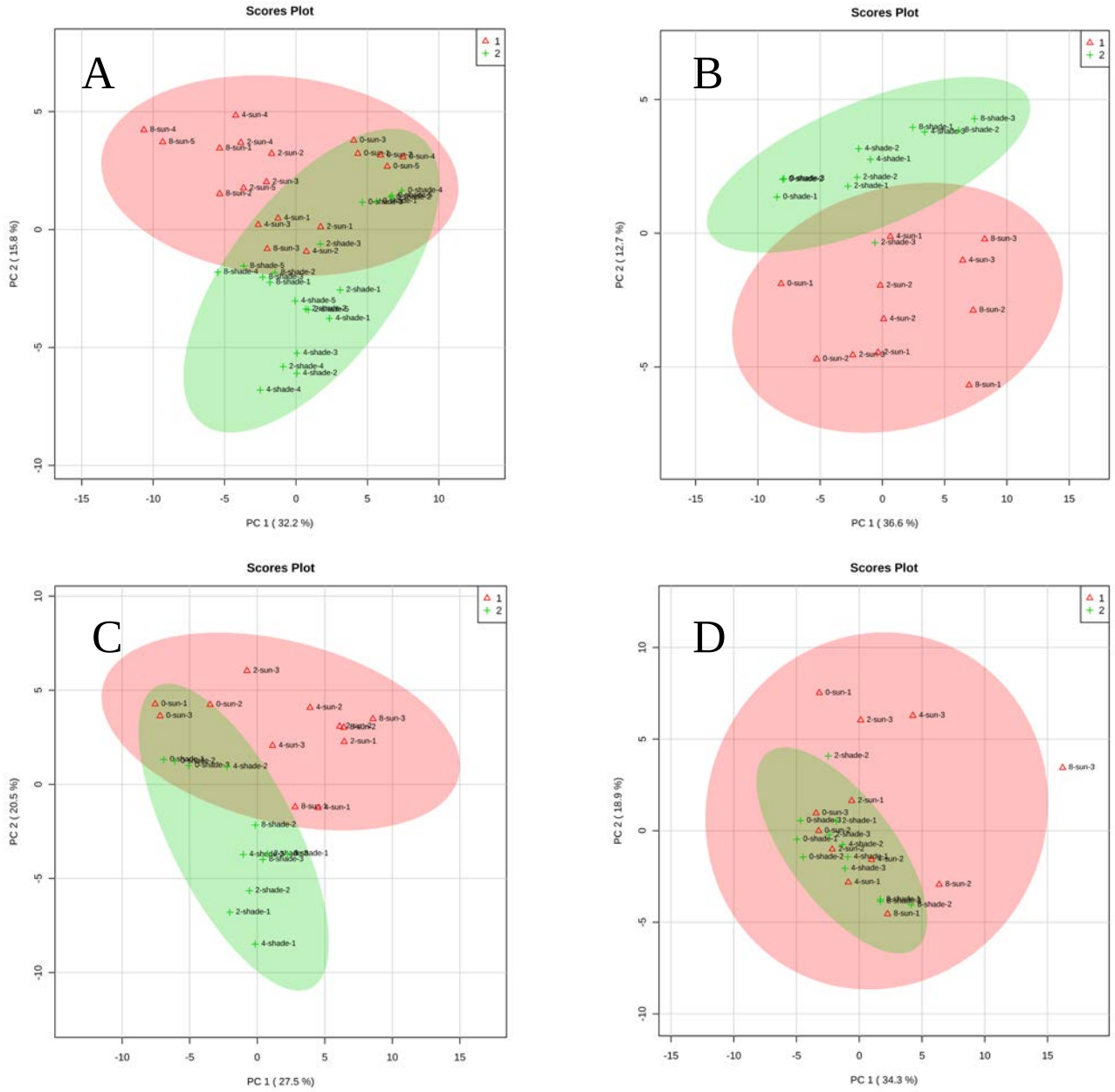

Fig. 4. Gala (A), Fuji (B), Honeycrisp (C), and Granny Smith (D) apple peel volatile aroma chemicals from exposed (red) and shaded (green) sides over the first 8 weeks of air storage (33 ºF). Each data point is a summary of the multiple volatile aroma compounds that comprise each cultivar. Data indicate that, with the exception of Granny Smith, peel chemisty among these metabolites is different at harvest and actually diverges in Gala and Honeycrisp with storage duration.

Table 1. Project milestones with anticipated products of "Risk assessment for delayed sunburn and sunscald". Current year's progress is emboldened.

Objective		1: Identify changes in apple peel chemistry associated with response to light prior to and during cold storage.		
Hypothesis		Apple peel metabolism following cold storage imposition is altered by pre-harvest light exposure.		
Team	Months	Milestone	Anticipated Product	Progress/Changes
DR,JM	12	Multi-cultivar harvest and storage experiment	An assessment of chemical changes associated with light and chilling regardless of injury outcome.	**Storage experiments and phenotypic and untargeted metabolic evaluations underway.**
	24	No work planned		
	36	No work planned		

Objective		2: Determine if changes in peel chemistry are specifically indicative of delayed sun scald and other sun-related postharvest peel disorder risk prior to symptom development.		
Hypothesis		Relative peel content of a subset of natural peel chemicals will be indicative of sunscald risk.		
Team	Months	Milestone	Anticipated Product	Progress/Changes
	12	No work planned		
DR,JM,CT	24	Established chemical changes associated with sunscald risk	A list of biochemical pathways impacted by conditions that enhance sunscald risk.	
DR,JM,CT	36	Validated changes associated with sunscald risk	A list of metabolites most strongly associated with sunscald risk.	

Objective		3: Develop protocols to establish tissue viability before and during cold storage.		
Hypothesis		Develop a system to check tissue viability at different time points before and during cold storage.		
Team	Months	Milestone	Anticipated Product	Progress/Changes
	12	No work planned		
DR, CT	24	Completed metabolic rate assessment based on tissue condition	New system for assessing tissue specific metabolic rate within multiple pathways	
	36	No work planned		

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-16-104

Project Title: Evaluation of fungicide application methods for improved fruit quality

PI: Achour Amiri
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Co-PI: R. Karina Gallardo
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City/State/Zip: Puyallup/WA/98371

Cooperators: Gebbers Fruit, Northern Fruit, McDougall Fruit. Richard Kim (Pace Int. LLC), Tim Mowry (Decco), and Mike Sandman, Syngenta.

Total Project Request: **Year 1**: $77,258 **Year 2**: **$80,067**

Other funding sources: *None*

WTFRC Collaborative expenses:

Item	**2016**	2017
RCA Room Rental	6,300	6,300
Shipping	0	0
Plot Fees	0	0
Miscellaneous	0	0
Total	6,300	**6,300**

Budget 1
Organization name: WSU **Contact Administrator:** Katy Roberts; Joni Cartwright
Telephone: 509-335-2885; 509-663-8181 x221 **Email:** arcgrants@wsu.edu;joni.cartwright@wsu.edu

Item	**2016**	**2017**
Salaries[1]	42,820	**44 533**
Benefits	16,886	**17 562**
Wages[2]	2,592	**2,696**
Benefits	260	**270**
Equipment	0	**0**
Supplies[3]	4,000	**4,000**
Travel[4]	3,400	**2 590**
Miscellaneous[5]	1,000	**2 116**
Plot Fees	0	**0**
Total	70,958	**73,767**

[1] Salaries are for a PostDoc (Vikas Koundal, 1.0 FTE) at 40.1% benefit rate and a Research Associate (6.25% FTE). Benefits calculated at 32.65%.
[2] Wages are for an hourly person for 12 weeks.
[3] Supplies include Petri dishes and microbiological media for lab use
[4] Travel in Year 1 is for meeting with individual packers in Yakima and Wenatchee. Travel in Year 2 is for meetings with scientists and industry stakeholders in Wenatchee to report the data gathered and economic analysis.
[5] Miscellaneous include residue level analysis, poster and publication of study results.

OBJECTIVES

1. Evaluate the efficacy of the different methods used currently to apply fungicides to control major postharvest pathogens including artificial and natural infections.
2. Investigate (a) residue levels related to each method and (b) impact on fungicide resistance development.
3. Evaluate the effect of different rates of fludioxonil and pyrimethanil on efficacy, residue levels, and resistance risks.
4. Evaluate the economic impact of each application method by estimating costs and benefits.

SIGNIFICANT FINDINGS:

Trials were initiated in October 2016 at Gebbers Fruit, Northern Fruit, and McDougall. Only partial preliminary results have been obtained yet and the later do not permit to make conclusive remarks. Full data will be shared with the industry in spring of 2017 and in next year report to the commission.

METHODS

OBJECTIVE 1: *Evaluate the efficacy of the different methods used currently to apply fungicides to control major postharvest pathogens including artificial and natural infections*

Three sets of trials were initiated in October of 2016 at Gebbers Fruit (Brewster) on Fuji, at Northern Fruit (East Wenatchee) on Red Delicious, and at McDougall (East Wenatchee & Quincy) on Gala. To study the efficacy of different methods on artificially wounded fruit, Fuji, Red Delicious and Gala fruit were wounded near the stem-end and inoculated with 20 µl of a spore suspension of *Penicillium expansum* (blue mold), *Botrytis cinerea* (gray mold), *Neofabraea perennans* (most widespread causal species of bull's eye rot in WA), or *Phacidiopycnis washingtonensis* (Speck rot) at 5 x 10^3 spore/ml. Four replicates of 10 fruit each were used for each pathogen/spore concentration combination. Because *N. perennans* and *P. whashingtonensis* are not typical wound pathogens, we used non-wounded fruit inoculated 6 days prior to fungicide treatments.

Fruit (each rep in separate mesh bags) were drenched with labeled rates of two fludioxonil formulations i.e. Scholar SC (Syngenta) and Shield-Brite FDL 230SC (Pace Int) and with pyrimethanil (Shield-Brite Penbotec), fogged with pyrimethanil (ecoFog^{TM}-160, Pace Int) or fludioxonil (ecoFOG^{80}, Pace Int.), or aerosoled with fludioxonil (Scholar EZ, Syngenta). Treatments were applied within 24 hours post-inoculation. Fruit were stored in a controlled atmosphere and verified for disease incidence and severity after 3 months for blue and gray molds, 4 months for bull's eye rot and speck rot (slow growing diseases). Trials will be repeated at the same warehouses during the 2017-18 season.

To investigate naturally infected fruit, trials were conducted using the same cultivars at Gebbers Fruit (Fuji) and Northern Fruit (Red Delicious). Fruit from the same lots for each cultivars were split into three rooms and either drenched, fogged or aerosoled with the fungicides described above. Fruit are stored in a controlled atmosphere and will be verified for disease incidence and severity after 6 months of storage. Fifteen bins from each lot taken at different positions in the rooms will be packed at each facility and disease incidence and diversity will be determined.

OBJECTIVE 2: *Impact of application methods on residue levels and resistance development*

Plugs including the wounded area (non-inoculated) as explained in objective 1 were taken from fruit treated with different fungicides and are being analyzed for residue levels on wounds. Samples were

taken immediately after the fungicide was applied (for drenching) and after one week for fogged or aerosoled fruits and will be taken again after 4 and 6 months of storage. To evaluate residue levels on commercial fruit (natural infection), sample fruit consisting of 10 fruit from three bins (one top, one in the middle and one from the ground) will be used to estimate in-bins and between bins variability. Wound tissues or whole fruit (commercial trial) are immediately stored in a cooler and transferred to analytical labs for residue analysis. The analyses will be repeated on fruit of the 2017-18 season trials (objective 1).

Decayed lesions form wounded fruit (objective 1) will be transferred to agar plates and will be tested for sensitivity to pyrimethanil and fludioxonil using protocol used in the lab. The sensitivity of isolates used for wound inoculation is known and potential shifts in sensitivity will be detected after 4 or 6 months of storage.
In fall of 2017, we will use agar plates and fruit from objective 1. The sensitivity of each fungal isolate to each fungicide will be determined prior to the beginning of the experiment and will be expressed as the effective concentration necessary to inhibit 50% mycelial growth (EC_{50}). If decay is not observed on fruit after the aforementioned incubation periods, fruit will be stored for an additional period at same temperature and CA conditions followed by a seven day period at room temperature. New fungal isolates will be made from fruit showing decay and their EC_{50} values will be determined for each fungicide and compared to the original values to detect potential change in sensitivity.

OBJECTIVE 3. *Evaluate the effect of different rates of dry applications of fludioxonil and pyrimethanil on disease control, residue levels, and resistance development*

Because no commercial room or RCA room (Stemilt) were not available, this objective will be conducted in 2017 at Pace and Decco facilities in Yakima. We will test the effect of reducing the rate of Flud and Pyr applied through fog or aerosol on efficacy and residue levels. Trials will be conducted in small cold rooms at Pace facility (Wapato) and at Decco facility (Yakima). An experiment will be conducted on wounded and inoculated fruit exactly as described in objective 1. For the semi-commercial trial using natural infections, five fruit bins will be used per room. Wounded and unwounded fruit will be placed in same room and fogged with pyrimethanil (ecoFog™-160, Pace Int) or fludioxonil (ecoFOG80, Pace Int.), or aerosoled with fludioxonil (Scholar EZ, Syngenta) at full, 75, and 50% label rate. Fruit will then be stored in a CA room at 1°C (33°F) for 3 months (wounded fruit) and 6 months (for unwounded fruit) before checking incidence and severity as explained in objective 1. Residue levels will be assessed on wounds and whole fruit as explained in objective 2.

OBJECTIVE 4. *Evaluate the costs and benefits related to different application methods.*

A partial budget approach will be used to estimate the costs of aerosol and fogging fungicide applications at packinghouses. A partial budget provides a method of calculating the net change in profit that can be expected from a specific change in operational procedures. This change can have one or more of the following effects: (1) incur new or additional costs; (2) reduce or eliminate current costs; (3) receive new or additional revenue; and/or (4) lose or reduce current revenue. The cost centers of aerosol and fogging fungicide application would be the chemical and the application costs. We will compare these costs with the status quo drenching, which costs centers are drencher, fungicide waste, and labor. We will measure as potential new or reduced revenues the different losses expressed as decay rates of three application methods.

RESULTS AND DISCUSSION
Results will be made available in spring of 2017 and will be fully reported in the 2017-18 report to the WTFRC.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-16-105

Project Title: Improved risk assessment and management of apple postharvest diseases

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Cooperators: Multiple packers in Washington. Decco, Pace, Syngenta.

Other funding sources: None

WTFRC Collaborative Expenses: None

Total Project Request: **Year 1**: $67,121 **Year 2**: **$67,534** **Year 3**: $67,635

Budget 1
Organization name: WSU-TFREC **Contact Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885;509-663-8181x 221 **Email:**arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	2017	2018
Salaries[1]	39,600	41,184	42,831
Benefits	15,721	16,350	17,004
Wages	0	0	0
Benefits	0	0	0
Equipment[2]	2,000	0	0
Supplies[3]	8,000	8,000	6,000
Travel[4]	1,800	2,000	1,800
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	67,121	**67,534**	67,635

Footnotes:
[1] Salaries are for PostDoc (Ali Emran, 1.0 FTE) at 39.7% benefit rate.
[2] Equipment will include costs for an Air-Sampler to monitor the airborne fungal population.
[3] Include costs for lab supplies i.e. sampling tubes, microbiological media and plates for fungal growth and fungicide sensitivity tests.
[4] Travel to packinghouses and orchards.

OBJECTIVES

5. Conduct a multiyear statewide decay survey program to detect and quantify decay risks.
6. Evaluate risks related to fungicide resistance
    a. Develop rapid and accurate methods for fungicide sensitivity evaluation.
    b. Conduct a multiyear statewide resistance monitoring program on fruit and in storage room atmospheres.
7. Evaluate the impact of storage conditions on resistance development in the blue and gray mold pathogens in relation to fungicide type.
8. Evaluate sensitivity/resistance to pre-harvest fungicides i.e. newly registered ones and non-target sprays in major pome fruit pathogens.
9. Evaluate pathogenicity and fungicide sensitivity of *Lambertella* and *Phacidium* rots, newly reported in pome fruit in WA.

SIGNIFICANT FINDINGS

Objective 1: *Conduct a multiyear statewide decay survey program to detect and quantify decay risks*

- 164 grower lots were surveyed across 10 counties in central Washington between February and June, 2016. Blue and gray molds were predominant and accounted for 48 and 25% of total decay, respectively.
- The "export" quarantine pathogens were found at about 10% of total decay with Bull's eye rot being the most predominant one in this group. Speck rot (Phacidiopycnis) was att about 1% whereas Sphaeropsis was found very sporadically.
- The newly reported Lambertella rot, now known as Yellow rot was found in 34% of lots surveyed with an incidence ranging from 2 and 40% per grower lots. The newly reported pathogen, Phacidium rot, was not found this year.

Objective 2: *Evaluate risks related to fungicide resistance*

2-a- Develop rapid and accurate methods for fungicide sensitivity evaluation

- The multi-wells plate assay was not suitable for fungicide sensitivity test using the inoculum (spores) directly from the fruit because the majority of fungi tested required additional incubation time (several weeks) for sporulation.
- Instead, we used 150 mm (5.9 inches)-diameter plates which allow to test for 60 isolates per plate at once and therefore to screen a high number of isolates quickly.

2-b- Conduct a multiyear statewide resistance monitoring program on fruit and in storage room atmospheres

- A total of 2,000 isolates of *Penicillium expansum* (blue mold) and 1,700 isolates of *Botrytis cinerea* (gray mold) were collected from different packinghouses. These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), pyrimethanil (Penbotec) and fludioxonil (Scholar) for both *P. expansum* and *B. cinerea* and to pyraclostrobin + boscalid (Pristine) and fluxapyroxad (Merivon) for *B. cinerea* only.
- Resistance of *P. expansum* to thiabendazole (Mertect) and pyrimethanil (Penbotec) was found in about 60% and 52% of the 164 lots surveyed, respectively.
- Resistance of *B. cinerea* to pyrimethanil, TBZ, Pristine, and fluxapyroxad (Merivon) was found in 55%, 46%, 38% and 15% of the 164 lots surveyed, respectively.

- Populations of *B. cinerea* and *P. expansum* with reduced sensitivity to fludioxonil were found in 51% and 46% grower lots, respectively. These populations are controlled by the label rate of the fungicide. However, continuous use of Scholar and related products can cause these populations to become actually resistant.
- A decay and resistance profile was created for each grower lot surveyed and results were sent to the participating packers and growers before the beginning of the new season to allow them change strategies and spray regimes based on decays and resistance found at their locations.
- We plan to conduct a decay and resistance monitoring in 2017 to detect variabilities among seasons and the impact of spray regime changes on resistance frequencies and distribution.

Objectives 3 & 4: Trials ongoing, results will be available next year

Objective 5: *Evaluate pathogenicity and fungicide sensitivity of Lambertella and Phacidium rots, newly reported in pome fruit in WA.*

- Nine major apple cultivars were tested for susceptibility to *Lambertella corni-maris* and *Phacidium lacerum*. All cultivars were infected when inoculated throughout wounds but some cultivars such as Honeycrisp, Fuji, Piñata and Gala were more susceptible.
- Fludioxonil and pyrimethanil controlled isolates of *L. corni-maris* on detached apple fruit whereas TBZ failed to provide any control. The preharvest fungicide Pristine provided only a moderate efficacy (30 to 50%).

