			APPLE HORTICULTURE AND POSTHARVEST RESEARCH REVIEW	
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FINAL PROJECT REPORT

Project Title: Assessment of Apple Packing for *Listeria* Risk

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Cooperators: Multiple industry partners. As of August 2015, Ines Hanrahan assumed PI status, since Dr. Killinger left WSU to take a position with FDA. Dr. Killinger remains as a co-PI on the project. The assistance of Tonia Green, Lauren Walter, Kyu Ho Jeong, and Andy Liao are acknowledged and greatly appreciated. Special thanks to Dr. Meijun Zhu (WSU) for help with oversight of WSU based budgets upon Dr. Killingers departure.

Other funding sources: None

Total Project Funding:

Budget History:

Item	2015
Salaries	23,525
Benefits	10,499
Wages	1,500
Benefits	33
Equipment	
Supplies	25,000
Travel	6,000
Plot Fees	
Miscellaneous	
Total	66,557

OBJECTIVES

1) Examine current industry standard practices for control of *Listeria* in packing environments and compare sampling and detection methods for *Listeria* spp.

2) Examine the prevalence of generic *Listeria* spp. and *Listeria monocytogenes* associated with Fuji apples stored under refrigerated storage and controlled atmosphere storage with and without ozone application

SIGNIFICANT FINDINGS

- The results from a single facility indicated that damaged walls and floors present challenges for normal cleaning procedures. Furthermore, bumpers and HVAC systems also appeared to be difficult to clean. After an aggressive cleaning, floors, bumpers, and damaged walls had reduced observations of presumptive positives for generic *Listeria* spp..

- Items associated with cleaning, including floor scrubbers and foggers, may also serve as sources of cross-contamination. This demonstrates the need for regular maintenance, cleaning and sanitizing of equipment and items used to clean facilities. A chemical fogger and floor scrubber both tested presumptive positive for generic *Listeria*.

- Results indicated that items such as wooden pallets and forklifts that move from Zone 4 (outside production areas) into cold storage or packinghouses areas (Zone 3) could serve as sources of contamination. A forklift was sampled and found to be presumptive positive for generic *Listeria*, 87.5% (7/8) positive and wooden pallets were also (100%).

- Apples that were directly contacted by open surface irrigation water had a higher percentage positive (12.3%) for generic *E. coli* compared to apples that were not contacted by irrigation water (3.3%).

- Further examination of refrigerated and controlled atmosphere storage on microbial levels is warranted as well as the use of ozone during controlled atmosphere storage.

METHODS

Objective 1: Environmental Listeria spp. risks in tree fruit packing operations

Partnering organizations and packing houses were identified for preliminary meetings to assess potential high risk areas for *Listeria* spp. contamination. Consideration was given to equipment design, materials used as food contact surfaces as well as cleaning and sanitation regimes. Data collected at each packinghouse were blinded for confidentiality. Each packinghouse was interviewed to understand current practices related to cleaning and sanitation practices as well as environmental monitoring to prioritize areas for environmental sampling.

Three facilities were interviewed for participation; based on responses, two were selected for sampling. Selection of target organisms, *Listeria* spp., total coliforms and generic *E. coli*, was discussed. The scope of sampling was explored, examination of food contact surfaces (Zone 1) as well as areas immediately adjacent to food contact surfaces (Zone 2), production areas (Zone 3) and areas outside of production (Zone 4). The approach taken for sampling and study design were different based on input from the facility and their specific needs. Aggressive environmental sampling was conducted in each facility on multiple days. In one facility, an evaluation of *Listeria*

prevalence was performed in the packing operation and cold storage rooms, including Zone 1-4 testing prior to cleaning and sanitation.

In one facility, a comparison of sanitation practices was performed. Two cold storage rooms were designated for the study. Cold storage rooms were sampled before and after typical sanitation versus before and after a more, aggressive sanitation protocol. Samples were collected from Zone 3 and some Zone 4 locations. In some cases, samples were composited to reduce the number of samples processed and increase the number of areas examined in the cold storage rooms. For example, all high wall samples were composited and all the low wall samples were compostied into one sample to create only two samples. Samples prior to cleaning and sanitation were collected from both rooms on one day. In another facility, samples were collected prior to sanitation from Zones 1-4 to evaluate areas that may require aggressive sanitation and potential routes of microbial contamination.