METHODS

Objective 1. Conduct a second-year statewide decay survey program.

To assess season variability due to changing weather conditions or different spray regimes, we will conduct a second-year survey. Fifty 50 decayed fruit will be sampled on the packing line from same 15 packinghouses surveyed in 2016 across the state. Ten grower lots (orchards) will be surveyed from each single packinghouse. Fruit will be sampled between February and June. Fruit will be placed in clamshells to avoid crashing and cross contamination and transported to the Pathology lab at WSU-TFREC for decay identification and culturing on agar media. Decay identification will be done based on symptoms, spore shape and colony morphology on agar plates. If needed, some pathogens will be identified using molecularly.

Objective 2b. Conduct a multiyear statewide resistance monitoring program.

Fruit collected for decay survey (objective 1) will be used to conduct the fungicide resistance monitoring. Fifty decayed fruit will be collected from the same lots surveyed in 2016. Additional lots may be included. We will test *Penicillium*, *Botrytis*, and *Neofabraea* (Bull's) isolates from each orchard lot. All *Botrytis* and *Neofabraea* isolates will be tested for sensitivity to boscalid, and fluxapyroxad (Merivon), from the same chemical group (FRAC7), and to difenoconazole, TBZ, pyrimethanil, and fludioxonil whereas *Penicillium* will be tested for the last four fungicides only. Results from the second year will be compared to those from 2016 to produce a map with location-specific resistance profiles to help understanding resistance development and spread. Because storage room can harbor tremendous amount of airborne fungal population, we will survey resistant population of *Penicillium* in storage room atmospheres across the State using an Air-Test sampler. This will help in understanding the buildup and spread of resistance inside storage rooms.

Objective 3. Evaluate the impact of storage conditions on resistance development in the blue and gray molds.

In November 2016, in vitro and in vivo experiments aimed to understand the impact of low temperatures and CA conditions on some of the biochemical and molecular mechanisms related to fungicide resistance development were started. Six *Penicillium* isolates and 6 *Botrytis* isolates having different sensitivity phenotypes were used. In vitro, isolates were inoculated to plates with a medium amended with a sub-lethal dose of fludioxonil, pyrimethanil, or thiabendazole. Plates are incubated in the lab at 0°C (33°F) for 6 months, or for 2 months at 22°C (68°F) and 28°C (84°F) or for 6 months at 0°C (33°F) in CA at Stemilt facility. A similar experiment was conducted on organic Fuji pre-wounded, sprayed with half and full label rate of each fungicide and inoculated with the same isolates used in vitro. This trial is currently ongoing. For each incubation period, the sensitivity of isolates will be assessed to all postharvest fungicides to check for shift in sensitivity. RNA will be extracted from some cultures from each treatment and used to evaluate the expression of ABC transporter gene and mrr1 genes, known to be overexpressed in resistant isolates. Based on the first-year results, we plan to reassess the impact of multiple temperatures and CA conditions on resistance development.

Objective 4. Evaluate sensitivity/resistance to pre-harvest fungicides and impact of non-target sprays.

A field trial was initiated in the mid-summer of 2016 at the Sunrise Research orchard to evaluate six different fungicide rotation programs on disease development in postharvest and potential for resistance development. Fruit were harvested in October and will be evaluated after 6 months of storage at 33°F. A second-year trial will be conducted in 2017 to start earlier in the season and include additional treatments based on results from 2016.

Objective 5. Fungicide sensitivity of Phacidium.

Phacidium is a newly reported pome fruit in WA. We will continue monitoring it as explained in objective 1. Herein, we will assess the sensitivity of 100 isolates previously collected (part of the collection at TFREC-Pathology lab) to boscalid, pyraclostrobin, TBZ, pyrimethanil, difenoconazole, and fludioxonil using mycelial growth inhibition assay on 10-cm Petri plates containing appropriate media amended with 0, 0.001, 0.01, 1, and 10 µg/ml for each fungicide. Plates will be incubated 4-5 days at 20°C and growth inhibition will be expressed compared to the control (0 µg/ml) and effective concentration necessary to inhibit 50% mycelial growth (EC_{50}) values will be determined. These values will serve as a baseline to monitor future sensitivity shifts. Efficacy of the aforementioned fungicides against *Phacidium* on fruit will be evaluated.

RESULTS AND DISCUSSION

Objective 1. *Postharvest diseases prevalence*

Blue and gray molds accounted for almost 72% of total decay observed with blue mold being predominant with 48% of total decay (Figure 1-left). Blue mold was detected in 157 of the 160 lots surveyed versus 132 lots for gray mold. A majority of lots had less than 20% incidence of gray mold, whereas a higher number of lots had between 40 and 80% blue mold (Figure 1-right). Besides these two mains decays, bull's eye rot was found in 52 lots at frequencies ranging from 1 to 75%, whereas the statewide frequency was 4.3%. The frequency of the "crabapple diseases" Speck rot and Sphaeropsis rot was 2.5 and 1.4%, respectively. It is possible that better management practices, including pruning and appropriate fungicide sprays, resulted in such low frequencies compared to those reported when these two pathogens were first described in the state. Additional minor pathogens

included Alternaria rot (2.9%) and the newly reported yellow rot (2%). Other minor or non-identified decays accounted for 14.3% of total decay.

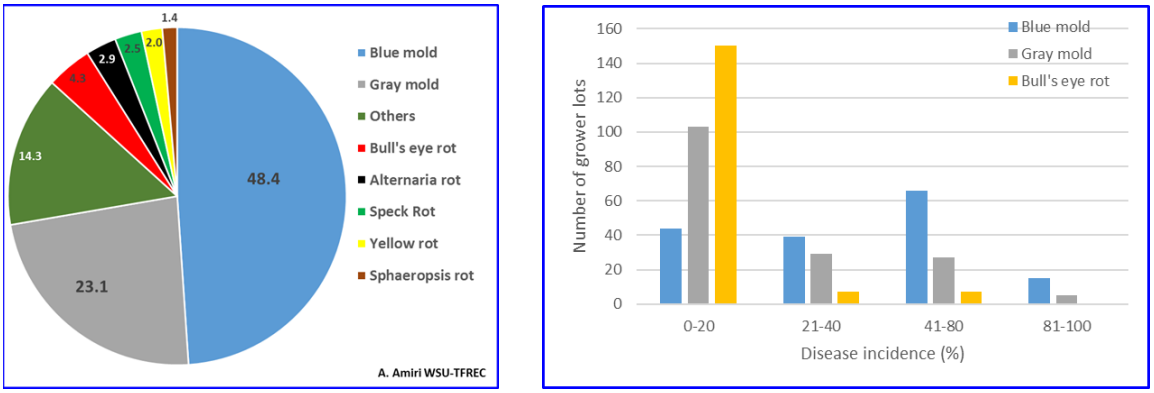

Figure 1. Overall incidence of major postharvest diseases found in Washington in 2016 (left) and incidence distribution of blue mold, gray mold and Bull's eye rot among grower lots (Right).

Objective 2. *Fungicide resistance occurrence and frequencies*

Over the 2,000 of *Penicillium expansum* (blue mold) isolates tested, 24% and 16% were resistant to TBZ and pyrimethanil (Penbotec), respectively. About 11% had reduced sensitivity to fludioxonil (Figure 2-left). Over the 1,700 of *Botrytis cinerea* (gray mold) isolates tested, 14% and 20% were resistant to TBZ and pyrimethanil (Penbotec), respectively, whereas 12% had reduced sensitivity to fludioxonil (Figure 2-right). Overall, 11% and 3% of *Botrytis* isolates were resistant to the pre-harvest fungicides Pristine and Merivon.

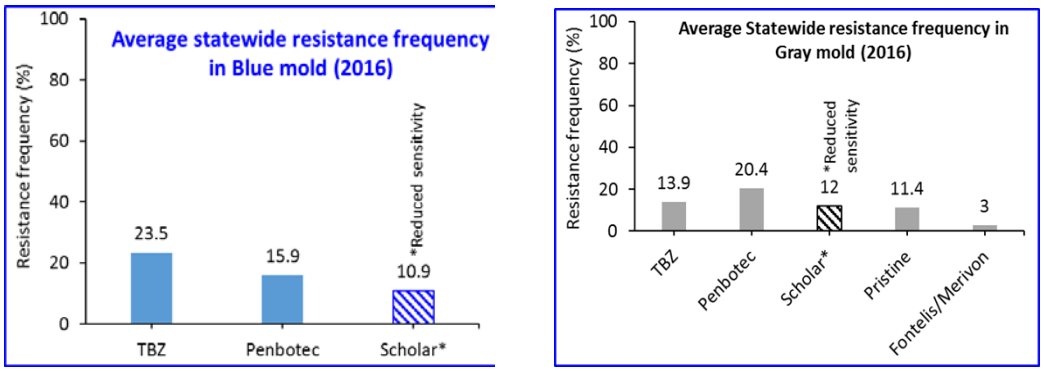

Figure 2. Overall resistance frequencies to major pre- and postharvest fungicides in blue mold (left) and gray mold (right) observed statewide in 2016.

In blue mold, about 66% of grower lots showed resistance to TBZ and more than 30% had a resistance frequency >40% (Figure 3, left). For pyrimethanil 55% of lots showed resistance with a largest portion having between 1 and 20% resistance. Interestingly, about 45% of lots surveyed showed reduced sensitivity to fludioxonil (Figure 3, left). Resistance was slightly lower in gray mold, compared to blue mold, with the highest frequency observed to pyrimethanil (Figure 2, right). In a non-negligible portion of lots surveyed, the same fungicide was used for more than one year which may explain their highest resistance frequencies compared to state average.

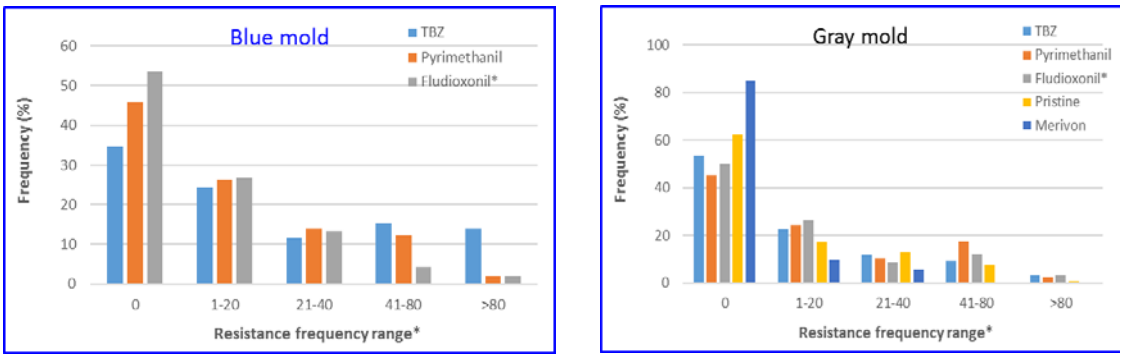

Figure 3. Resistance frequency distribution in blue mold (left) and gray mold (right) observed statewide in 2016. * for fludioxonil, it is only reduced sensitivity.

Objective 3: *Evaluate the impact of storage conditions on resistance development in the blue and gray mold pathogens in relation to fungicide type (**ongoing**)*

- An experiment has been started in the lab at WSU-TFREC and at Stemilt facility to evaluate the impact of different temperatures and CA regimes on the metabolism and ability of *P. expansum* and *B. cinerea* to adapt to fungicides and develop resistance to three postharvest fungicides. Results will be available next year.

Objective 4: *Evaluate sensitivity/resistance to pre-harvest fungicides i.e. newly registered ones and non-target sprays in major pome fruit pathogens (**ongoing**)*

- A field trial was initiated in the summer of 2016 at the Sunrise Research orchard to evaluate different fungicide rotation programs on disease development in postharvest and potential for resistance development. Fruit were harvested in October and will be evaluated after 6 months of storage at 33°F.

Objective 5: *Prevalence of yellow rot and its sensitivity to pre- and postharvest fungicides*

Yellow rot (Lambertella, Figure 4) was found in 34% of lots surveyed with an incidence ranging from 2 and 40% per grower lot. Overall, 96% of the lots surveyed had an incidence of 2 to 10% whereas only 4% showed a yellow rot incidence higher than 10%.

Figure 4. Symptoms of naturally occurring yellow rot on Gala apple from a conventionally-managed orchard after 7 months of storage at 0°C (33°F) in a controlled atmosphere.

The susceptibility of 9 apple cultivars to yellow rot (*L. corni-maris*) shown in Figure 4 indicates that Red Delicious is the least susceptible cultivar together with Cameo and Granny Smith being significantly less susceptible than the remaining cultivars. On the other hand, Honeycrisp and Gala are among the most susceptible ones (Figure 5).

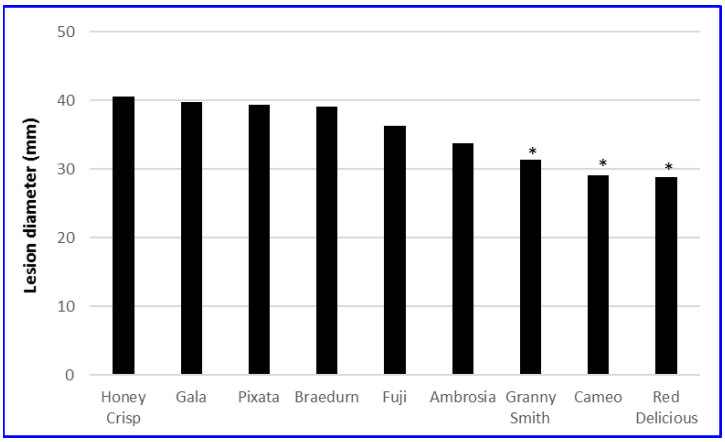

Figure 5. Susceptibility (expressed as lesion diameter) of most common apple cultivars to yellow rot (Amiri et al. Plant Disease, 2017, *In Press*). * indicate cultivars significantly less susceptible.

Yellow rot is totally controlled by fludioxonil at label rate while pyrimethanil provided a high efficacy (>94% control) on fruit wounded and inoculated with the fungus (Figure 5). To the contrary, TBZ failed to provide any efficacy against yellow rot. This is not due to fungicide resistance but rather to inherent inefficacy of this group of fungicide against yellow rot. It is not clear yet if *L. corni-maris* infect fruit pre- or postharvest, however the preharvest fungicides Topsin-M (same group as TBZ) and Pristine (pyraclostrobin + boscalid) may not provide adequate control if fruit are infected in the orchard.

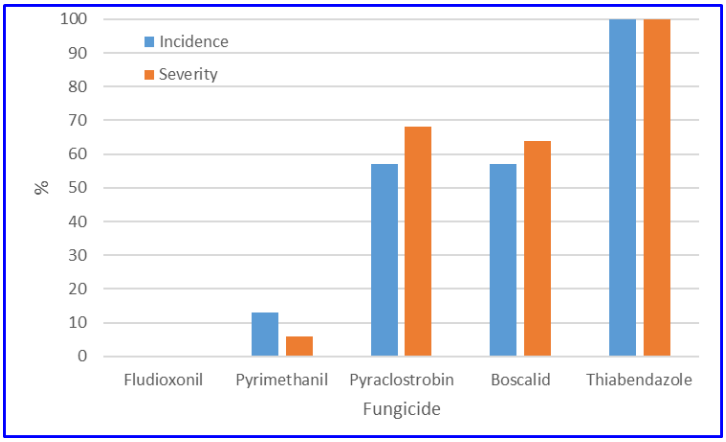

Figure 6. Efficacy of pre- and postharvest fungicides against yellow rot on apple fruit (Amiri et al. Plant Disease, 2017, *In Press*).

CONTINUING PROJECT REPORT **YEAR:** 2016
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
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City/State/Zip: Union Gap, WA, 98903

Cooperators:
Scientists: Stefan Roeder, WSU; Suzanne Niemann (Allan Brothers)
WTFRC internal program: Manoella Mendoza, Tory Schmidt, Sandy Stone, Kyle Tynan
Others: Grower collaborators, WTFRC seasonal crew and interns, Glade Brosi (Stemilt)

Other funding sources
Supplies and fruit were donated by industry suppliers.

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

Item	2016	2017
Salaries	6,785	6,785
Salary benefits	3,401	4,301
Wages	15,734	7,800
Wage benefits	5,156	2,643
RCA rental	1,800	1,800
Equipment + supplies	268	300
Travel	167	500
Total net costs	**33,311**	**24,129**

Footnotes:
Entire budget is based on the calendar year 2016.
Salaries: incl. proportional time spent on outlined projects for Hanrahan, Mendoza, Schmidt, Tynan
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, if applicable

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

OBJECTIVES*

1. Document Honeycrisp fruit quality in local store displays.
2. Test new tools to determine fruit quality parameters (NEW in 2016).
3. Serve on WSU Tree Fruit Extension team (NEW in 2016).
4. Field test methods to induce bitter pit in Honeycrisp (NEW in 2016).
5. Evaluate Honeycrisp storage performance of fruit exposed to near harvest rain events (NEW 2016).
6. 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: Honeycrisp were available year-round in local stores for the first time since starting this evaluation. Consistently good eating quality in the last part of the storage season (May – August) remains of concern.

Objective 2: Several new devices to help growers and packers to accurately, fast and economically determine common maturity parameters, have become available in recent years. Some devices, such as the Accuvin Titratable Acidity Test Kit, may be implemented successfully without any problem. Others, like the Felix F-750 are very promising, but will need further refinement in order to be easily adoptable.

Objective 3: Collaboration to develop an updated disorder guide for apple is underway. Hanrahan team provided pictures and helped with content update.

Objective 6: 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.

METHODS

Supermarket survey: Eight Yakima supermarkets were visited monthly from February until August 2016. Visual and sensory quality of fruit was determined.

New tools for fruit maturity determination: Two tube tests (Accuvin Titratable Acidity and BSG Wine Acid Test) and the Atago PAL-BX|ACID5 were compared to an automated titrator (Methrom). The Felix F 750 was tested for: chlorophyll quantity, SSC, and DMC.

Induced bitterpit: Honeycrisp was harvested from three orchards with varying degree of bitterpit symptom expression at harvest. Bitterpit prevalence in the orchard and after 3 month storage will be determined and combined to account for total bitterpit symptom expression. Three methods were utilized: hot water immersion, treatment with ethylene, and passive treatment (storage at room temperature).

HC storage performance vs. preharvest rain events: Fruit from one orchard near Gleed was harvested sequentially (four times) to include two periods of preharvest rain events. Storage performance is currently being evaluated.

RESULTS & DISCUSSION

The fruit quality program has continued to focus part of its effort on Honeycrisp fruit quality in 2016, but the general scope was scaled back based on industry direction.

Supermarket survey: Eight Yakima area supermarkets were visited eight times from February 10 until August 1, 2016. All locations carried 100% local fruit until July 1, then one store switched to Chilean supplies (not shown). Fruit eating quality was good until April, but started to fluctuate after that (not shown). If consumers are having inconsistent eating experiences from good to off flavor, repeat sales could be impacted negatively. It appears that this issue has remained a constant struggle over the past four storage seasons (Figure 1).

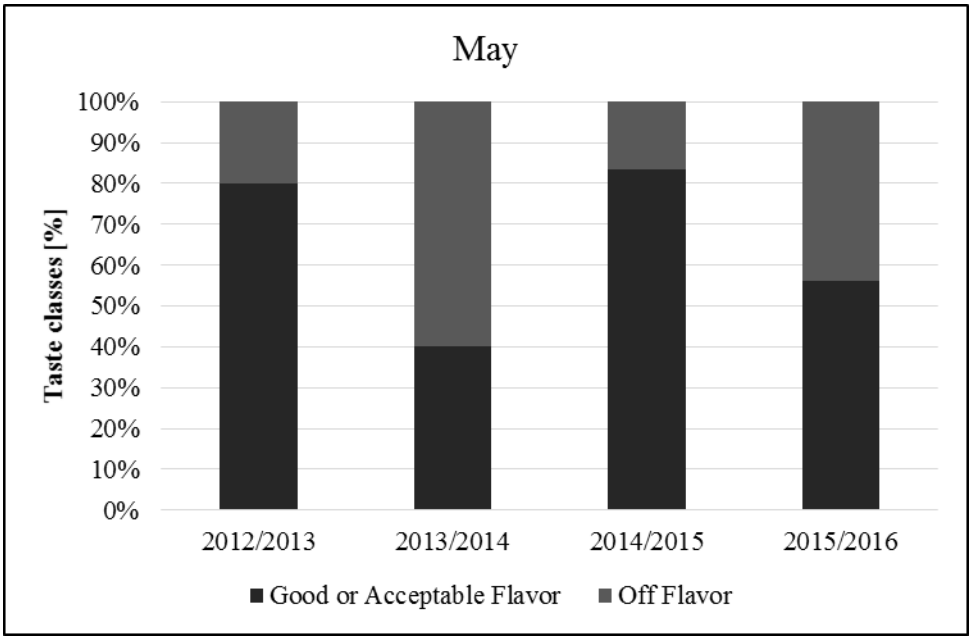


Figure 1: Taste of fruit (8 stores total) for Honeycrisp purchased in Yakima area supermarkets between May 2012 - 2016.

New tools for fruit maturity determination: In order to be useful for any operation, a new instrument should be accurate, preferably more accurate than the standard method. Secondly, instruments need to be reliable, especially when operated under field conditions or by personnel with minimum training. In addition to the initial purchase price the cost of labor (training, performing of task, maintenance etc.) needs to be considered. WTFRC continued in 2016 to evaluate new tools, as they became available. A summary is provided in Table 1.

Accuvin Titratable Acidity Test Kit: Currently, the Accuvin acidity tube test is the fastest and easiest method to determine apple fruit acidity levels of all test kits evaluated by WTFRC to date. Due to the lower accuracy, this test can be used if the knowledge of the acidity range is more important than an exact value, for example when trying to group lots of Honeycrisp apples based on high or low TA levels for different storage durations. We noted that this test requires minimum training and can be performed in the field or at receiving.
BSG wine acid test: In general, the acquisition costs of the wine acid test from BSG are lower than that of the Accuvin tube test. However, due to the additional equipment needs and the higher time requirement to perform the actual test, the overall operating costs are higher. Also, this test is not suitable for field use.

Atago PAL-BX|ACID5: Atago's PAL-BX|ACID5 is able to measure the SSC and the TA one after another from the same juice sample. However, in order to be able to measure the TA content after the SSC measurement, a 1:50 dilution has to be made. This step can lead to calculation errors. Overall, the device showed an accuracy of approximate 78%.
Felix F750: The F750 allows the non-destructive measurement of certain parameters. However, a model has to be created for each parameter and each lot of fruit separately. During a proof of concept study the WTFRC attempted to develop a firmness, DMC, TA, SSC and DA model for Fuji, Honeycrisp and Cosmic Crisp™ apples. Our results showed the possibility to create DA and dry matter models. However, no model could be created for firmness, SSC and TA with the current software. More specific research is required to see if there is a need to develop models for individual varieties, pre-and postharvest measurements and to work out specific recommendations for the measuring procedure. Right now the device is not ready for field application.

We reported our findings in the Good Fruit Grower (http://www.goodfruit.com/new-tools-to-determine-fruit-quality-parameters/) and developed a more comprehensive 'How to' guide for the WSU Tree Fruit Extension webpage (http://treefruit.wsu.edu/news/new-tools-to-help-determine-maturity-of-tree-fruit/).

Collaborative research

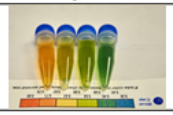
We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to pre-and postharvest fruit quality management (Table 2).

Table 2. 2016 Hanrahan/WTFRC collaborations on pre-and post- harvest fruit quality projects.

COLLABORATOR(S)	PROJECT	HANRAHAN ROLE
2016 (continuing and new)		
Evans/Auvil	WSU Breeding: P3	Collaborator storage evaluation
Univ. of Talca*	Superficial Scald control	Contract project
SCS*	Weight loss in storage	Contract project
2017 pending		
Blakey	Losses in storage	Collaborator
Gallardo et al.	Market potential for Cosmic Crisp	CO-PI

*project costs completely covered by companies/external projects

Table 1: New tools for fruit maturity determination

	DA Meter	Metrohm Titrator	Accuvin Titratable Acidity Test Kit	BSG wine acid test	Atago PAL-BX| ACID5	Felix F-750
						
Parameter	chlorophyll quantity (aka background color)	titratable acidity	titratable acidity	titratable acidity	soluble solid content, titratable acidity	chlorophyll quantity, dry matter content, (soluble solid content)
Price for device ($)	$ 3,000.00	$ 30,000.00	-	-	$ 1,260.00	$ 6,950.00
Price per sample ($)	-	-	from $ 1.82 ($ 52 for 20 test/kit) ($ 103 for 50 test/kit) ($ 182 for 100 test/kit)	$ 0.25 ($ 10 for test kit - approx. 40 test)	-	-
Time requirement per sample	3 sec.	3 min.	1 min.	3 min.	3 min.	5 sec.
Accuracy	100 %	100 %	73 %	87 %	69 - 88 %	-
Training	Easy-intermediate	difficult	easy	intermediate	intermediate	difficult
Advantages & disadvantages	✓ quick calibration ✓ for field use ✓ easy to use ✓ hand held device ✗ low correlation between DA value and other fruit quality parameter ✗ difficult to download data with software provided by company	✓ exact value ✓ high reliability ✓ auto sampler for high sample trough put ✗ acquisition and maintenance cost ✗ training required ✗ requires lab conditions	✓ for field use ✓ fast & easy to use ✓ no training needed ✗ no exact value ✗ less accurate	✓ cheap method ✓ good accuracy ✗ not comfortable for field use ✗ additional tools (electric pipette and glass beaker) are useful when analyzing larger sample set (> 15)	✓ SSC & TA estimation from the same sample ✗ error susceptibility when making the 1:50 dilution ✗ low accuracy for TA reading	✓ measures up to 3 parameters at the same time ✓ hand held device ✓ high future potential ✗ models have to be developed ✗ many open research question regarding the specifics for models ✗ not ready for field application

CONTINUING PROJECT REPORT **YEAR**: 1/3
WTFRC Project Number: 1087 (internal account, general food safety)

Project Title: WTFRC internal program – food safety efforts

PI: Ines Hanrahan
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Telephone: 509 669 0267
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Address: 2403 S.18th St., Suite 100
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Cooperators: Laura Grunenfelder and Kate Woods (NHC), Jacqui Gordon (WSTFA), Manoella Mendoza and Mackenzie Perrault (WTFRC), Lauren Walter and Kyu Jeong (WSU), Missy Partyka, Ronny Bond, Jennifer Chace, Jayanti Das (UC Davis), Bonnie Fernandez-Fenaroli (CPS)

Acknowledgement: WTFRC seasonal crew efforts are acknowledged and appreciated. We would like to thank Harold Schell, Jake Gutzwiler, and Brent Milne (all WTFRC board members) for their assistance in reviewing CPS grant proposals.

Other funding sources

Agency Name: WA SCBGP
Amt. requested/awarded: $ 216,682 Title: Enhanced food safety education and training for tree fruit producers (awarded to WSTFA with Jacqui Gordon as PI)
Notes: In 2016 a total of six workshops (three topic areas) were organized for tree fruit producers, with WTFRC participation

Agency Name: FDA
Amt. requested/awarded: $243,651 for FY17 awarded to WCFSS (Atwill et al.) Title: Facilitating implementation of FSMA regulations for agricultural water quality
Notes: This budget covers sampling in both California and Washington and includes staff salaries. The budget for Washington alone is estimated at ~$140K. WTFRC participated in site selection, experimental design, and planning for 2017

Agency Name: CPS
Amt. requested/awarded: $334,252 Title: Evaluation of an alternative irrigation water quality indicator; PI: Trevor Suslow, UC Davis
Notes: In 2016 WTFRC sampled 14 locations and filtered samples (Mohr swabs) in monthly intervals from May-Sept. as part of a multi-state study (CA, AR, WA)

Agency Name: WSU/UI School of Food Science
Amt. requested/awarded: $30,000 (awarded)
Notes: Funds were utilized to continue food safety research in a microbiology lab in Pullman, former Killinger lab, and to help develop industry training

WTFRC internal program expenses:

Item	2016	2017[3]
Salaries	27,146	27,689
Benefits	5,322	5,428
Wages	2,257	2,584
Benefits	855	979
RCA Room Rental		
Shipping		
Supplies[1]	177	200
Travel[2]	1,622	5,000
Plot Fees		
Miscellaneous		
Total	**37,379**	**41,880**

Footnotes:

[1]Supplies include three posters (2 for IAFP, 1 for ASHS)

[2]Travel includes: CPS in Seattle, trip to WSU in Pullman, in state day travel to attend trainings, IAFP in St. Louis, NW Food Safety and Sanitation Conference in Portland; projection of 2017 travel costs is significantly higher, since Dr. Hanrahan cannot guarantee that she will be an invited speaker and/or can purchase flight tickets on WTFRC credit card miles; an effort will be made to reduce the projected costs similar to 2016

[3]Wages and salaries have been calculated as follows: salaries = 2% increase, wages = 14.5% increase based on projected state minimum wage increase and assuming that workload does not further increase

OBJECTIVES

1. Enhance collaboration in all areas of food safety (research, policy, FSMA implementation)
    a. Participate in development of training for industry
    b. Develop effective food safety outreach program

SIGNIFICANT ACCOMPLISHMENTS IN 2016

Research: Dr. Hanrahan started her year with a six-week ***sabbatical at UC Davis***. From February 4 until March 13, 2016, she spent time as a visiting scholar at the Western Center for Food Safety (WCFS) with the team of Robert Atwill (Center Director; www.wcfs.ucdavis.edu). Primarily, Dr. Hanrahan received basic and advanced microbiology training and participated in ongoing projects (including a trip to Mexico to investigate the cause of *Salmonella* infection in Papaya). A second focus of her leave was the chance to network and foster or expand professional connections in California, explore ways to provide training and answer questions related to FSMA implementation, and to deepen existing and forge new collaborations with several research teams (Suslow, Linke, Walse, Holcroft). A full report and detailed ppt descriptions of activities are available upon request.

Secondly, Dr. Hanrahan continued to lead the former Killinger lab staff at WSU in Pullman, and successfully finished all on-going projects, while transitioning staff to new positions. Kyu Ho Jeong remains on the team until July 2017 to help finish data analysis and preparation of manuscripts.

Third, we participated in a number of on-going collaborative projects, funded by WTFRC, CPS, and FDA (see Table 1).

Lastly, the WTFRC, under leadership of Ines Hanrahan, has served as a partner in research for the Center for Produce Safety (CPS). Tree fruit specific research priorities are developed and integrated into the annual CPS call for proposals, with the help of the NHC Food Safety Committee. As a result, six proposals addressing industry needs were originally submitted, and ultimately one was funded: 'Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention' (Zhu/Suslow; $290,000). WTFRC staff (Hanrahan) was also involved in planning of the annual CPS conference held in Seattle this year. As a result, CPS devoted a half day of the two-day conference to the experience and research needs of tree fruit (specifically *Listeria*). Dr. Hanrahan was invited as a speaker to explain our industry response to the *Listeria* outbreak (Washington Tree Fruit Industry Response to *Listeria monocytogenes* Caramel Apple Outbreak).
Further, we supplied pictures of our crops to be used in advertising material, supplied names of local students (including Manoella Mendoza) to help at the meeting, and WTFRC designed and assembled centerpieces themed around tree fruit for the VIP dinner. Following the meeting, Hanrahan organized a visit of the entire staff of the Produce Safety Alliance (PSA) to the Yakima Valley for a combination of field trips (orchards and warehouses) and discussions (incl. NHC, WSTFA). The PSA is responsible for providing training for the Produce Rule.

Table 1: Summary of WTFRC collaborations* in food safety research in 2016 and pending research for 2017

Keyword	PI's	Affiliation(s)	Funding Source	Amount
Continuing in 2016				
Evapor. cooling	Hanrahan/Zhu	WSU, UW, WTFRC	WTFRC	190,000
List. packing	Hanrahan/Suslow	WSU, WTFRC, UC Davis	WTFRC	66,000
Imp. Dryer	Ganjyal/Zhu	WSU	WTFRC	57,000
Bacteroides	Suslow	UC Davis; UoA, WTFRC	CPS-SCBG	336,000
New in 2016				
Listeria storage	Zhu/Amiri/Hanrahan	WSU, WTFRC	WTFRC	195,414
Water sampling	Partyka/Bond	UC Davis, WTFRC	FDA	243,651
Pending for 2017				
List. cleaning	Zhu/Hanrahan	WSU, WTFRC	WTFRC	306,285
Water sampling	Atwill/Partyka/Bond/Hanrahan	UC Davis, WTFRC	CPS	367,000
Equipment	Linke/Das/Chase/Hanrahan	UC Davis, WTFRC	WTFRC	66,000
Education	Ganjyal	WSU	WTFRC	95,000
Brush beds	Blakey	WSU	WTFRC	55,000

*collaborations may involve a WTFRC internal budget or utilize Dr. Hanrahan as a consultant/co-PI or collaborator

FSMA implementation: In order to best serve the needs for timely information distribution related to new laws pertaining to food safety, WTFRC staff (Hanrahan) lead an effort to coordinate all outreach activities by the various industry organizations. Specifically, NHC (policy), WSTFA (education) and WTFRC (research) efforts were combined and talking points coordinated to prevent further confusion, when learning how to implement the already complicated laws. The entire team (Grunenfelder, Woods, Gordon, Hanrahan) has developed a uniform slide set to be used by each group member when addressing groups. This is a living document and has been updated numerous times.

Development of industry training modules: In collaboration with WSTFA, NHC, and WSU we developed and executed two workshops with a total of 112 participants in 2016:

a. Putting Cleaning and Sanitation Programs into Practice (two locations)
b. Verification of Cleaning and Sanitation for Tree Fruit Packinghouses

These workshops provided a combination of classroom and hands-on activities and took place in collaborating packing facilities (Table 2). Dr. Hanrahan's contributions to these workshops included: leading of general curriculum development, being a trainer, delivering talks, and helping with logistical support (including staff). Secondly, the same group, in collaboration with UC Davis held three workshops named: FSMA water quality testing. This module was also the first of it's kind in the nation to address practical considerations for water testing under FSMA. Workshops were designed to give participants theoretical background in combination with outdoor activities geared towards learning based on examples coupled with hands on training (Table 2). After conclusion of the workshop, a permanent document was prepared collaboratively between Melissa Partyka, Ronny Bond, and Ines Hanrahan to detail considerations for water testing and placed onto the WSU Tree Fruit Extension team webpage as a living document (http://treefruit.wsu.edu/news/water-sampling-for-fsma-compliance-done-simply/). For 2017, we plan on repeating all workshops as needed, or as soon as guidance for water sampling is released by FDA. In addition, WTFRC is collaborating with the WSFTA to develop hand washing training materials including a video and practical training material.

Table 2: WTFRC staff involvement in WSTFA sponsored food safety trainings in 2016

Name of Workshop/Training	**Date**
2016 FSMA Water Quality Testing Workshop Wenatchee	5/17/2016
2016 FSMA Water Quality Testing Workshop Selah	5/18/2016
2016 FSMA Water Quality Testing Workshop Yakima	5/19/2016
Putting cleaning and sanitation programs into practice - Zillah	7/20/2016
Putting cleaning and sanitation programs into practice - Wenatchee	7/21/2016
Verification of cleaning and sanitation programs for tree fruit packinghouses: a hands-on environmental monitoring workshop for food safety managers	11/1/2016

Food Safety outreach: Ines served as the session manager for the food safety session during the WSTFA 112th Annual Meeting (HortShow) in December 2016. As an added service to conference attendees, the team (incl. Jacqui Gordon, Mackenzie Perrault and Ines Hanrahan) collected all questions asked during the session that did not get answered, prepared written responses and sent them out via email.

Dr. Hanrahan also served on the search committee for the WSU Food Safety Extension position. To date, the position remains open, because no suitable candidate has been found yet. In addition, Ines was asked to join the WSU School of Food Science as an adjunct faculty member. She is currently serving on one MsSc. Committee.

Further, Dr. Hanrahan is serving on the stirring committee of the PNW Food Safety and Sanitation Conference, a regional conference with 450 attendees annually. Other outreach activities included: three posters at national/international meetings, and ten invited talks. The press covered WTFRC food safety activities in five Good Fruit Grower articles and three blogs/videos.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-16-106

Project Title: Managing rhizosphere/soil microbiology via apple rootstock biochemistry

PI: Mark Mazzola
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Co-PI: Rachel Leisso
Organization: USDA-ARS Tree Fruit Research Laboratory
Telephone: (509) 664-2280 ext. 206
Email: Rachel.Leisso@ars.usda.gov

Cooperators: David Rudell, James Mattheis, USDA-ARS, Wenatchee, WA

Total Project Request: **Year 1**: $48,000 **Year 2**: **$50,000** **Year 3**: $52,000

Other funding sources

USDA-ARS base funds; $35,000 per year for salary support of postdoctoral research associate.

Organization Name: USDA-ARS **Contract Administrator: Chuck Myers**
Telephone: 510-559-6019 **Email address:** Chuck.Myers@ars.usda.gov

Item	2016	2017	2018
Salaries[1]	$30,000	$31,000	$32,000
Benefits	$10,000	$10,200	$10,400
Wages			
Benefits			
Equipment			
Supplies	$7,500	$8,300	$9,100
Travel	$500	$500	$500
Miscellaneous			
Plot Fees			
Total	$48,000	**$50,000**	$52,000

Footnotes: [1]Salary support is requested from 0.5 FTE of a postdoctoral research associate.