Samples were collected using PUR-Blue ™ swabs (World Bioproducts), Quick swabs ™ (3M™), Swab Samplers™ (3M™) or EZ Reach ™ (World Bioproducts) sponge samplers that were appropriate for each type of sampling site. Previous results from our laboratory indicated that some surfaces, such as wood bins, would benefit from aseptic sampling with small pieces of 3M Scotch-Brite™ pads, so in some cases, samples were collected with commercially available swabs and using treated 3M Scotch-Brite™ pads. Efforts were made for most commerical sampling supplies to use Dey-Engley (D/E) neutralizing broth, but in some cases, supplies using letheen broth were used. Environmental samples were collected and held on ice for transport to the laboratory. Liquid from the sampling device was collected and brought up to a 10ml volume of D/E neutralizing broth to align sampling volumes among swabbing devices.

Several methods of analysis were used for isolation of presumptive positive generic *Listeria* spp. The FDA-BAM method (FDA-BAM, 2011) with some modifications was used. The same pre-enrichment steps were used for all methods; samples were pre-enriched in Buffered *Listeria* Enrichment Broth (BLEB) for 24 hours at 30°C; after 4 hours of incubation, acriflavin HCL (10mg/L), nalidixic acid (40mg/L) and natamycin (25mg/L) were added to reduce background flora levels. Serial dilutions of the pre-enriched sample were inoculated onto Environmental *Listeria* Petrifilm™ (3M™) for determination of generic *Listeria* spp.. At 24 and 48 hours incubation, the pre-enriched sample was plated onto Modified Oxford *Listeria* selective agar. At 24 hours incubation, the pre-enriched sample was plated onto HardyChrom *Listeria* agar for differentiation of generic *Listeria* spp. from *Listeria monocytogenes* and *ivanovii*. Results from these methods should be considered presumptive positive, not confirmed, for generic *Listeria* or *Listeria monocytogenes*. Some isolates remain to be tested for further confirmation using methods described below.

Selected samples were processed as duplicate swabs for comparison and examination of other methods, including the use of HardyChrom *Listeria* agar, a proprietary test for *Listeria* spp., qPCR and immunomagnetic separation followed by qPCR.

Objective 2: Examine the prevalence of generic Listeria and Listeria monocytogenes on Fuji apples stored under differing conditions

There is a need to understand the prevalence of *Listeria* spp. on fruit upon arrival from the orchard and after storage. Apples were harvested from WSU orchards and were not intended for the commercial market, so there were no negative commercial implications associated with the results. Microbial tests performed at harvest in October of 2014 did not include *Listeria* spp., but did examine fruit that was directly contacted by water and fruit that was not directly contacted by irrigation water from an open surface water source. Apples were examined for the presence of total coliforms, generic *E. coli* using 3M™ E. coli/Coliform Petrifilms™. The need to include *Listeria* spp. was identified and approved for funding, which allowed for examination of apples held under refrigerated storage and controlled atmosphere storage with and without ozone treatment. With input from post-harvest experts, a chemical supplier and a partnering facility, apples were stored refrigerated storage

for 2 months and 3 months and in controlled atmosphere storage with and without ozone application for 6 months and 8-9 months. The presence of presumptive *Listeria* spp. and *Listeria monocytogenes* were examined as described above. Apples were also examined for pathogenic *E. coli* O157:H7 and *Salmonella*. The isolation of *E. coli* O157 was performed using immunomagnetic separation (IMS), standard plating techniques, and latex agglutination (LeJeune et al., 2001; Wright et al., 1994). *Salmonella* spp. were isolated by standard plating techniques and latex agglutination (FDA-BAM 2011). Confirmation of presumptive positive samples for *E. coli* O157, *Salmonella* spp., generic *Listeria* and *Listeria monocytogenes* will be performed by third-party laboratory serotyping.

RESULTS AND DISCUSSION

Objective 1. Examination of Current Sanitation Practices for control of Listeria.

The examination of cleaning and santizing procedures at one facility focused on cold storage rooms. The facility requested that interpretation of presumptive positives be reported as positive if any media (1 of 3 media utilized) presented presumptive positive colonies; in this way, the most conservative approach to potential comtamination was captured. Samples were collected after a typical cleaning strategy and samples were collected after a more aggressive cleaning and sanitizing procedure. Two rooms were sampled on the same day before cleaning. Areas sampled were categorized into the following: walls, damaged walls, floors, damaged floors, bumpers, door and HVAC systems. Prior to cleaning, the cold storage rooms had some similarities and some differences regarding prevalence of generic *Listeria*. Both rooms had a high prevalence of generic *Listeria* (greather than 80%) for both floors and damaged floors. In one room, both door samples (100%) were positive for generic *Listeria*, while one sample from the other door was positive (50%). However, one room had 100% (2/2) presumptive positive wall samples and the other had none detected (0/2) for wall samples. One room had over 87.5% of bumpers test positive for generic *Listeria* while the other room had a lower percentage (62.5%). Before cleaning, 100% of wooden pallets (4/4) were presumptive postive for generic *Listeria*.

After the normal cleaning procedure, the walls exhibited the the greatest difference in generic *Listeria* prevalence; from 100% (2/2) positive before cleaning to 6.3% (1/16) detected after cleaning (Figure 1). However, generic *Listeria* prevalence on the floors were only sligthly affected by cleaning (from 100% (8/8) before cleaning to 75% (9/12) after. Furthermore, some areas had more observed generic *Listeria* after cleaning, including damaged floors, HVAC, and bumpers. The results from a single facility indicated that damaged walls and floors present challenges for normal cleaning procedures. Furthermore, bumpers and HVAC systems also appeared to be difficult to clean. Cleaning equipment was also examined after normal cleaning, and appeared to be a potential source of cross-contamination. The chemical fogger and floor scrubber both tested presumptive positive for generic *Listeria* (100%; 3/3 and 75%; 9/12, respectively. A forklift was also sampled and found to be presumptive positive for generic *Listeria*, 87.5% (7/8) positive.

In addition to some equipment in Zone 3, outdoor areas (Zone 4) were also examined. The docking areas immediately outside of the rooms were sampled, and some distant outdoor area where culled apples are kept and vehicle traffic was relevant. High traffic loading dock areas infront of both cold rooms were 100% (8/8) positive and the high forklift traffic in front of the packinghouse were 100% (4/4) positive. All samples taken in or near the culled apples were also 100% (8/8) positive; trucks travel through these areas toward the packinghouses and cold room loading docks.

The cold storage room designated for aggressive cleaning had some differences in presumptive generic *Listeria* spp. presence before cleaning. Presumptive *Listeria* spp. were not detected on the walls prior to cleaning. After an aggressive cleaning, floors, bumpers, and damaged walls were greatly affected. The observation of presumptive generic *Listeria* spp. associated with floors was lower after aggressive cleaning (87.5% before versus 7.7% after). For bumpers, a similar

obervation was made (87.5% before versus 5.3% after). Also, damaged walls had a lower observation of presumptive generic *Listeria* spp. after aggressive cleaning (50% to none detected). There was a moderate decrease in positives for the door (50% to none detected) and damaged floor samples (100% before to 37.5% after). The HVAC systems sampled had 25% presumptive generic *Listeria* spp. before cleaning, and decreased to 13.3% after cleaning. Although aggressive cleaning was performed, visual observations noted challenges with cleaning, as the presence of fruit debris in difficult to clean areas of the cold rooms was noted during sampling (between bumpers and the wall). Items associated with cleaning were also found to be presumptive positive for generic *Listeria*, including a chemical barrel (100%; 1/1).

In the facility where the packing line and cold storage rooms were examined for *Listeria* prevalence prior to cleaning and sanitation, for any individual sample, results varied when methods were compared within laboratories for presumptive generic *Listeria* (Tables 2-3). Table 3 provides presumptive positive results in three main areas of the facility for presumptive generic *Listeria* and *Listeria monocytogenes* using three methods. Zones 1-4 demonstrated presumptive positive results prior to cleaning and sanitation. Some sampling sites were swabbed in adjacent locations and the duplicate swab was examined for comparison and examination of other methods as well as confirmation. The results of twenty-five samples involved in duplicate testing and confirmation are provided (Table 2). Although individual sample results varied by method, in 6 of 9 categories (line equipment, non-line equipment, line support, drain, drencher, drencher parts and cold room floors) presumptive positive generic *Listeria* results were confirmed by qPCR or immunomagnetic separation followed by qPCR. Furthermore, samples testing presumptive positive for generic *Listeria* or *Listeria monocytogenes* were confirmed positive for *Listeria monocytogenes* in the following categories: non-line equipment, drain, drencher and drencher parts.

Objective 2. Examination of the prevalence of Listeria on Fuji apples during storage.

Fuji apples were harvested and apples that were contacted by surface water from under-tree irrigation were observed and collected for comparison with apples that were not directly contacted by surface water. The apples were stored for 2 and 3 months in refridgerated atmosphere (RA) storage and in controlled atmosphere (CA) storage for 6 and 8-9 months, with and without ozone. At harvest, *Salmonella* spp. and *E. coli* O157:H7 were not detected, *Listeria* spp. and *Listeria monocytogenes* were not tested at that time (Table 1). Apples that were directly contacted by open surface irrigation water had a higher percentage positive (12.3%) for generic *E. coli* compared to apples that were not contacted by irrigation water (3.3%).