OBJECTIVES

This research project addresses several priorities of the tree fruit industry as identified in the 2016 Apple Horticulture and Postharvest research needs assessment, including i.) Understanding and management of soil health and productivity in conventional and organic systems (critical priority) ii.) Soil health and productivity – Interaction of rootstocks with rhizosphere microbiology (high priority) and contributes to additional priorities including iii.) apple replant (high priority) and iv.) improved scion and rootstock genetics (medium priority). The project objectives outlined in the original proposal are as follows:

1. Characterize the effect of apple rootstock genotype on composition of the rhizosphere and orchard soil-inhabiting microbial community (microbiome).
2. Define differences in the natural chemical compound profile produced by rootstock cultivars that differ in inhibiting deleterious (pathogenic) or attracting beneficial rhizosphere micro-organisms.
3. Test the composite and independent (single compound instead of natural suite of compounds) impacts of natural chemical compounds on specific microbes or the soil microbiome to verify functional role in inhibition of deleterious microbes or attraction of beneficial microbes.
4. Determine effects of apple rootstock genotype on rhizosphere soil pH, contrasting rootstocks harboring different rhizosphere microbiomes of functional importance.

Activities in year one focused on aspects associated with objectives 1, 2, and 4. A convergent strategy for initiating studies relative to objective 3 in the upcoming year has been outlined based on current results. The strategy for performing objective 3 is:

- **Specific metabolites that differ among apple rootstocks have been defined and will be tested individually for effects on growth activities of known beneficial or deleterious microorganisms.**
- **The metabolic profile of apple root exudates / rhizodeposits includes unidentified metabolites. However collection of the complete rootstock exudate profile can be obtained through gnotobiotic (microbe-free) or hydroponic cultivation of commercial-age rootstocks and subsequent micropore filtration of the composite exudates.**
- **In our initial studies we have demonstrated the effects of environmental conditions and rootstock ontogeny (developmental stage) on the metabolic profile of rootstock exudates, and further experimentation will be developed accordingly.**

Preliminary results from research pertaining to objective 4 indicate that not only do rootstocks influence the rhizosphere / bulk soil pH, but also that the initial pH and quality of the water applied to the trees (for trees grown in pure sand) influences rhizosphere pH. Studies pertaining to objective 4 will be expanded to specifically examine the impact of rootstock genotype in determining rhizosphere soil pH when established in soil of high (greater than 7.5) and low (less than 5) pH.

SIGNIFICANT FINDINGS

In greenhouse experiments, exudates collected from sand-based systems planted to apple rootstocks included metabolites of non-tree origin. This finding was based on development of a tree-specific metabolic library that was generated in experiments that utilized sterilely micropropagated (tissue culture) derived apple rootstocks. Follow-up assessment indicated the presence of bacteria in the

rhizosphere, and bacterial populations detected in the rhizosphere increased in a manner that corresponded with increases in estimated leaf area of the rootstock.

- **When cultivated in the same orchard soil, plant biomass, disease severity, and rhizosphere microbiome composition of apple seedlings differed depending upon genotype of the rootstock cultivated in this orchard soil.**
- Metabolic composition of root exudates differed among apple rootstock genotypes. The genotypes assessed in the present study were G41, G935, M9Nic29, and M26. Results suggest that G41 and G935 are the most similar in terms of root exudate metabolite profiles.
- The quantity of exudates released into the rhizosphere generally corresponded to rootstock vigor.
- pH of both soil and water infiltrated through the tree rhizosphere were altered by the presence of a rootstock but did not differ significantly among rootstocks.

METHODS

Objective 1: Initial assessment of rootstock genotype effects on the soil microbiome were conducted using plant bioassays. The previous rootstock was found to determine composition of the microbiome in a subsequent planting which affected apple seedling growth. Microbial community composition was characterized by terminal restriction fragment length polymorphism analysis (T-RFLP) of the bacterial and fungal community. In the coming year a more comprehensive analysis of rootstock effects on the soil microbiome will be conducted through use of NextGen sequencing methods. Rhizosphere or root exudate treated soil samples will be collected and DNA will be extracted and amplified by PCR using bacterial specific (16S rDNA, 515f/806r) or fungal-specific (ITS1F-Bt1/ ITS4Rbt) primer pairs ((Mazzola et al., 2016). Pooled and purified PCR products will be used to prepare a DNA library and NextGen sequencing will be performed using the Illumina MiSeq platform (Mazzola et al., 2015). Explicet software (Robertson et al., 2013) will be used to conduct statistical analysis and visualization of microbiome data. Analyses will enable comparison across soil systems and to assess how rootstock genotype impacted the microbiome.

Objective 2. Further analysis of exudates will be conducted as to identify potentially functional metabolites that structure composition of the rhizosphere microbiome. Information will also be useful to garner a preliminary appraisal of genetic control of this plant attribute. Exudates will be collected as outlined in **Fig. 1**. In this system, rootstock liners were planted into sterile sand with watering conducted in a manner that allowed infiltration through the sand column (**Fig. 1**). A portion of the filtrate will be collected for metabolite analysis with the balance allowed to pass through onto the surface of an orchard soil system suspended below. Samples will be analyzed using our targeted and untargeted metabolomic profiling techniques that are well established in this research unit (Hewavitharana et al., 2014; Leisso et al., 2016; Rudell et al., 2009). A metabolic library containing nearly 800 unique mass spectral tags has been established and availability of a liquid chromatography–mass spectrometry accurate mass QTOF enables determination of mass accuracy to four decimal places. Uncharacterized natural chemicals will be discovered and added to our metabolic library so that they can be routinely evaluated in further tests.

Objective 3. Differential rootstock chemical compounds will be tested in vitro for capacity to sustain/diminish growth of target microorganisms and/or in situ to attract/repel specific microbes to the rhizosphere. In microbial growth assays, minimal media possessing glucose as a sole carbon source will be supplanted with individual unique or differentially abundant root exudate compounds.

Objective 4. Studies pertaining to objective 4 will be expanded to specifically examine the impact of rootstock genotype in determining rhizosphere soil pH when established in soil of

high (pH greater than 7.5) and low (pH less than 5). Studies will include M.9Nic29, M.26, G.41, G210 and G.935, and will monitor differential rhizosphere pH generated in these soils in relation to rhizosphere microbiome composition.

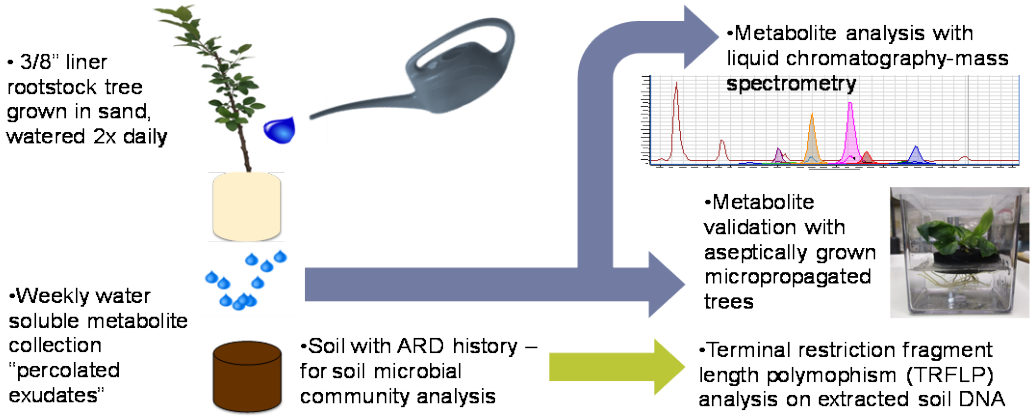

Fig. 1. Experimental systems for assessing composition and quantity of apple rootstock root exudates.

RESULTS & DISCUSSION

Significant Finding 1: Previous rootstock genotype influenced composition of the rhizosphere microbiome recovered from apple seedlings, disease development and plant growth. Soils were collected from the rhizosphere of G.41, G.935 and M.9 rootstocks. Soil was subsequently used in bioassays using Gala seedlings as the test plant. Disease severity, seedling biomass and composition of the seedling rhizosphere microbiome differed with the rootstock genotype from which soil was obtained. Seedling growth in soil previously cultivated with G.935 rootstock was equivalent to that obtained in pasteurized soil, which was utilized as a surrogate for soil fumigation (**Fig. 2**). Biomass was lowest for seedlings grown in soil previously cultivated to M.9 rootstock. Composition of the bacterial community recovered from Gala seedlings also differed in a rootstock dependent manner. These data demonstrate that previous rootstock will influence disease severity in replant orchard and is associated with specific, though as yet unknown, changes in the rhizosphere microbiome.

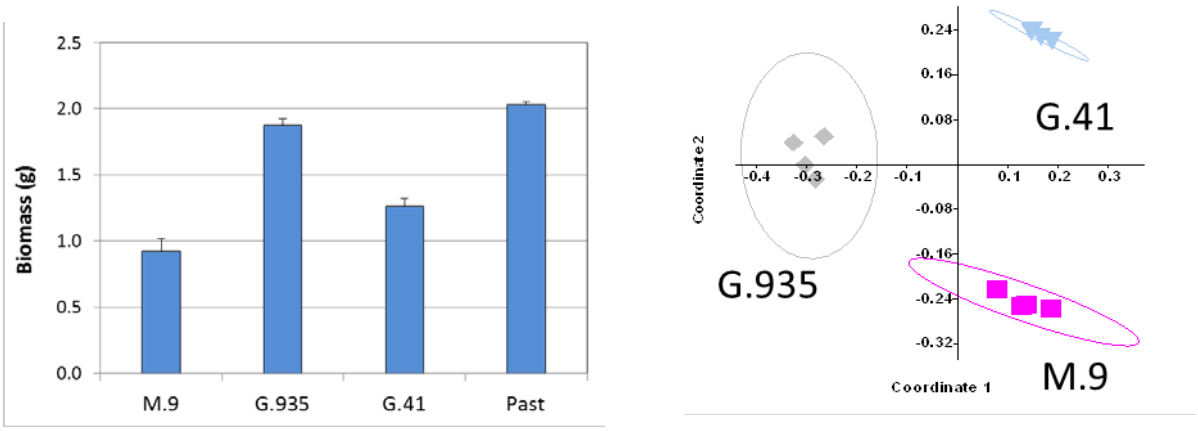

Fig. 2. Biomass of 'Gala' apple seedlings grown in soils previously cultivated to the specified apple roostock (left panel). Relative relatedness of bacterial communities recovered from the rhizosphere of

apple grown in soils previously cultivated to G.41, G935 and M.9 rootstock as determined by principal coordinate analysis of bacterial 16S ribosomal DNA T-RFLP derived data (right panel).

Significant finding 2: In greenhouse experiments, metabolites collected in water infiltrated through the root zone of apple rootstocks (**Fig. 1**) included metabolites of non-tree origin, based on development of a tree-specific metabolic library utilizing sterile micropropagated (tissue culture generated) rootstocks (**Fig. 3**). Follow-up assessment affirmed populations of bacteria in the rhizosphere with bacterial densities increasing in a manner corresponding to estimates of leaf area (data not shown).

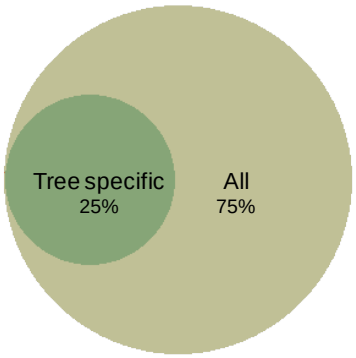

Fig. 3. Venn diagram illustrating the relative portion of metabolites derived from apple rootstock root system and those of non-tree origin. Those of non-tree origin possess several plausible sources, including from associated or introduced microorganisms.

Significant finding 3: Metabolic exudate quantity differed among rootstocks, and generally corresponded to rootstock vigor (**Fig. 4**). A potential implication of this result is that more vigorous rootstocks have a more profound effect on the soil metabolite and microbial composition.

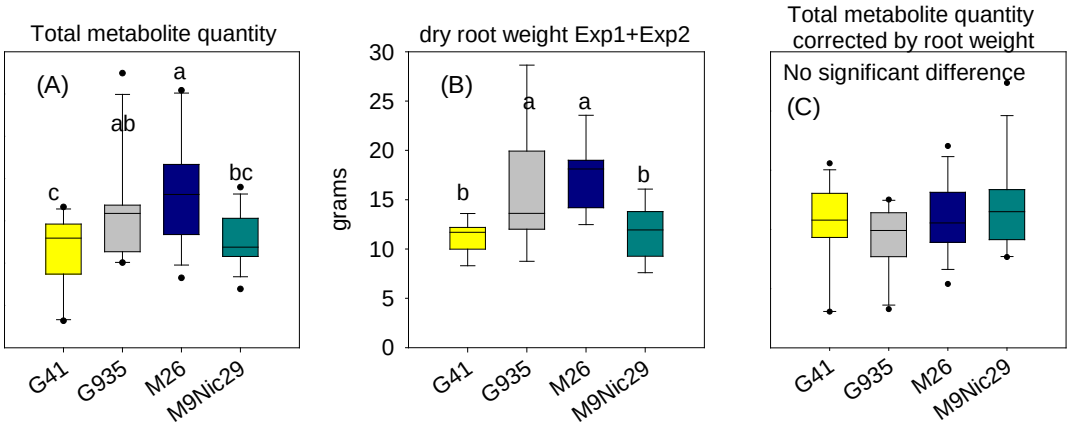

Fig 4. Total metabolite quantity (A) corresponded to rootstock vigor (B), but when corrected for dry root weight (C), metabolite quantity did not differ significantly among the rootstocks. Data summarizes the final metabolite collection point for two greenhouse experiments. Pearsons' correlation between total metabolite quantity (A) and dry root weight (B) is r = 0.40, p = 0.0029. Exp1 = experiment 1, Exp2 = experiment 2.

Significant finding 4: Metabolic composition of exudates differed among apple rootstock genotypes. The genotypes assessed in the present study were G.41, G935, M.9, Nic29, and M.26. Results suggest that G41 and G935 are the most similar in terms of composition (**Fig. 5**). Potential implications of different exudate metabolite profiles according to rootstock genotype include both differences in the attraction or inhibition of potential pathogens, as well as the creation of niche environments that specifically recruit and support beneficial microbes with activities including plant growth promotion and nutrient acquisition.

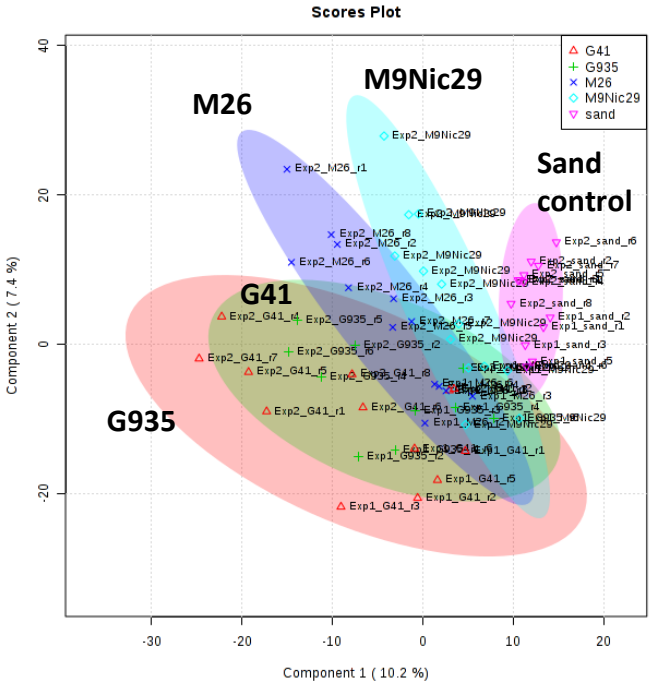

Fig. 5. A multivariate data analysis of tree-specific root exudate metabolites recovered at the end of two greenhouse experiments indicates that metabolic profiles of apple rootstock cultivars G41 and G935 root exudates are most similar to each other in these studies. These two cultivars have exhibited greater field tolerance to apple replant disease (ARD) than either M26 or M9Nic29. This result could have implications for breeding or management of ARD.

Significant finding 5. pH of both soil and water percolated through the tree rhizosphere were altered by the presence of a rootstock but did not differ among rootstocks (**Fig. 6**). pH changes in the soil can affect nutrient availability.

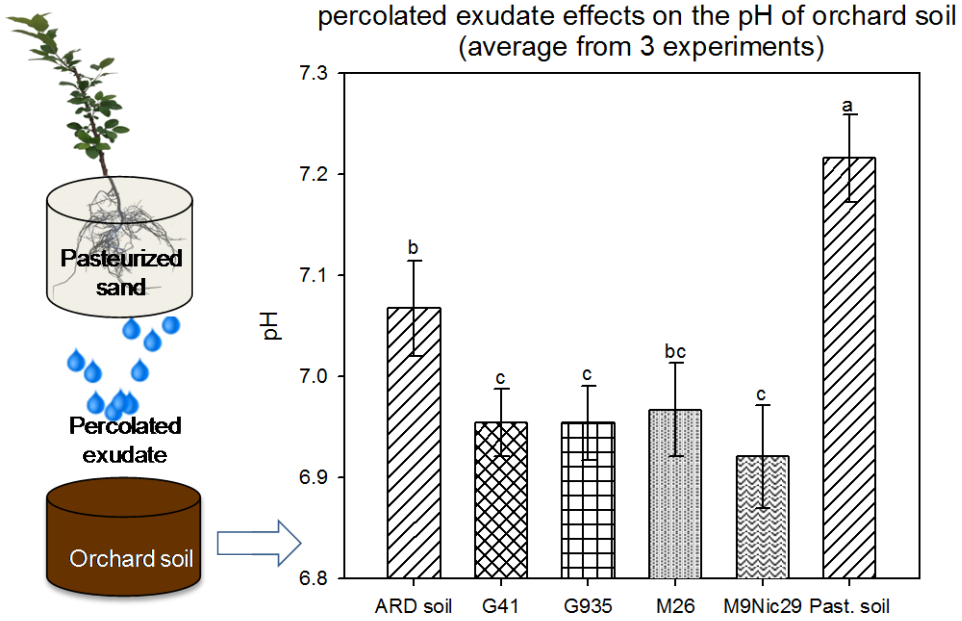

Fig. 6. The average pH of orchard soil after 2 months of treatment with root exudates from four different apple rootstock cultivars differed from the controls (ARD soil and pasteurized soil).

References:

Hewavitharana SS, Ruddell D and Mazzola M (2014) Carbon source-dependent antifungal and nematicidal volatiles derived during anaerobic soil disinfestation. *European Journal of Plant Pathology* 140:39-52.

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Mazzola M, Hewavitharana SS and Strauss SL (2015) Brassica seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation. *Phytopathology* 105:460-469.

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Robertson CE, Harris JK, Wagner BD, Granger D, Browne K, Tatem B, Feazel LM, Park K, Pace NR and Frank DN (2013) Explicet: graphical user interface software for metadata-driven management, analysis and visualization of microbiome data. *Bioinformatics* 29:3100-3101.