Apples in 2 months RA storage were presumptive postive for *Salmonella* (7%), *E. coli* O157 (3%), *Listeria* spp. (11-21%), and *Listeria monocytonegenes*(5%) (Table 1); these results are not confirmed for *Listeria* spp. or pathogen presence. Additional testing will be performed for confirmation. After 6 months in CA storage, observed total coliform levels were lower in both the untreated and ozone treated apples. At 8-9 months CA storage, observed total coliforms were lower from the ozone treated apples (14% versus 29.6%). Further examination of refrigerated and controlled atmosphere storage on microbial levels is warranted as well as the use of ozone during controlled atmosphere storage.

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Figure 1: Percentage of presumptive positive samples for generic *Listeria* before and after normal cleaning and aggressive cleaning of apple storage cold rooms. Samples were organized within categoreis and number of samples collected before and after each type of cleaning provided in parenthesis. † denotes samples that were composited (8 samples into each sample). Samples were considered presumptive positive if any media (1 of 3 media utilized) presented presumptive positive colonies.

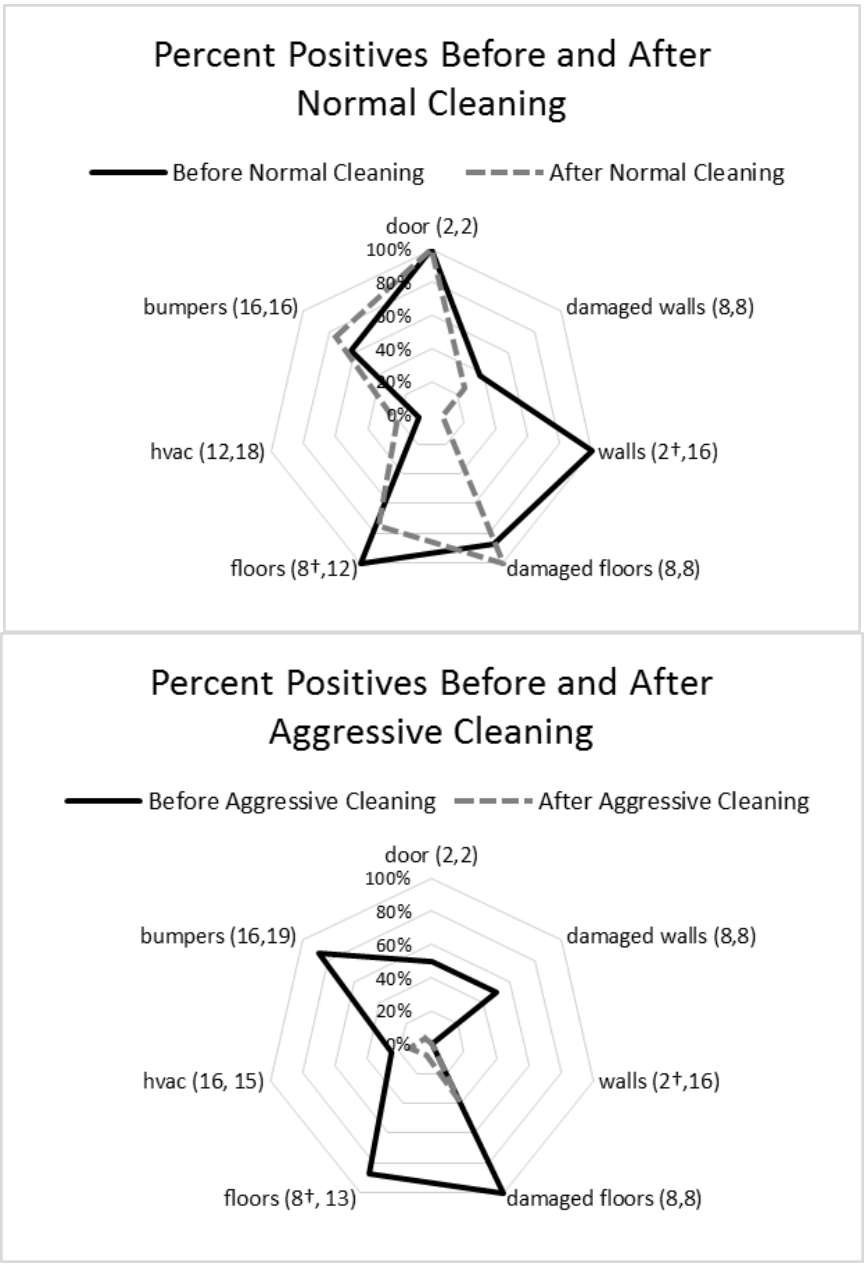

Figure 2: Percent positives of supplementary Zone 3 and 4 samples taken in addition to the before and after cleaning samples. Cleaning equipment includes a fogger, a riding floor scrubber, and chemical barrel used to clean the rooms. Outdoor samples include ground samples taken in the loading dock area outside of each room as well as infront of a high traffic forklift door of the packing house and an apple cull area. Other includes wooden pallets, forklifts, and fruit debris.

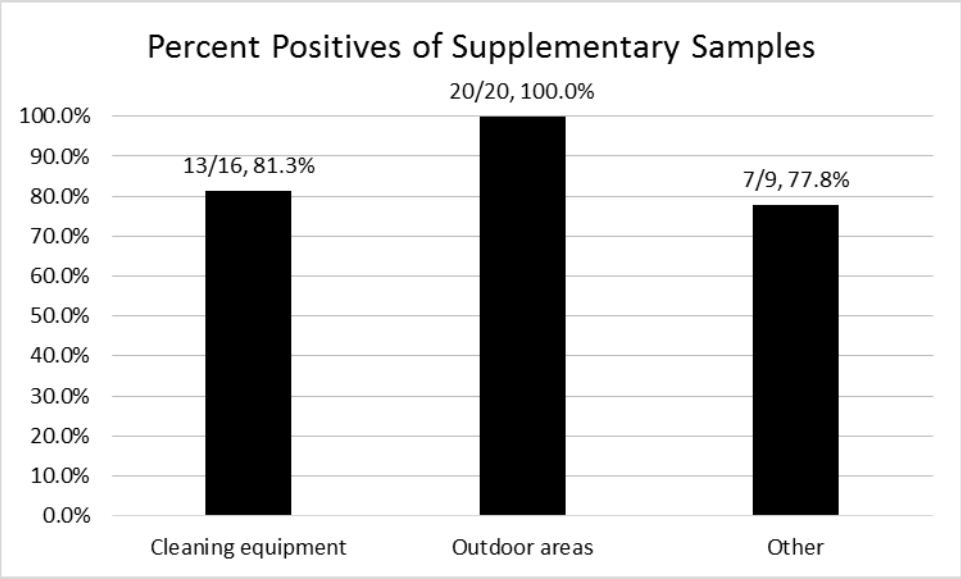

Table 1. Percentage of positive and presumptive positive samples among apples tested at harvest (with and without direct water contact from a surface irrigation source) after refrigerated atmosphere (RA) storage (2 and 3 months) and controlled atmosphere (CA) storage (with and without ozone, 8-9 months).

Microorganism Type	**Harvest** No direct water contact (n=181)	**Harvest** Direct surface water contact (n=155)	**RA** 2 months (n=100)	**RA** 3 months (n=100)	**CA** 6 months No Ozone (n=100)	**CA** 6 months With Ozone (n-118)	**CA** 8 months No Ozone (n=98)	**CA** 8 months With Ozone (n=100)
Coliform	61.3%	61.9%	21%	19%	12%	10.2%	29.6%	14%
Generic *E. coli*	3.3%	12.3%	2%	ND	ND	ND	1%	ND
Generic *Listeria**	NT	NT	11-21%	20-21%	9-30%	2.5 – 25%	ND – 23%	ND – 5%
E. coli O157:H7*	ND	ND	3%	1%	ND	ND	ND	ND
*Salmonella**	ND	ND	7%	ND	ND	ND	ND	ND

*Presumptive positive test results, further confirmation necessary and being performed.
n=number of samples collected
ND= none detected
NT= not tested

Table 2. Percentage of positive samples within sampling categories by testing media or method for presumptive (†) or confirmed generic *Listeria* or *Listeria monocytogenes*.

Category (number of samples)	*Listeria* spp. MOX†	*Listeria* spp. Petri film†	Proprietary *Listeria* spp. †	*L. mono* CHR 1†	*L. mono* CHR 2†	*Listeria* spp. qPCR	*L.* mono qPCR	*Listeria* spp. IMM qPCR	*L.* mono IMM qPCR
Packingline									
Line equipment (2)	50%	50%	50%	ND	50%	50%	ND	NT	NT
non-line equipment (3)	33%	67%	33%	ND	100%	33%	33%	NT	NT
drain (5)	60%	60%	100%	20%	100%	100%	100 %	NT	NT
line support (1)	100%	ND	100%	ND	ND	ND	ND	NT	ND
walls (1)	100%	ND	100%	ND	ND	NT	NT	ND	NT
Outdoors									
drencher (4)	50%	100%	100%	25%	75%	75%	75%	NT	ND
Cold Rooms									
drencher parts (5*)	100%	ND	100%	ND	20%	NT	NT	60%	33%
floors (2)	50%	50%	100%	ND	ND	NT	NT	50%	0%
HVAC (1)	ND	ND	100%	ND	ND	NT	NT	ND	NT

*One sample was composited and the composite was positive**.**

ND= none detected

NT= not tested

Table 3. Percentage of positive samples within sampling categories by testing media or method for presumptive (†)generic *Listeria* or *Listeria monocytogenes*. Further confirmation testing is being performed.