Rudell D, Mattheis J and Hertog M (2009) Metabolomic change precedes apple superficial scald symptoms. *Journal of Agricultural and Food Chemistry* 57:8459-8466.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-16-107

Project Title: At-harvest protocols for apple fruit disorder and quality management

PI:	Jim Mattheis	**Co-PI:**	Dave Rudell
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Address:	USDA, ARS	**Address**:	USDA, ARS
Address 2:	1104 N. Western Avenue	**Address 2**:	1104 N. Western Avenue
City/State/Zip:	Wenatchee, WA 98801	**City/State/Zip**:	Wenatchee, WA 98801

Cooperators: Glade Brosi, Stemilt Growers, Wenatchee; Lee Kalcsits, Corina Serban, WSU TFREC

Total Project Request: **Year 1:** $53,441 **Year 2: $53,956** **Year 3:** $54,476

Other funding sources: USDA, ARS ($25,462 salary and benefits, GS-9 technician; $27,608 CA chamber refurbishment)

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

Item	2016	2017	2018
Salaries	$35,135	$35,239	$35,828
Benefits	$17,306	$17,716	$17,647
Wages			
Benefits			
Equipment			
Supplies	$1000	$1000	$1000
Travel			
Miscellaneous			
Plot Fees			
Total	$53,441	**$53,956**	$54,476

Footnotes: 0.75 Salary, benefits for GS-7 technician

OBJECTIVES

1) Identify optimum controlled atmosphere conditions during 'Honeycrisp' conditioning.
2) Determine impacts of CA established during temperature conditioning on fruit quality and disorder development of 'Gala', 'Golden Delicious', and 'Granny Smith' apples.
3) Compare how 1-MCP and rapid CA establishment during temperature conditioning impact disorders and fruit quality.

SIGNIFICANT FINDINGS

- 'Honeycrisp' bitter pit incidence was reduced by one to four weeks in CA followed by storage in air, or by 1-MCP treatment the day after harvest followed by CA or air storage.
- No external CO_2 disorders developed through 3 months storage on 'Honeycrisp' apples stored in CA with O_2 initially at 3% then reduced to 2% with up to 2% CO_2.

METHODS

Fruit will be obtained from commercial orchards at commercial maturity. At harvest, maturity analyses (starch index, firmness, soluble solids content, titratable acidity, weight, color, internal ethylene content, dry matter) will be performed. Fruit from each orchard will be conditioned 7 days at 50 ^{o}F or cooled immediately to 33 ^{o}F (all except 'Honeycrisp'). 1-MCP treatment will be applied the day fruit is received. CA will be initiated 1 or 7 days after receipt. Fruit chlorophyll fluorescence will be monitored during initiation of 'Honeycrisp' CA and also as space and time permits for other varieties. One storage atmosphere will be at the oxygen concentration where a change in chlorophyll fluorescence is observed, one atmosphere will be 0.2-0.4% O_2 higher, and another 1-2% higher. Fruit will also be stored in air. All fruit will be stored in the CA cold storage facility in the USDA, ARS Wenatchee laboratory.

Objectives 1,3, 'Honeycrisp': Experiments will be conducted in years 1 and 2 with additional work as needed in year 3. Our goal is to identify CA conditions that fail to reduce bitter pit or induce other physiological disorders. Previous studies have used 2% O_2, 0.5% CO_2 as the final CA set points. The proposed work with add oxygen concentrations below 2%. Fruit will be stored for up to 8 months. An additional trial will examine CA duration impacts on bitter pit development. A previous study indicated 1-MCP plus CA storage for one week or more reduces bitter pit development compared to untreated fruit stored in air (Figure 1). This study will be repeated using multiple lots. After receipt, fruit will be treated or not with 1-MCP at 50 ^{o}F. CA (2% O_2, 0.5% CO_2) will be established the day after receipt, and fruit will remain in CA for 1, 2, 4, or 8 weeks followed by storage in air for up to 4 months. The final storage temperature will be 37 ^{o}F.

Objectives 2,3: Studies with 'Gala' apples will be conducted in years 1 and 2, and in year 3 for 'Golden Delicious' and 'Granny Smith'. Fruit will be obtained from orchards with a history of peel and/or internal disorders. Apples will be cooled to 33 or 50 ^{o}F and CA (1% O_2, 1% CO_2) established 1 or 7 days after receipt. Application of 1-MCP will be on the day fruit is received. Fruit will be evaluated for external disorders monthly and internal disorders and quality after 2, 4, 6 ('Gala') and 8 ('Golden Delicious', 'Granny Smith') months plus 7 days at 70 ^{o}F. 'Granny Smith' studies will include CA settings determined using chlorophyll fluorescence monitoring similar to that described for 'Honeycrisp'.

RESULTS & DISCUSSION

'Gala' and 'Fuji' experiments: Delayed cooling. Two Gala lots, one with a history of internal browning development, one netted, were obtained at commercial maturity. Fruit were held at 50 ^{o}F

for 7 days then at 33 °F. During the week at 50 °F, some fruit were treated with 1-MCP and/or CA (1% O_2, 1% CO_2) was established. CA was also established after the storage temperature was reduced to 33 °F. As internal browning typically develops slowly, fruit will be assessed after 6 months.

Ultra-low O_2 storage. The same two lots as above were cooled to 31 or 33 °F. CA was established three days after receipt with O_2 held at either 0.5 or 1.0% O_2 and 0.5% CO_2. Fruit disorders and quality will be assessed after 7 months in storage.

Orchard	weight g	ground color 1-5	starch 1-6	SSC %	TA %	lbs	IEC ppm
Vantage	202±14	3.9±0.2	1.7±0.5	10.5±0.3	0.355±0.015	19.0±1.8	0.88±0.62
Saddle Mtn	197±26	3.8±0.5	2.6±1.6	13.5±0.7	0.323±0.013	18.0±2.3	3.4±1.4

Table 1. 'Gala' maturity at harvest. Values are mean ± standard deviation for 18 fruit. Ground color: 1=green, 5=yellow; starch: 1=full, 6=none; SSC: soluble solids content; TA: titratable acidity; IEC: internal ethylene content.

weight g	ground color 1-5	starch 1-6	SSC %	TA %	lbs	IEC ppm	watercore %	watercore severity 1-4
233±33	2.8±0.7	5.9±0.1	12.5±0.7	0.274±0.015	14.0±1.2	1.1±1.4	83	2.2±0.7

Table 2. 'Fuji' maturity at harvest. Values are mean ± standard deviation for 18 fruit. Ground color: 1=green, 5=yellow; starch: 1=full, 6=none; SSC: soluble solids content; TA: titratable acidity; IEC: internal ethylene content; watercore %: incidence of watercore; watercore severity: 1=none; 4=severe.

'Honeycrisp' experiments. **Short-term CA.** Fruit from two commercial orchards near Quincy, WA was obtained and held at 50 °F for 7 days. Fruit was stored in air or CA (2.5% O_2, 0.5% CO_2) established 1 or 9 days after receipt. 1-MCP was applied to some fruit the day of receipt. After 1, 2, or 4 weeks, fruit where CA was established the day after receipt was removed from CA and held in air. Through 3 months after harvest, storage in CA for 1-4 weeks reduced bitter pit development compared to fruit stored continuously in air (Figures 1,2). Treatment with 1-MCP reduced bitter pit development in fruit stored in air or CA. Disorders and fruit quality will be assessed after 4 and 7 months storage.

Orchard	weight g	ground color 1-5	starch 1-6	SSC %	TA %	lbs	IEC ppm
A	323±66	1.2±0.4	5.0±1.0	14.3±0.6	0.546±0.062	13.4±1.2	21±15
B	237±38	4.0±1.4	4.0±1.4	12.1±0.2	0.477±0.014	13.5±0.9	2.9±3.9

Table 3. 'Honeycrisp' maturity at harvest. Values are mean ± standard deviation for 18 fruit. Ground color: 1=green, 5=yellow; starch: 1=full, 6=none; SSC: soluble solids content; TA: titratable acidity; IEC: internal ethylene content.

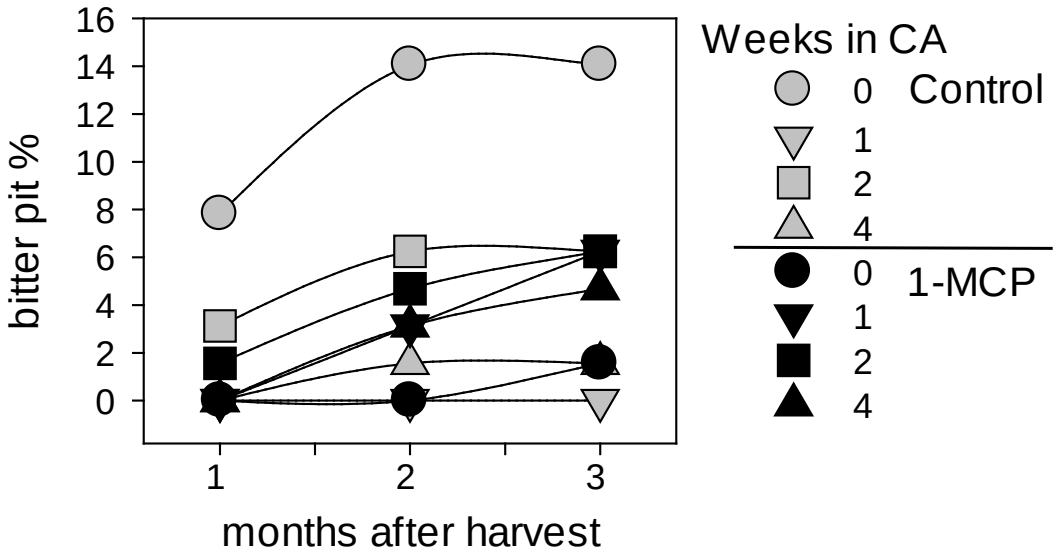

Figure 1. Bitter pit incidence in 'Honeycrisp' apples through 3 months in storage (orchard A). All fruit were held 7 days at 50 °F then at 37 °F. Fruit were held in air or a CA (2.5% O_2, 0.5% CO_2) established 1 day after harvest for 1,2, or 4 weeks then in air at 37 °F.

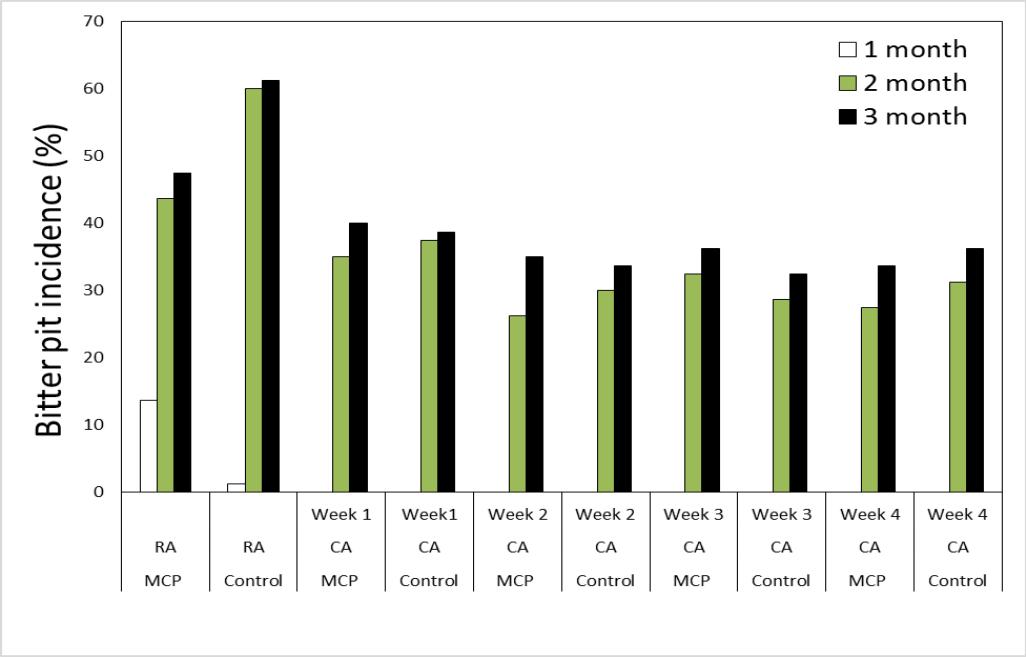

Figure 2. Bitter pit incidence (%) in 'Honeycrisp' apples (Orchard B) treated with 1-MCP or an untreated control and stored 0, 1,2 3, or 4 weeks in CA before air storage. Results of Corina Serban and Lee Kalcsits.

DPA and Rapid CA. 'Honeycrisp' apples can develop internal browning during low O_2/high CO_2 storage. DPA can prevent this type of injury in 'Honeycrisp' and other varieties but has not been evaluated when CA is established during conditioning. Fruit were treated with 1000 ppm DPA and/or

1-MCP after harvest then stored in air or CA established during conditioning. Disorders and fruit quality will be evaluated after 8 months storage.

CA Settings during Conditioning: CA during conditioning to date has reduced bitter pit development and not caused development of other disorders. In addition to the CA protocol used previously (3% O_2 for 2 days then 2%, 0.5% CO_2), fruit were stored in 2% O_2, 0.5% CO_2 continuously, or the 3% O_2 for 2 days then 2% protocol with 1 or 2% CO_2.
Overall 1-MCP treated fruit had less bitter pit, more peel blotch, but the same total number of fruit with an external disorder.

CA	1-MCP	BP %	BP severity 1-4	peel blotch %	disorders %
2% O_2, 0.5% CO_2	no	31	1.4	0	31
3/2% O_2, 0.5% CO_2		44	1.5	0	44
3/2% O_2, 1% CO_2		44	1.7	0	44
3/2% O_2, 2% CO_2		41	1.7	0	41
2% O_2, 0.5% CO_2	yes	36	1.4	23	36
3/2% O_2, 0.5% CO_2		37	1.4	17	37
3/2% O_2, 1% CO_2		25	1.2	21	25
3/2% O_2, 2% CO_2		24	1.2	11	24

Table 4. 'Honeycrisp' external disorders after 3 months storage. BP: bitter pit; BP severity: 1=none; 2=1-25% peel with bitter pit; 3=26-50% peel with bitter pit; 4=51-100% peel with bitter pit. Peel blotch=peel lesions larger than bitter pit.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-16-108A

Project Title: Validation of the Red Delicious pollen tube growth model

PI: Keith Yoder
Organization: Virginia Tech
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Cooperators: Leon Combs, Research Specialist, Virginia Tech AHS-AREC; Winchester, VA
E-mail: lecombs@vt.edu
Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA

Total Budget: Year 1: $16,640 **Year 2: $16,640**

Other funding sources: None

WTFRC Collaborative expenses:

Item	2016	2017
Salaries	2000	2000
Benefits	600	600
Wages	1000	1000
Benefits	250	250
Shipping	50	50
Supplies	50	50
Travel	250	250
Total	$4,200	**$4,200**

Budget:
Organization Name: Virginia Polytechnic Institute and State University (Va. Tech)
Contract Administrator: Eric James Dinwiddie, Pre-Award Administrator
Telephone: 540-231-9368 Email address: EricJD@VT.edu

Item	2016	2017
Salaries*	8000	8000
Benefits	4080	4080
Supplies	360	360
Total	12,440	**12,440**

*Note: Salary for Research Specialist Leon Combs.

OBJECTIVES

Objective 1

1. **Validation Testing of Red Delicious Pollen Tube Growth Model in Washington Orchards. (Virginia Tech & WTFRC)**
   Pollen tube growth model validation will include criteria from three tests in 2016 and/or 2017:

   Test 1: Commercial use of the pollen tube growth models. In this test, grower-participants use the models made available to them through the AgWeatherNet website. These growers (beta-testers) trained in the use of the models then monitor the blocks start times and bloom thinning application timings. At the end of harvest, the beta-test participants rate their actual crop relative to their ideal expected yield. Comparing the desired yield with the actual harvested yield demonstrates that the beta-test participants understand the principles of the model and that it is working to their satisfaction. This harvest data will be cross-referenced with application timings as done with other models in previous years.

   Test 2: Validation test 2 includes flower samples collected in Washington orchards after thinning chemicals were applied, by comparing model-predicted pollen tube growth versus actual growth in flowers. Flower samples from beta-test blocks will be evaluated microscopically to determine if fertilization occurred on the segment of the flower population that was intended to be the harvested crop. Bloom thinning applications can then be re-applied to reduce unwanted additional cropping.

   Test 3: We will request harvest data from selected Washington orchard blocks that will be bloom thinned using the pollen tube growth model in the 2016 growing season. This data, if available for validating the Red Delicious model, will come from selected beta-testers who had access to the Red Delicious beta test model for the 2016 growing season.

SIGNIFICANT FINDINGS

- Working with 75 test sites in Washington State in 2016 (Graph 2), we are evaluating the Red Delicious pollen tube growth model as a precision bloom-thinning tool.
- Microscopic evaluation of the model in the laboratory of sampled flowers from the field to determine the percent of flowers that had been fertilized shows predictive effectiveness of Red Delicious model.
- Reported harvest data results show that the pollen tube growth model is helping growers achieve their targeted crop load.

METHODS:

Objective 1 (Virginia Tech):

Flowers will be removed from trees and placed in a solution of 5% sodium sulfite in labeled glass containers. Samples will be refrigerated at 38°F until processing. Flower samples are prepared by boiling for 15 minutes. Pistillate organs are dissected from the remaining flower tissue, rinsed with distilled water, stained with 0.01% aniline blue in 0.067M K_2HPO_4, and then squashed between a coverslip and slide. Slides are stained for 24 hours before examination at 100X under fluorescent light using a Zeiss HBO-50 high-pressure mercury vapor light source and a Nikon Optiphot microscope. Collected data include the abundance of pollen germination/tube growth on the stigma surface using a 0-10 rating scale, number of tubes penetrating the stigma base, mean length of the longest pollen tube,

mean style length, and number of pollen tubes reaching the base of the style. This procedure was modified from procedures used by Embree and Foster (1999).