	Listeria spp. MOX†	*Listeria* spp. Petrifilm†	*L. mono* CHROM†
Packingline			
line-equipment (96†)	22%	35%	ND
line support (9)	89%	56%	ND
floors (4)	100%	50%	ND
non-line equipment (8)	38%	38%	ND
drain (4)	50%	100%	ND
walls (12)	50%	50%	8%
Outdoors			
loading area (10)	60%	100%	ND
drencher (2)	100%	100%	ND
Cold Rooms			
floors (15)	13%	20%	7%
drencher parts (8†)	88%	13%	13%
walls (22†)	36%	14%	ND
door (4)	25%	25%	ND
hvac (11)	ND	9%	ND
non-line equipment (1)	ND	ND	ND

†Some samples were composited to reduce the total number of samples.
ND= none detected

EXECUTIVE SUMMARY

This study identified several areas of apple packinghouses and cold storage rooms that are important to evaluate in environmental monitoring programs. Floors, damaged floors, and concrete bumpers were challenging to clean unless aggressive cleaning and sanitizing practices were followed. Although HVAC systems did not frequently test positive for presumptive generic *Listeria*, they appeared to be challenging to clean with normal cleaning procedures. Loading and drenching areas may harbor generic *Listeria* or *Listeria monocytogenes*; therefore, consideration of vehicle and worker traffic patterns are important to consider to reduce contamination risk in production areas. Results indicated that items such as wooden pallets and forklifts that move from Zone 4 (outside production areas) into cold storage or packinghouses (Zone 3) could serve as sources of contamination. Items associated with cleaning, including floor scrubbers and foggers, may also serve as sources of cross-contamination. This demonstrates the need for regular maintenance, cleaning and sanitizing of equipment and items used to clean facilities. This may also explain why some areas had more observed positives for generic *Listeria* after cleaning, especially floors and damaged floors. Other equipment in Zone 3 also appeared to harbor generic *Listeria* or *Listeria monocytogenes*, emphasizing the importance of cleaning and sanitizing not only direct food contact surfaces but also other equipment in production areas as well as drains. Incoming fruit may also harbor microbial contamination. Apples that were directly contacted by open surface irrigation water had a higher percentage positive (12.3%) for generic *E. coli* compared to apples that were not contacted by irrigation water (3.3%). After 2 months in refrigerated storage, a low percentage of the apples tested presumptive positive for pathogens; however, presumptive positive samples for pathogens were not observed with further controlled atmosphere storage (6-8 months) of apples harvested at the same time. After 8 months of controlled atmosphere storage, percentage positive for total coliforms were lower among apples treated with ozone. Further examination of refrigerated and controlled atmosphere storage on microbial levels is warranted as well as the use of ozone during controlled atmosphere storage. The potential exists for microbial contamination to enter packinghouses on fruit at a low prevalence rate or through routes of contamination associated with vehicles, equipment and worker foot traffic. Therefore, emphasis on cleaning and sanitizing in production areas is necessary to prevent establishment of biofilms and long-term sources of *Listeria* contamination.

FINAL PROJECT REPORT **PROPOSED DURATION**: 1 Year

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

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

Total Project Request: Year 1: $56,743

Note: A new 3-year proposal was funded to continue the work on this idea. This report is the conclusion of the 1-year project that was funded in 2014. The continuing report for the 3-year proposal has been submitted separately.

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

Budget 1
Organization Name: WSU **Contract Administrator:** Ben Weller
Telephone: 509-335-0052 **Email address:** wellerb@wsu.edu

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

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

RECAP OF ORIGINAL OBJECTIVES

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

The more specific objectives of the proposal were:

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

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

SIGNIFICANT FINDINGS

All the objectives set forward for this project have been completed. Below are the list of key tasks completed and the key findings from the work.