Objective 2:

WTFRC:

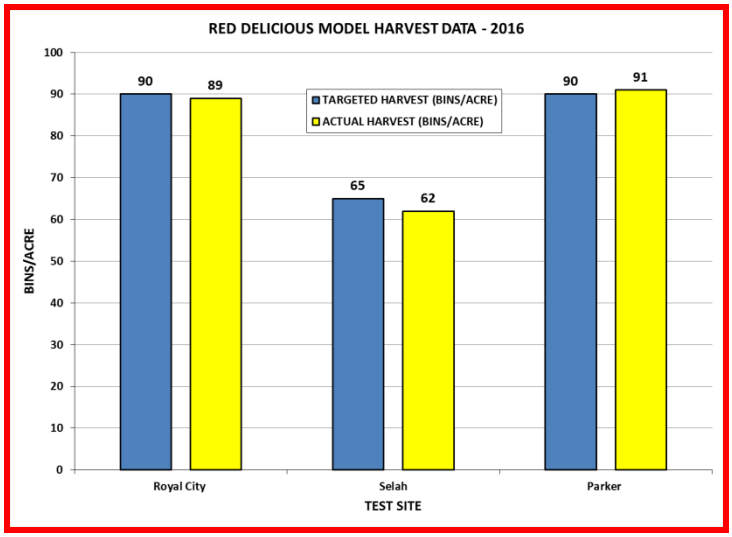
WTFRC staff will work with commercial growers to select several orchard blocks for these tests. In each block, they will randomly flag four trees (border and unhealthy trees will be avoided). On the flagged trees, they will tag or flag six flower clusters (with the king bloom open) that are part of target crop load. In other words, these are the flowers that should become fertilized before the first chemical thinning application. Forty-eight hours after first bloom thinning spray, whole flagged clusters will be removed from the tree. Petals will then be removed and the king bloom marked with a permanent marker to distinguish it from the lateral blooms. The whole cluster will then be placed into a plastic bottle containing a 5% sodium sulfite (5 g/100 ml distilled water) solution. After all samples are collected, the samples will be shipped to Virginia Tech AHS-AREC for histological evaluation.

Virginia Tech

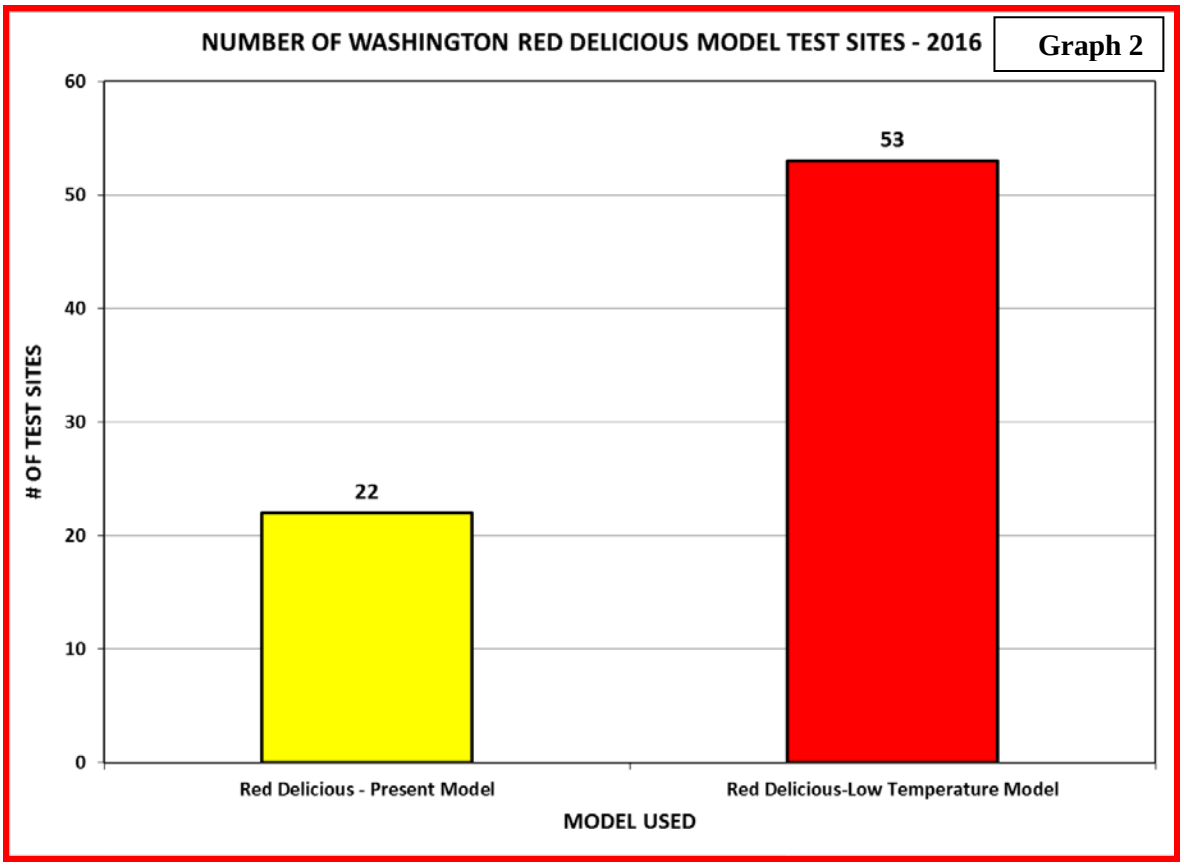
Upon receipt at the Virginia Tech facility, samples will be refrigerated at 38°F until processing. The flowers will be prepared and examined as described for Objective 1. Collected data will be the same as described for Objective 1.

RESULTS AND DISCUSSION

As stated in previous reports on models presently being used by growers, tracking actual harvest totals versus desired cropping is needed to verify the models effectiveness. Comparing desired crop load with actual harvest data (Graph 1, Table 1) demonstrates either understanding of beta-testers in implementation or need for further training in initiation of the modeling program at the proper time. Results from field evaluations of desired bins/acre vs actual bins/acre harvested show that the model helps beta-testers/growers achieve their targeted crop. Evaluation of the model in 2017 will include sampling flowers from the field as in 2016 (Graph 3, 4) to determine the percent that have been fertilized, which will further validate model predictions. Comparing average style length determined in the field and in the laboratory is an integral part of evaluating and refining the model to actual field conditions as well.



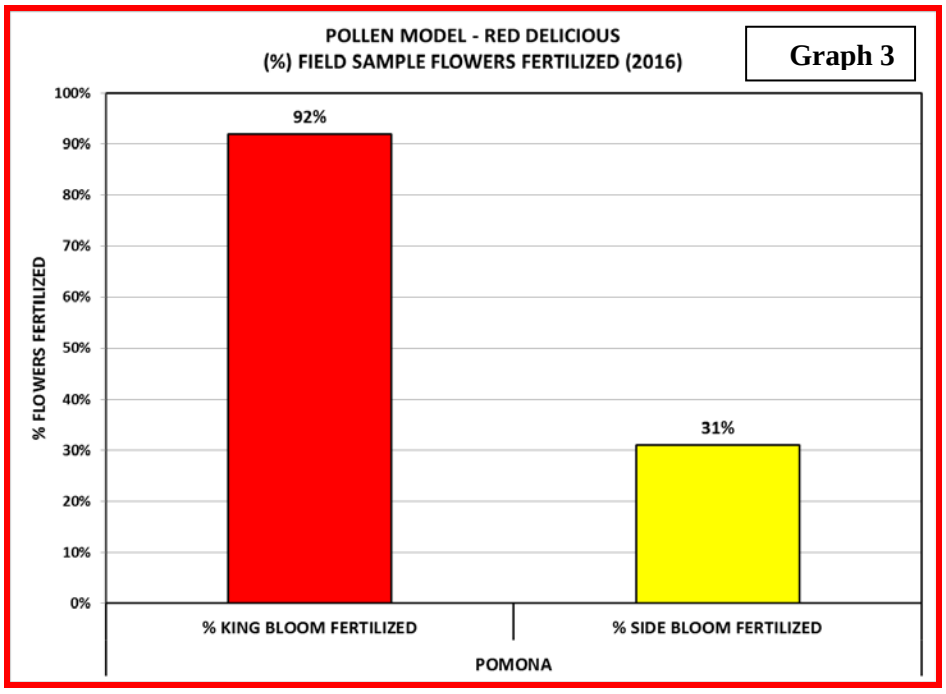
Graph 1. Comparison of targeted crop to actual harvested crop demonstrates the effectiveness of the model in predicting thinning applications and understanding of model concept by users.

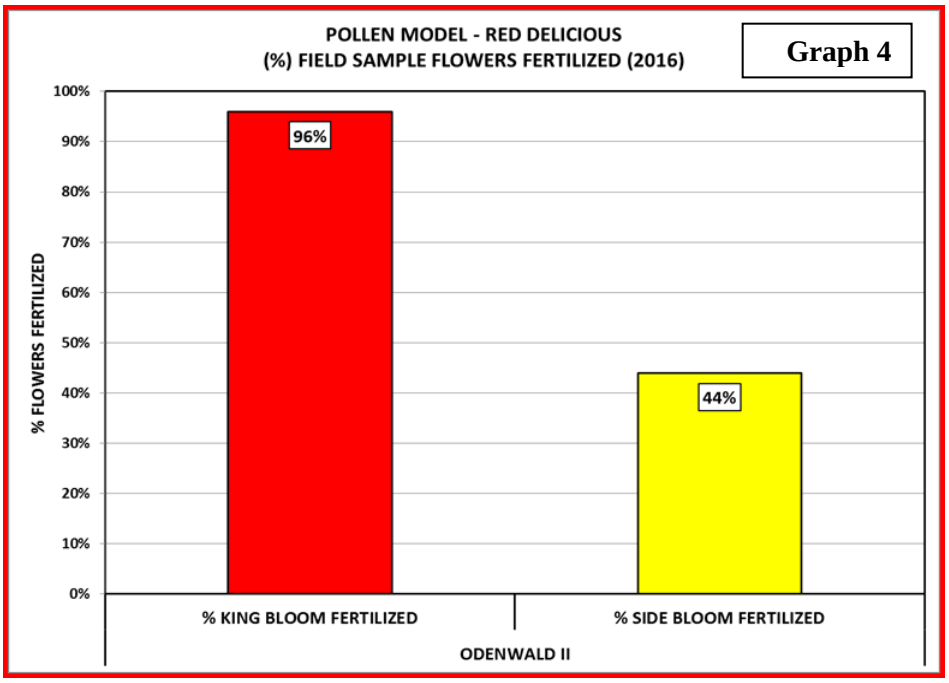


GRAPH 2. 75 registered Red Delicious test sites in Washington State in 2016. 22 sites used present model and 53 sites used new model incorporated with low temperature growth rates.

LOCATION	AVERAGE STYLE LENGTH (MM)	TIMING APPLIED (MM)	TIMING APPLIED (% of style length)	TARGETED HARVEST (BINS/ACRE)	ACTUAL HARVEST (BINS/ACRE)
BLO 6 STRIPE	8.21	7.64	93%	35.7	33.9
BLO 7 STRIPE	9.12	8.18	90%	36.5	43.5
BLO 8 STRIPE	8.59	7.20	84%	36.3	56.8
CAS 8	9.20	8.00	87%	32.4	21.3
FRO 5 STRIPE	8.72	6.75	77%	50.5	55.5
ODO 2	7.94	7.17	90%	40.9	46.8
WIN 5	8.99	7.77	86%	45.9	54.2
CAS 10	8.30	7.30	88%	36.8	36.0

Table 1. Harvest and application timing results from tests conducted in 2014 at Washington beta-test sites.

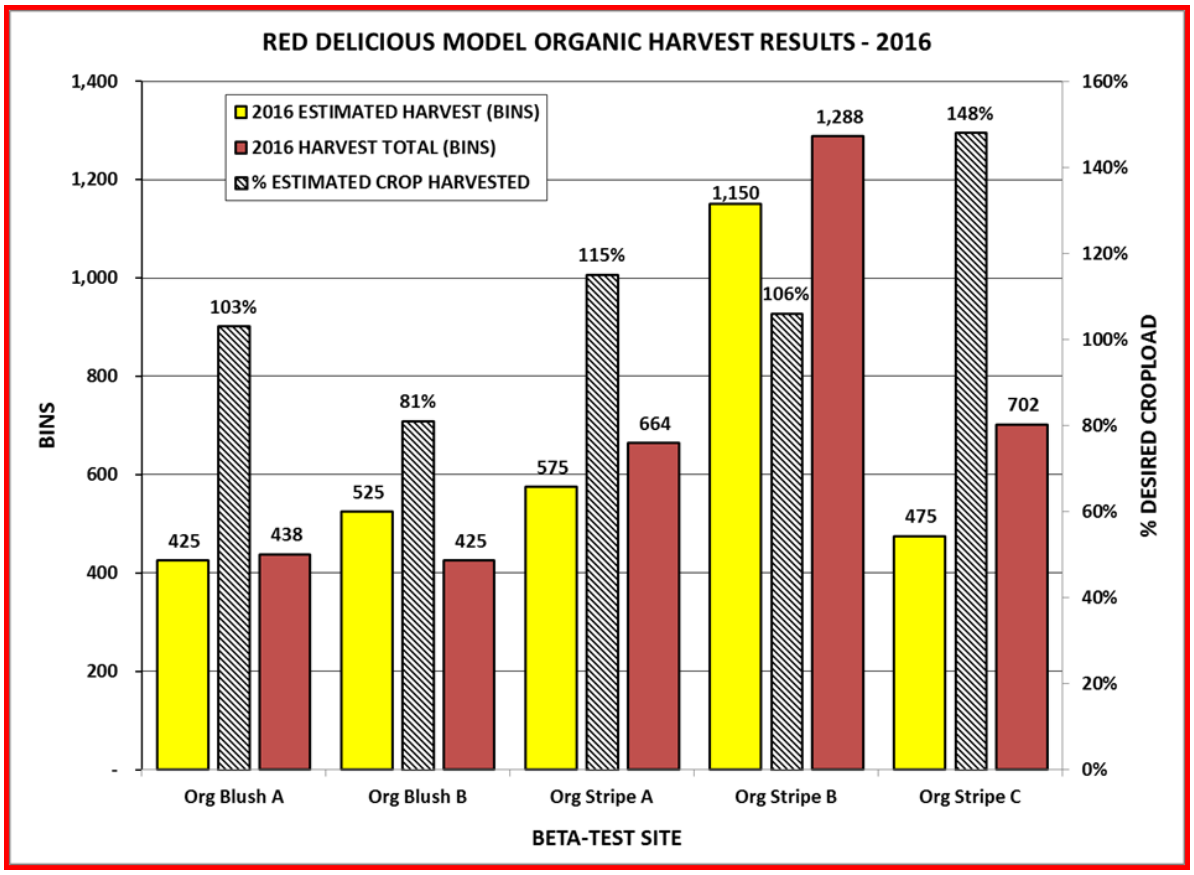




Graphs 3 and 4. Microscopic evaluation of model predicted fertilization of sampled flowers from the field beta tests in 2016 shows a high percent of king bloom fertilized and a relatively lower percent of side bloom fertilized.

As was stated in our proposal, an in-orchard study at Winchester, VA in 2007 showed that there may be as much as a three-fold difference in pollen tube growth rates among cultivars, with 'Red Delicious' standing out as being the slowest of seven cultivar models presently or soon to be available to the public for use as a bloom thinning tool. This delayed pollen tube growth in Red Delicious could lead to serious over-thinning if one ignored these differences and based the timing of Red Delicious bloom-thinning applications on the models for other cultivars currently available

through AgWeatherNet rather than on a fully validated Red Delicious model. In all models the user will need to implement the models according to thinning factors of governing whether thinning for conventional crops or organic. The options for thinning are more restricted in organic crops so those growers need the Red Delicious model tested and validated rigorously. As shown below in Graph 5 crop load results for 2016 at beta-test sites in Washington showed help in crop load management in all but one test site.



Graph 5

We would like to thank the Washington Tree Fruit Research Commission for its continued support of this project. We would particularly like to thank Tory Schmidt, whose help on the project has been essential to the project's success. Lastly, we would like to thank the beta-testers, growers, and others who are providing critical feedback on the pollen tube growth model.

CONTINUING PROJECT REPORT **YEAR**: 2016

Project Title: Apple rootstock evaluation
PI: Tom Auvil
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WTFRC Staff cooperators: Ines Hanrahan, Mano Mendoza, Tory Schmidt, Jim McFerson, Kyle Tynan
Cooperators: Mark Wilcox, Jim Divis, Scott McDougall, Dave Taber

Collaborators: Dr. Gennaro Fazio, USDA-ARS, Geneva, New York

Total Project Request: **Year 1:** 57,900 **Year 2: 110,165** **Year 3:** 118,040

WTFRC Expenses:

Item	2016	**2017**[3,4]	2018[4]
Salaries[2,3]	30,500	40,500	41,600
Benefits[2,3]	10,000	13,365	14,560
Crew Wages[3,4]	5,000	33,000	36,000
Crew Benefits[3,4]	1,000	4,900	5,480
Stemilt RCA room	8,400	8,400	8,400
Shipping			
Supplies			
Travel[1,4]	3,000	10,000	12,000
Miscellaneous			
Total	57,900	**110,165**	118,040

[1]Fuel and maintenance plus hours for time slip to travel to plots
[2]Salaries and benefits for Auvil, Hanrahan, Schmidt, and Mendoza apportioned to this project
[3]2017 will see large increase in field activity, fruit harvest, storage and lab activity. 4 new, small trials to evaluate 3 new rootstocks from New Zealand are planned
[4]Minimum wage increase

Note: WTFRC work on Phase 3 trials of the apple breeding program is available in the apple breeding program report along with the WTFRC collaborative budget for the scion project.

OBJECTIVES:

1. Evaluate performance of replant tolerant Geneva apple rootstocks in new ground and replant sites compared to commercial standards, in commercial settings in Washington State. Three new rootstocks from New Zealand will be placed in grower trials with Honeycrisp scion.
2. Conduct outreach activities to provide opportunity for nurseries and industry to see new commercially available rootstocks in the Geneva family as the trees grow canopy and become productive.

Rootstock findings and activities:

- Field days were held in October with participation from the rootstock liner producers, finished tree nurseries and Gennaro Fazio, the national apple rootstock breeder with USDA located at Geneva, New York.
- Rootstock information is updated and is on the treefruit.wsu.edu website.
- The overall grouping of canopy volume by rootstock in the 2015 trials are similar to previous trial results.
- G.969 continues to look very promising in many aspects including nursery propagation, yield, woolly aphid resistance and replant tolerance.
- G.935, G.30, G.11 and CG. 4011 are NOT woolly aphid resistant. We encourage growers and nurseries to pursue the woolly aphid resistant genotypes.
- Availability of G.30 is declining due to its unreliable propagation performance.
- For the 2015 rootstock trial plantings, G.890, G.935, G.214, G.969, G.11 had good propagation success across the four scion varieties in trials. G.210 and G.41 did well except with Honeycrisp in terms of liners planted and finished trees delivered. G.41 also had some transplant issues with Honeycrisp and Red Delicious.
- G.41 has encountered broken unions especially with large caliper trees from some, but not all nurseries. ½" and smaller caliper trees have very minimal union breakage. ¾" caliper and larger trees on G.41 can have serious losses, especially with Cripps, Honey Crisp, Scilate/Envy and Jonagold.
- G.41 has issues in transplanting which may be related to the number of roots on the plant being transplanted (fewer roots = less transplant success). Tissue culture sourced liners seem to have more consistent and higher root count.
- G.969 has good to excellent finished tree propagation traits. The initial year of yield data indicates G.969 will be similar to other members of the replant tolerant Geneva's in productivity: equal to or better than Mark and M.9

Figure 1. Brewster Pazzaz Trunk Cross Sectional Area (TCSA)

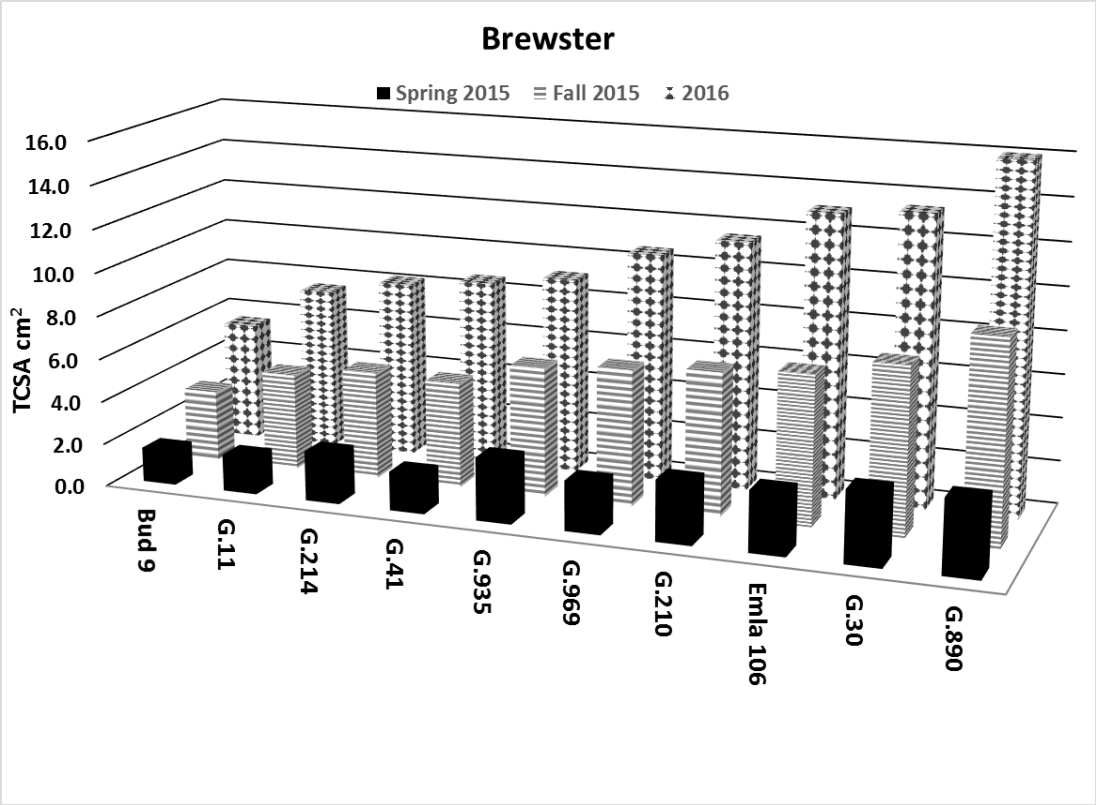
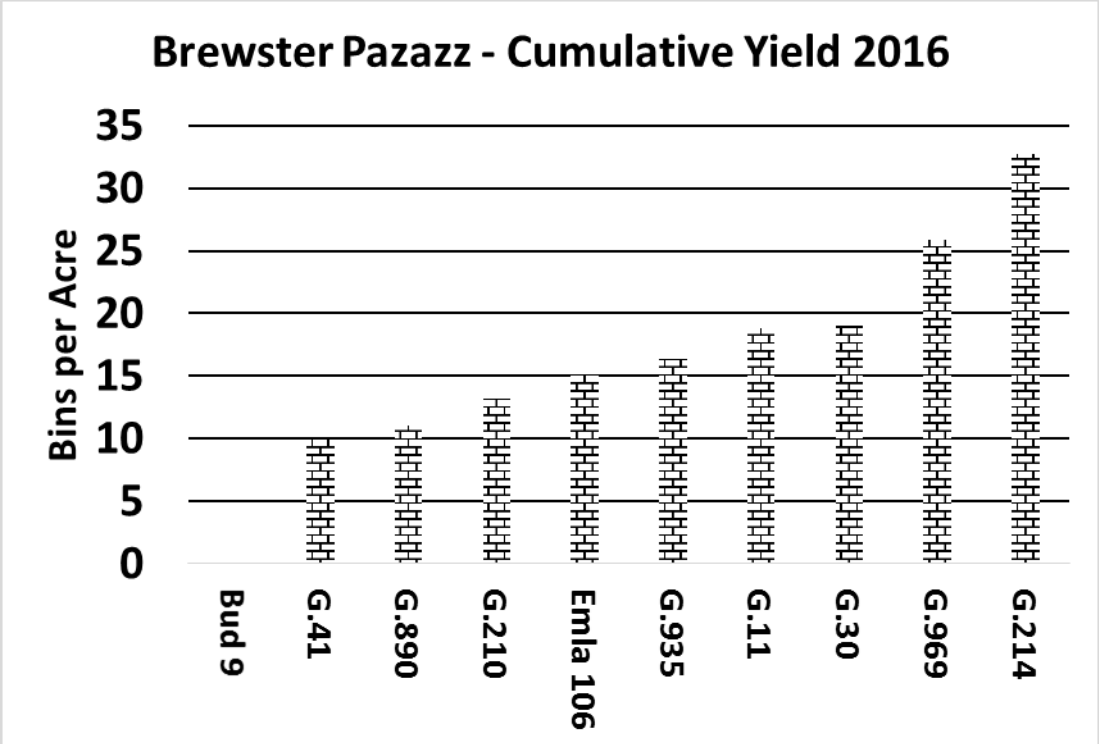


Figure 2: Brewster Pazzazz yield in bins per acre



The Pazazz trial was chemically thinned (accidentally) with the larger, more vigorous rootstocks (G.890, G.210, EMLA 106) overthinning. The Bud 9 were deflowered prior to bloom and the G.41 were heavily thinned prior to bloom..

Figure 3: Oroville Honeycrisp trunk cross sectional area (TCSA)

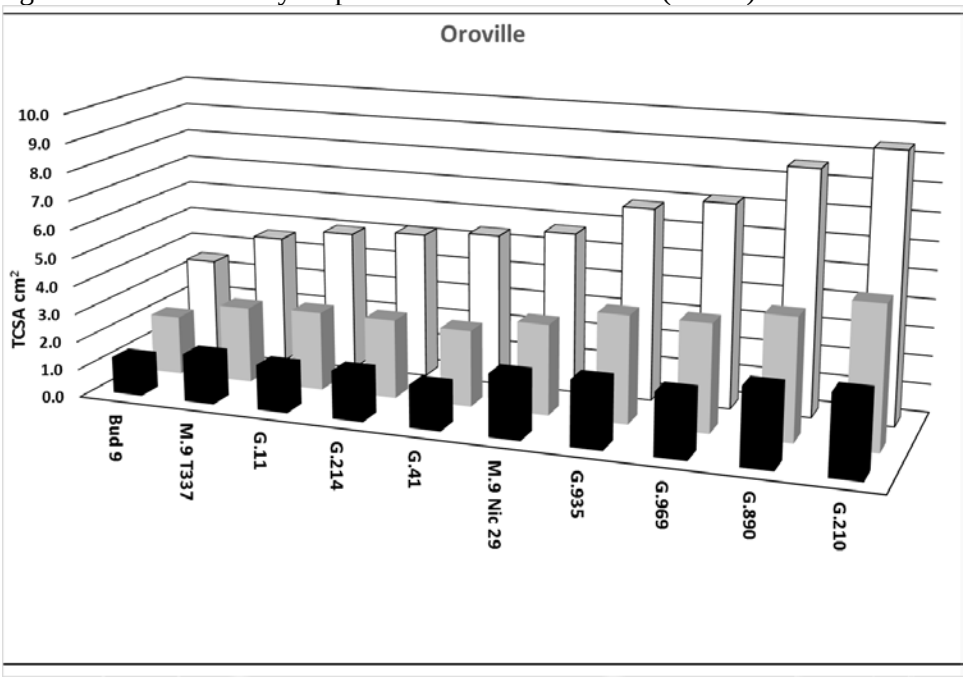


Figure 3: East Wenatchee Honeycrisp trunk cross sectional area (TCSA)

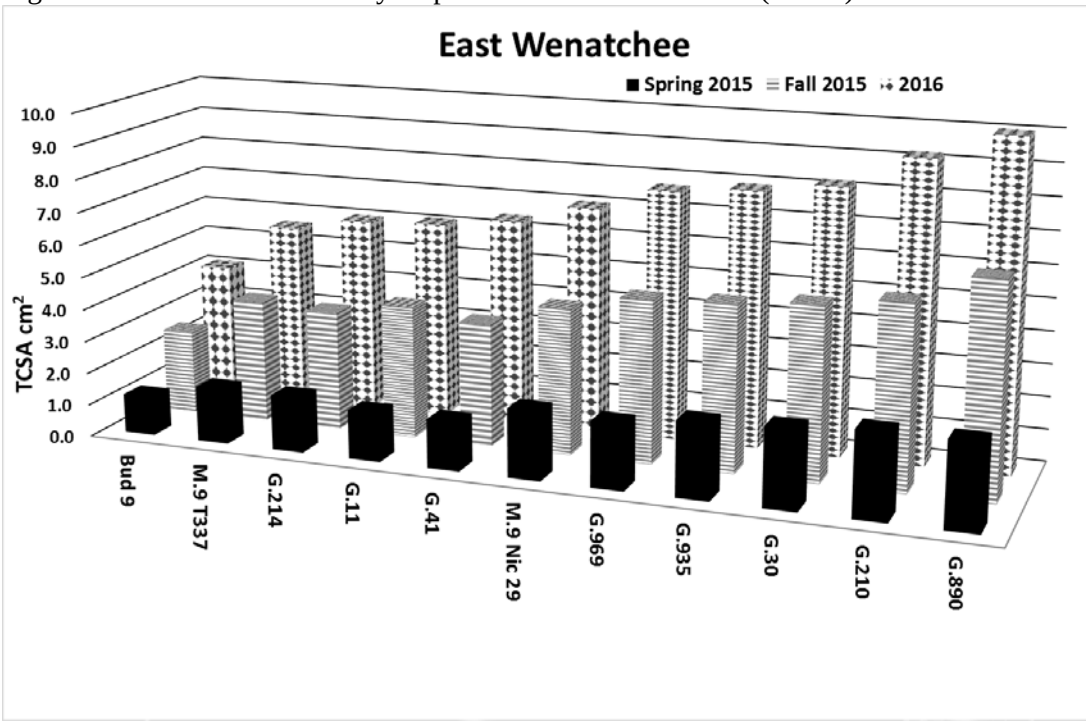


Figure 4: Wapato Red Delicious replacement tree trial TCSA

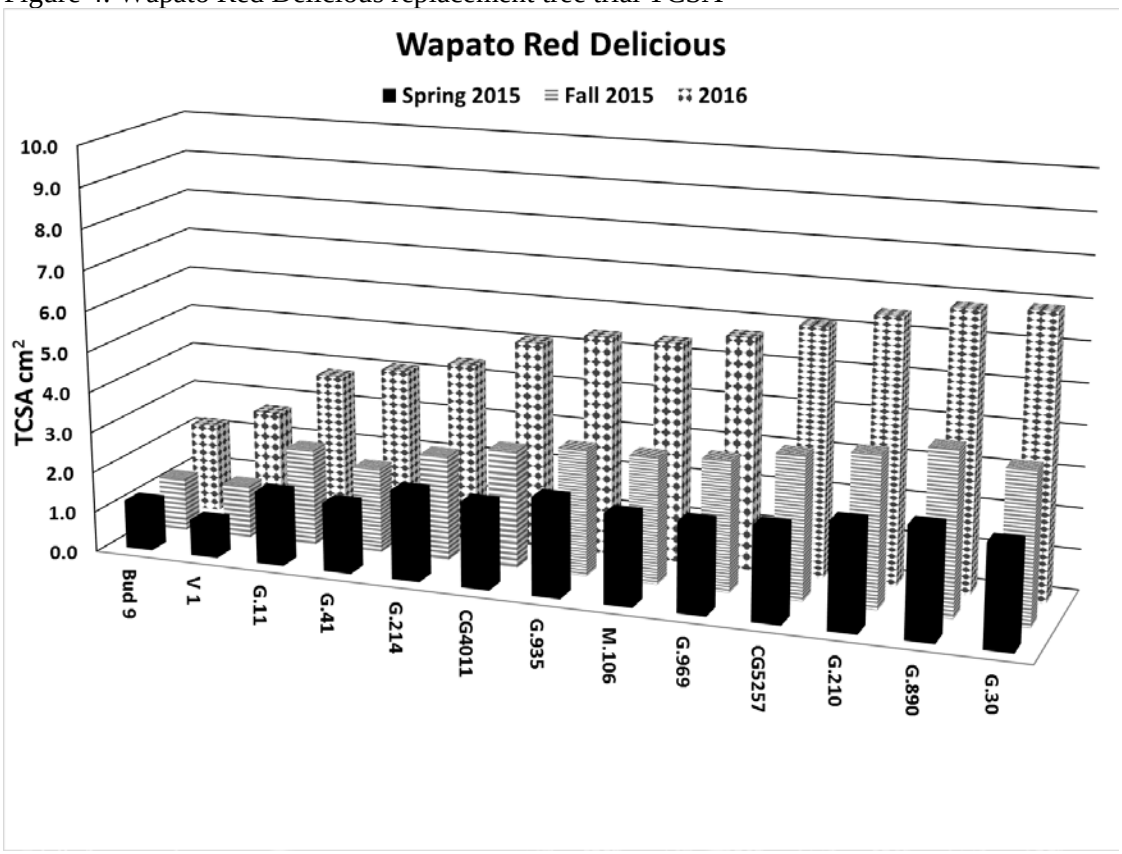
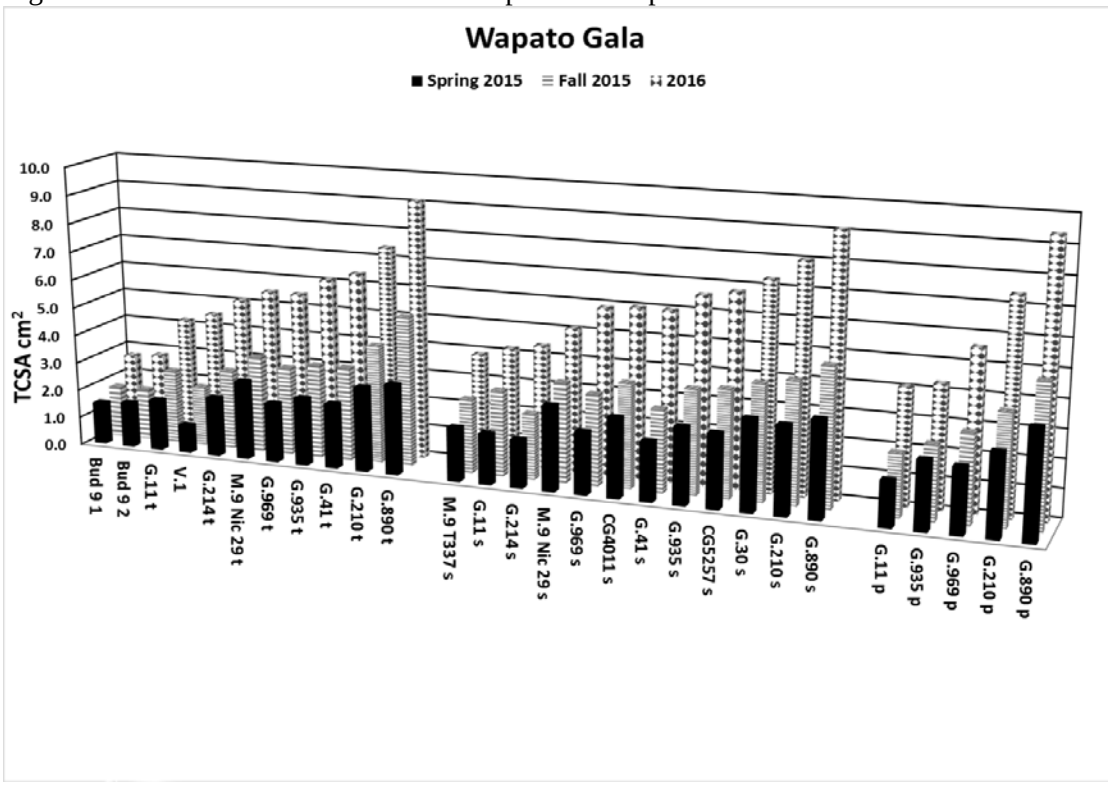


Figure 5: Trunk cross sectional area of Wapato Gala replacement tree trial.



S= all feathers stubbed to 4 inches, reducing bloom therefore fireblight potential- Feathers regrew by the end of the season.
T= all feathers tipped by removing 4 buds from terminal- significant blind wood
P= feathers pruned off and central leader headed at 30 inches

Statistical groupings across trials:

- G.11, G.214 and M.9 T337 are statistically similar.
- B.9 was consistently the smallest.
- G.890, M.106, G.210 and G.30 are consistently the largest trees. Based on previous trials, the cropping potential of the Geneva rootstocks will keep the tree canopy size in the 'M.26' class, significantly smaller than M.106. G.210 is not as vigorous as G.890, though can be more vigorous than G.969 and G.935.
- M.9 Nic 29, G.969, G.935 are statistically in the middle, or Large M.9 category.
- In the Wapato Gala trial, the pruning treatments revealed the severe heading was indeed horticulturally 'dwarfing' reducing canopy volume compared to other pruning treatments.
- The very light tipping did not reduce blind wood. The stubbing, at least initially, appears more effective at reducing the blind wood. Third leaf yields should determine if blind wood is a production problem.
- Stubbing and the severe heading reduced bloom and risk of fireblight infection.
    - Fireblight in the Wapato Gala trial is extremely high in the established M.9 trees. About 10% of the trees planted in 2010 had dead rootstocks in 2015. Fire blight management was intense in spring 2016 and control was very successful.
- Crop load will play a significant role in managing vigor of the Geneva rootstocks.
- B.9, M.9 337 and M.9 Nic29 show replant stress in the Gala and Red Delicious trials. M.106 is showing replant stress in the Red Delicious trial. V.1 is tolerating the replant conditions in both Gala and Red Delicious in Wapato.