- Rheological properties of two kinds of waxes (Carnauba and Shellac) were studied.
- An assessment of the current drying conditions in different packing houses was conducted by more than 12 plant visits.
- Drying characteristics of apples coated with both Carnauba and Shellac waxes with convection air drying method were thoroughly studied at a drying air temperature range of 100 to 200^{o}F and drying times of 1 to 3 minutes, as determined by assessment of current packinghouse conditions.
- Quality assessments were conducted for apples dried with the (conventional) convection drying method over a three week period.
- An impingement dryer was purchased after thoroughly researching the options available in the market.
- The new impingement dryer purchased was modified to:
    - Increase the clearance for drying bigger apples
    - Conveyor belt was modified to suit the apple drying process
- Plant trials were conducted in a packing facility to determine the potential effectiveness of the impingement drying of waxed apples.
- Both types of wax (shellac and carnauba) behave differently under higher heat conditions.
- Viscosity of both waxes reduces significantly with increase in temperature.
- Both Carnauba and Shellac waxes can be effectively dried at higher temperatures (up to 200^{o}F), in a regular convection air dryer.
- The Shellac wax tends to have flaking issues around 100^{o}F. However, with an increase in temperature beyond 150^{o}F, it provides a high level of gloss on apples.
- Carnauba wax provides good performance in the temperature range of 100 to 200^{o}F.
- In general, at higher temperature, shorter drying time was better for maintaining gloss.
- The overall quality of the apples (as determined by measurement of total solids, moisture loss and pH) were comparable with the control apples (without waxing, but similar drying treatment) over the 3 week storage period.
- In-plant impingement drying tests provided very encouraging results on the drying of waxed apples.

- We observed that the drying temperatures could be increased to 300°F for both the Carnauba and Shellac waxes without negatively affecting the glossiness on the dried apples.
- With higher temperatures of 250°F and 300°F, shorter drying times (less than 1 min) was found to be more beneficial.
- Longer drying times at higher temperatures had negative effects on the wax quality.

Overall, we had very encouraging results from the first year of the project. A new 3 year project was funded during last year to explore this idea further. A continuing report for the first year of the work has been submitted for that project separately. In that project we have done extensive testing of the apples in the plant for the quality assessment and some preliminary work on the impacts on the microbiological load has been started.

METHODS

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

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

Waxes were tested using a rheometer to understand the effects of their viscosity behaviour as an effect of temperature. Viscosity and melting characteristics helped us to understand the spread-ability of the waxes. The drying characteristics of the waxes were also tested using the waxing sheets. These drying characteristics of the waxes helped us to determine the best drying conditions for the impingement drying system.

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

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

RESULTS & DISCUSSION

i) Current drying conditions in packing houses:

We visited various apple packing houses and assessed the drying conditions (more than 15). Thanks to all the packing houses that provided tours of their facilities and helped us with drying

temperature readings and information regarding drying conditions. We found that the drying conditions range very broadly, with the air temperatures ranging from 80°F to 150°F. The drying times were narrow, ranging from 2 ½ minutes to 3 minutes.

ii) Convection air drying of waxed apples:

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

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

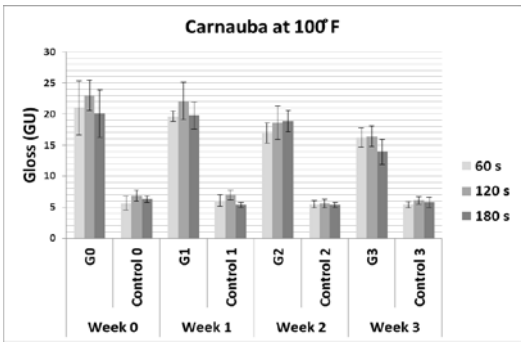

Fig 1. Gloss data for Fuji Apples coated with Carnauba.

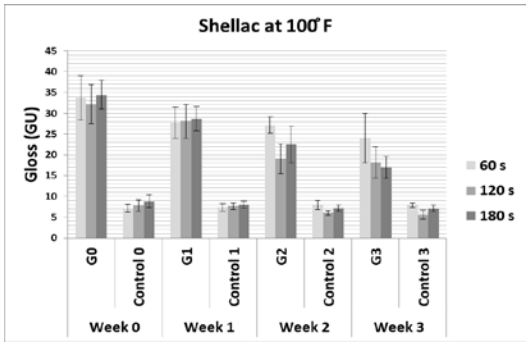

Fig 2. Gloss data for Fuji Apples coated with Shellac.