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

Project Title: Crop load and canopy management of apple

PI: Tory Schmidt
Organization: WTFRC
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State/Province/Zip WA 98801

Cooperators: Jim McFerson, Ines Hanrahan, Manoella Mendoza, 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	30,000	20,000
Benefits	10,000	9,000	6,000
Wages	50,000	35,000	26,000
Benefits	17,000	12,000	8,600
Equipment			
Supplies	1,000	500	500
Travel	3,000	2,500	2,000
Stemilt lab fees	2,000	1,500	500
WSU plot fees			6,400
Statistical consulting	1,000	0	0
Total gross costs	119,000	90,500	70,000
Reimbursements	(119,000)	(87,000)	(70,000)
Total net costs	0	3,500	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

2014-2016 CONCLUSIONS:

K-Pax, an alternative lime sulfur formulation, performed similarly to Rex Lime Sulfur in two years of thinning studies (Table 2)

Spray oil + lime sulfur programs are the most efficacious options for chemical bloom thinning of apple (Table 3)

Metamitron can effectively reduce fruit set and boost fruit size in WA conditions when used aggressively (Tables 4, 5)

Metamitron efficacy can be promoted by tank mixing with non-ionic surfactants, lightweight summer petroleum oils, or NAA (Table 4)

Aggressive metamitron programs can induce phytotoxicity in apple trees experiencing carbon stress, but effects are largely temporary

High temperatures combined with low light conditions following applications of postbloom thinners can amplify treatment effects, potentially resulting in over-thinning (Table 4)

BA + NAA programs are as effective as any postbloom thinning program featuring carbaryl (Table 5)

Addition of calcium phosphite to postbloom thinning programs demonstrated no clear effects (see 2015 and 2016 project reports)

Multiple formulations of prohexadione calcium significantly reduced shoot growth of Fuji; efficacy increased with acidification of spray tanks with ammonium sulfate (data not shown)

2014 summer applications of new NAA formulations were as ineffective at promoting return bloom as multiple standard NAA and ethephon programs evaluated by WTFRC in the mid 2000's (data not shown)

Multiple applications of 100-200 ppm GA_3 have effectively reduced return bloom in apple over several years of study, including 2015 trials (Table 6)

Shade netting improved fruit size and packouts (reduced sunburn and hail damage) without loss of yields in Granny Smith (see 2014 project report)

Collaborative research efforts continue to help develop new information and technologies to improve crop load management of WA apples

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 wholly by third party companies that contract our services to independently evaluate their products alongside industry standard programs. We continue to evaluate the relative success of 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.

Chemical thinning programs evaluated in 2016 are listed in Table 1. Due to the potentially risky nature of many of our treatments, we conducted all but one of our trials at WSU's Sunrise Research Orchard, which also allowed us to ensure no other thinning applications were superimposed on our plots. Historically, however, additional bloom or postbloom chemical thinning applications have been left to the discretion of individual commercial grower-cooperators, provided that each experimental plot received the same programs.

Table 1. Chemical thinning programs evaluated. WTFRC 2016.

BLOOM THINNERS (applied in 100 gal water/A @ 60% & 100% bloom)
4 & 8% Rex Lime Sulfur (LS)
4 & 8% K-Pax II
2% Crocker's Fish Oil (CFO) + 1.5-3% K-Pax II
2% Crocker's Fish Oil (CFO) + 3% Rex Lime Sulfur (LS)
POSTBLOOM THINNERS (applied in 100 gal water/A at PF & 12mm, or 8mm & 15mm)
300-800 ppm Brevis (metamitron)
400-600 ppm Brevis + 1% Wilbur Ellis Supreme Oil (WES)
300-600 ppm Brevis + 16 oz Regulaid/A
600 ppm Brevis + 5 oz Fruitone L/A
600-800 ppm ADA 46342
400-600 ppm ADA 46342 + 16 oz Regulaid/A
24 oz Exilis 9.5SC + Fruitone L/A
122 oz Exilis Plus + 4 oz Fruitone L/A
48 oz Carbaryl 4L + 4-5 oz Fruitone L (NAA)/A
128 oz MaxCel + 4-5 oz Fruitone L/A

BLOOM THINNING:
2016 marked the first full scale bloom thinning trial with K-Pax, a new alternative formulation of lime sulfur being developed by Orcal Inc., the registrant of Rex Lime Sulfur. K-Pax has been engineered to produce a higher yield of H_2S, theoretically making it more efficacious against fungi including powdery mildew. Preliminary trials in 2014 and 2015 demonstrated reduced fruit set with no phytotoxicity with applications of K-Pax as a stand-alone product. In 2016, we expanded the treatment list to include tank mixes of K-Pax with a spray oil (Crocker's Fish Oil) at typical commercial rates; unfortunately, we were unable to observe thinning effects from any treatment,

including a standard program of CFO + Rex Lime Sulfur (Table 2). We had no difficulties in handling or mixing and observed no phytotoxicity to leaves or fruit from any treatment.

Table 2. Crop load and fruit quality effects of bloom chemical thinning programs. WTFRC 2016.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	*%*	*g*		*%*
Gala / M.9 Nic.29 – Rock Island						
2 gal CFO + 1.5 gal K-Pax II	93 a	44 ns	32 ns	152 ns	119	93 ns
2 gal CFO + 3 gal K-Pax II	88 ab	49	29	152	119	97
2 gal CFO + 3 gal Rex LS	61 b	57	29	164	111	93
4 gal K-Pax II	65 ab	56	28	160	114	87
8 gal K-Pax II	72 ab	49	34	151	120	95
4 gal Rex LS	73 ab	52	31	154	118	100
8 gal Rex LS	62 b	57	29	158	115	85
Control	67 ab	55	28	153	119	98

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 1999-2016.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	15 / 60 (25%)	10 / 63 (16%)	4 / 55 (7%)
NC99	15 / 32 (47%)	7 / 34 (21%)	2 / 28 (7%)
Lime sulfur	26 / 58 (45%)	12 / 52 (23%)	9 / 51 (18%)
CFO + LS	62 / 115 (54%)	27 / 106 (25%)	22 / 104 (21%)
JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	15 / 30 (50%)	5 / 29 (17%)	4 / 29 (14%)
ThinRite	7 / 22 (32%)	0 / 23 (0%)	0 / 12

[1]Does not include data from 2016 trials.

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

POSTBLOOM THINNING:

Our main focus for postbloom thinning continues to be metamitron, a sugar beet herbicide that has been recently registered by Adama under the trade name “Brevis” as a postbloom thinning agent in several countries including Italy, France, Spain, and South Africa. We have worked with small quantities of metamitron since 2011, finding it to be a promising chemistry when used aggressively in our relatively low plant stress environment. While trials in Europe and the Eastern US have found single applications of 200-400 ppm metamitron to be efficacious, our results indicate that two applications of 600-800 ppm are necessary to produce similar effects in Washington conditions. With these aggressive use patterns, we continue to produce trial results which indicate metamitron can be a viable thinning chemistry for our industry, particularly if carbaryl eventually loses its registration.

This year, we evaluated Brevis, the commercial formulation of metamitron used in Europe, alongside a numbered formulation (ADA 46432) from Adama which contains a different package of inert ingredients. As in 2015, our 2016 metamitron treatments were generally equal to or better than industry standards like carbaryl and BA in terms of reducing fruit set and/or promoting fruit size across sites and cultivars (Table 4). Generally speaking, we have found that metamitron can pair well in tank mixes with a non-ionic surfactant (Regulaid), a summer oil (Wilbur Ellis Superior Oil), or NAA (Fruitone L); in most instances, a reduced concentration of metamitron in a tank mix with one of those partner chemistries has produced similar results to those of higher rates of metamitron alone. Previous WTFRC studies found that adding silicone-based surfactants or heavier-weight dormant oil to metamitron produced significant levels of phytotoxicity.

Table 4. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2016.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	*%*	*g*		*%*
Fuji / M.9 337 – Othello						
Brevis 300ppm	116 bc	42 abc	23 ns	264 ns	69	64 b
Brevis 300ppm + Regulaid	82 c	54 a	22	259	70	48 b
Brevis 600ppm	86 c	51 ab	25	269	68	70 a
Carbaryl 4L + Fruitone L	154 a	32 c	19	269	68	78 a
Exilis 9.5 SC + Fruitone L	142 ab	38 bc	18	254	71	73 a
ExilisPlus + Fruitone L	113 bc	42 abc	24	267	68	76 a
MaxCel + Fruitone L	121 ab	41 abc	22	257	71	71 a
Control	116 bc	44 abc	22	264	69	74 a
Golden Delicious 3D / M.9 – Rock Island						
ADA 46342 600ppm	13 bc	90 bc	8 bc	261 a	70	35 ns
ADA 46342 800ppm	6 c	95 a	4 c	279 a	65	31
Brevis 600ppm	10 c	92 ab	7 bc	280 a	65	38
Brevis 600ppm + Fruitone L	10 c	92 ab	6 bc	267 a	68	26
Brevis 800ppm once	25 b	84 c	10 b	268 a	68	36
Brevis 800ppm twice	11 c	93 ab	5 c	259 a	70	30
MaxCel + Fruitone L	5 c	96 a	4 c	289 a	63	40
Control	87 a	48 d	30 a	189 b	96	55
Granny Smith 9A / M.9 337 – Rock Island						
ADA 46342 400ppm + Regulaid	35 def	67 cde	32 bcd	289 ab	63	51 ns
ADA 46342 600ppm	20 efg	81 bc	18 de	258 b	70	56
ADA 46342 600ppm + Regulaid	29 def	72 bcd	26 cde	270 ab	67	66
Brevis 400ppm	74 ab	29 f	52 a	292 ab	62	58
Brevis 400ppm + Regulaid	38 cde	64 cde	34 bcd	287 ab	63	60
Brevis 400ppm + WES	56 bc	51 ef	44 ab	273 ab	67	33
Brevis 600ppm	43 cd	59 def	40 abc	242 b	75	54
Brevis 600ppm + Regulaid	36 def	67 cde	31 bcd	252 b	72	50
Brevis 600ppm + WES	25 defg	76 bc	23 cde	295 ab	62	65
Carbaryl 4L + Fruitone L	17 fg	83 b	17 de	326 a	56	51

MaxCel + Fruitone L	6 g	95 a	5 e	293 ab	62	38
Control	79 a	44 f	38 bc	238 b	76	50

In more than 300 replicated chemical thinning trials since 1998, our research program has seen only a few cases of legitimate over-thinning, but 2016 will be remembered as a season when several of our thinning treatments were clearly too aggressive in trials on Granny Smith and Golden Delicious at the WSU Sunrise Research Orchard. Weather conditions for several days after our second sprays on May 2 featured heavy cloud cover, daytime temperatures in 70s and 80s, and nighttime temperatures in the high 50s and low 60s, creating considerable carbohydrate stress in test trees and setting them up for strong thinning responses. Dramatic reductions in fruit set and increases in harvest fruit size were observed across nearly all treatments, especially in Golden Delicious (Table 4). Treated trees were in visible shock for several days after the 15mm applications, particularly those that were sprayed with NAA, whether it was partnered with carbaryl, BA, or metamitron (Figures 1, 2). In fact, trees in several plots treated with NAA continued to feature wilted, curled leaves and poor shoot growth through most of the growing season. More typical Central Washington weather conditions bracketed the spray applications in a commercial Fuji orchard near Othello, and the thinning responses in that trial were far more subtle (Table 4).

Figure 1. Untreated control Granny Smith trees (L) and leaves (R). May 4, 2016.

Figure 2. Granny Smith trees (L) and leaves (R) 48 hours after 15mm BA + NAA application. May 4, 2016.

Several plots treated with metamitron programs also featured some phytotoxicity commonly associated with that chemistry. Mild chlorosis and marginal burn on primary leaves similar to effects observed in 2015 (Figure 3) were sprinkled throughout treated areas, but as has been the case in previous studies, those trees grew out of those conditions within a few weeks and no long-term harm to trees or fruit occurred.

Figure 3. Mild (L), moderate (C), and severe (R) leaf damage caused by metamitron applications. WTFRC 2015.

Our confidence in the potential of metamitron as a thinner in WA conditions continues to grow as we gain more experience with this chemistry. Table 5 demonstrates that after several years of testing, our success rates for producing satisfactory results from metamitron thinning treatments are comparable or superior to any standard industry programs; when metamitron is partnered with materials like a non-ionic surfactant, a summer oil, or another thinner such as NAA, our results have consistently improved. Even though metamitron is unlikely to complete registration with the EPA within the next 5 years, WA growers should be able to achieve satisfactory results with currently available products. We continue to find good results in postbloom thinning programs that feature tank mixes of carbaryl, BA, and/or NAA (Table 5).

Table 5. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2016.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	3 / 23 (13%)	0 / 24 (0%)	0 / 22 (0%)
Carb + BA	33 / 91 (36%)	10 / 89 (11%)	13 / 86 (15%)
Carb + NAA	18 / 65 (28%)	12 / 65 (18%)	6 / 61 (10%)
BA + NAA	17 / 39 (44%)	8 / 38 (21%)	5 / 32 (16%)
Metamitron	7 / 16	3 / 15	1 / 13

[1]Does not include data from 2016 trials.

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

GIBBERELLIC ACID FOR BLOOM INHIBITION:

Despite the annual cropping tendencies of modern dwarfing rootstocks and improved chemical thinning programs, biennial bearing continues to present a major challenge to many apple growers, especially in organic production systems which have limited options for postbloom thinning and plant growth regulators (PGRs). Over the years, we have investigated a number of PGR programs to promote bloom, but had very poor results with industry standards such as summer applications of ethephon and/or NAA. Consequently, we shifted our focus to investigate cost-effective PGRs, namely gibberellic acids (GAs), which could help excessive cropping in an “on” year of an alternate bearing cycle by inhibiting flower formation after a season of light bloom (i.e. the “off” year). Our work showed that several isomers of GA can reduce return bloom in WA conditions, but our primary

focus was on GA_3 due to its potential for use in organic orchards and effective rates of that isomer would potentially be less expensive to growers than effective rates of more potent isomers.

After many years of studying product rates and timings, we determined that 2-4 applications of 100-200 ppm of GA_3 in the month after petal fall yielded the most consistent reductions in return bloom across numerous sites and cultivars. Single applications of higher concentrations of product were also sometimes effective, but not as reliably as multiple applications at 7-14 day intervals. Table 6 reports results from GA trials launched in 2015 which primarily featured Falgro 2XLV, a commercial formulation of GA_3 registered for use on cherry to promote size and delay maturity.

Table 6. Effects on tree vigor, fruit size, and return bloom of GA applications. WTFRC 2015.

Treatment	2015 shoot length	2015 harvest fruit weight	2015 relative box size	2016 return bloom	2016 return bloom per CSA
	cm	*g*		*%*	*clusters/cm²*
Fuji / M.7 w/Red Del & Cameo interstems - Bridgeport					
Falgro 2XLV 100ppm	21.4 ns	nd	nd	339 ns	0.6 ns
Falgro 2XLV 200ppm	20.7			421	0.9
Falgro 2XLV 400ppm	20.0			263	0.7
Control	19.3			661	1.0
Fuji / Multiple leader grafts - Brewster					
Falgro 2XLV 100ppm	32.7 ns	223 ns	81	532 ns	2.7 b
Falgro 2XLV 200ppm	36.5	219	83	807	2.7 b
Falgro 2XLV 400ppm	34.6	216	84	902	4.0 b
Control	32.2	222	82	1071	6.0 a
Fuji / M.9 - East Mattawa					
Falgro 2XLV 100ppm	23.1 ns	190 ns	96	130 ns	0.8 ns
Falgro 2XLV 200ppm	20.9	206	88	139	0.5
Falgro 2XLV 400ppm	20.9	196	93	84	0.8
Control	23.2	198	92	112	0.9
Fuji – M.26 / Rock Island					
Falgro 2XLV 100ppm	40.0 ns	184 ns	99	45 ns	0.4 ns
Falgro 2XLV 200ppm	39.2	200	91	378	0.4
Falgro 2XLV 400ppm	36.3	199	91	95	0.1
Control	36.1	202	90	179	0.4
Golden Delicious / Seedling – South Mattawa					
Falgro 2XLV 100ppm	25.3 a	201 ns	90	404 ns	2.2 ns
Falgro 2XLV 200ppm	24.6 ab	205	89	337	2.0
Falgro 2XLV 400ppm	21.9 b	208	87	438	2.6
FAL 477	22.3 ab	206	88	375	2.4
Novagib 10L	25.2 a	214	85	765	2.4
Control	24.7 ab	216	84	601	2.3

As in the past, our recent trials demonstrate the inherent challenge of generating statistically significant results due to pronounced variability within return bloom data; even though a grower would consider trees with either 2 or 20 flower clusters to have insufficient bloom, results like those still reflect a 10X degree of variability, which can thoroughly confound an analysis of variance. Despite these mathematical challenges, roughly half of our GA_3 trials through the years have produced statistically significant reductions in return bloom. Further, another 20-30% of our studies have yielded results similar to those from our 2015 Bridgeport and Rock Island Fuji trials (Table 6), where numeric reductions in return bloom were observed without statistical significance.

The fundamental question remaining for these programs is not their efficacy, but whether or not registrants of GA_3 products will decide to amend their labels to accommodate this use pattern on apple. Several companies manufacture GA_3 for use in tree fruit, and we have lobbied the key PGR suppliers for the Pacific Northwest tree fruit market for years to consider relevant label expansions. Unfortunately, these companies can find little financial incentive to assume the costs and potential liabilities for doing so given the availability of several other analogous competitor products in the market.

Based on the relatively consistent performance of these GA_3 programs, it seemed of little marginal value to continue demonstrating their efficacy, so we decided in 2016 to limit any new trial work to evaluation of new GA formulations. As such, we launched two studies this spring to evaluate a new product with a unique profile of GA isomers; return bloom data will be collected this coming spring. If this formulation shows promise, the company that is developing it hopes to have it registered specifically to reduce bloom in apple.

COLLABORATIVE CROP LOAD MANAGEMENT RESEARCH:

"Effects of physiology of apple under photoselective anti-hail nets" (AP-15-104; PI: Kalcsits) – support for labor intensive data collection, harvest sampling, and postharvest fruit quality analysis; also support for project leadership team including sharing of relevant WTFRC projects and protocols, as well as editing of project manuscripts

"Pollen tube growth model validation & utilization for flower thinning" (AP-15-105; PI: Yoder) – local support for coordination with WSU-AgWeatherNet, beta testers, and flower sample collection for shipment to VTU for microscopic analysis; leadership of extension/education efforts regarding industry adoption of models

"Validation of Honeycrisp and Granny Smith pollen tube growth models" (AP-15-103; PI: Yoder) – local support for coordination of beta testers and flower sample collection for shipment to VTU for microscopic analysis

"Validation of the Red Delicious pollen tube growth model" (AP-16-108; PI: Yoder) – local support for coordination of beta testers and flower sample collection for shipment to VTU for microscopic analysis

"Development and validation of a precision pollination model" (TR-16-102; PI: Rafferty) – coordination of local data collection for bee foraging, bloom phenology, and fruit sampling activity at sites near Yakima and Chelan; active member of project leadership team (project funded through WTFRC technology committee)

CONTINUNING PROJECT REPORT **YEAR**: Ongoing

Project Title: Pesticide residues on apple

PI: Tory Schmidt
Organization: WTFRC
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Cooperators: Mike Willett, Gerardo Garcia, Ensa Ceesay – WTFRC
Laura Grunenfelder, Kate Woods – NW Hort Council
Steve Thun, Rick Jordan – Pacific Agricultural Labs

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

Year	2016	2017
Salaries	3500	3500
Benefits	1000	1000
Wages	1000	1000
Benefits	300	300
Equipment	200	200
Supplies	200	200
Travel	1000	1000
Analytical lab fees	5670	5670
TOTAL	$12,870	**$12,870**

Footnotes: Supply costs primarily covered by private industry cooperators
Travel includes costs for hauling spray equipment to trial site and delivery of samples to Portland

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