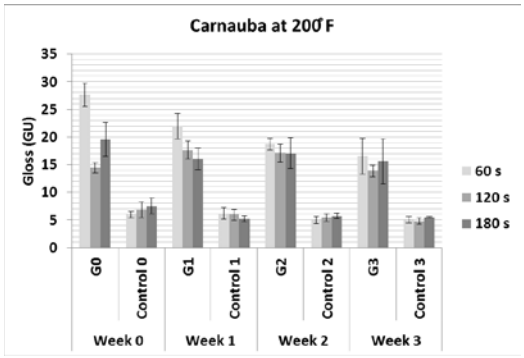

Fig 3. Gloss data for Fuji Apples coated with Carnauba.

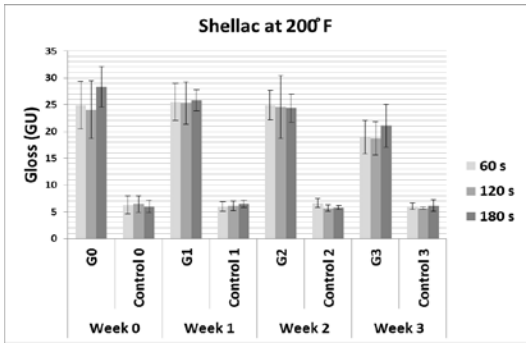

Fig 4. Gloss data for Fuji Apples coated with Shellac.

Figures 1 through 4 show the gloss results of the apples coated with either Carnauba or Shellac wax and dried at different drying conditions. From these results we saw that there were no statistically significant differences in the Apple glossiness at different temperatures and times of drying.

However, we generally observed that the Shellac wax has more glossiness at higher temperatures than the Carnauba wax. “G” in the figures above represent the wax treated samples.

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

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

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

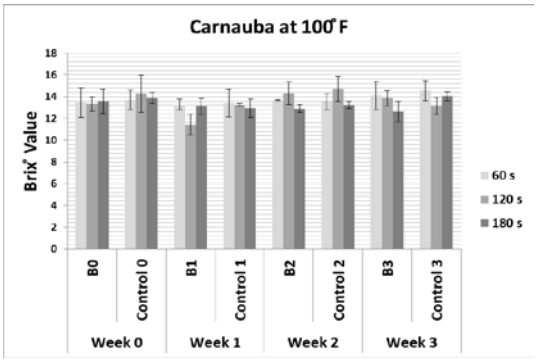

Fig 6. °Brix data for Fuji Apples coated with Carnauba.

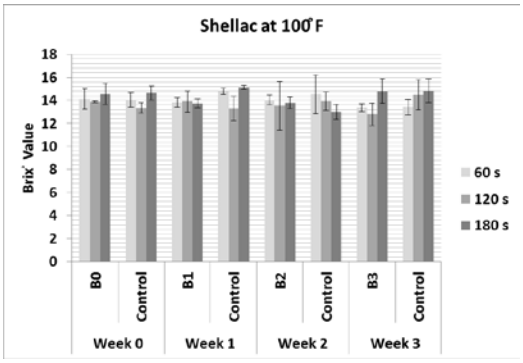

Fig 7. °Brix data for Fuji Apples coated with Shellac.

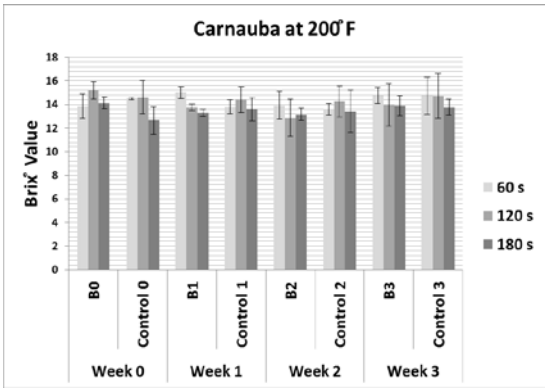

Fig 8. °Brix data for Fuji Apples coated with Carnauba.

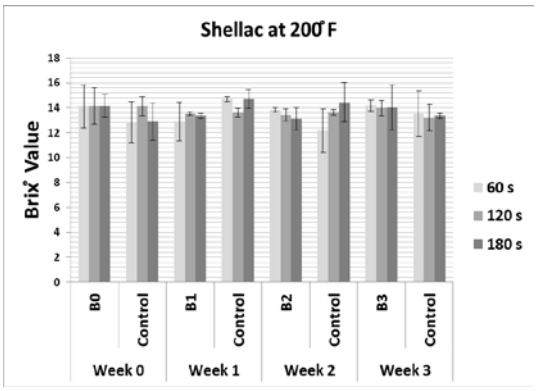

Fig 9. °Brix data for Fuji Apples coated with Shellac.

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

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

iii) Impingement air drying of waxed apples:

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

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

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

Fig 10. Modified impingement dryer in use in packing house

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

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

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

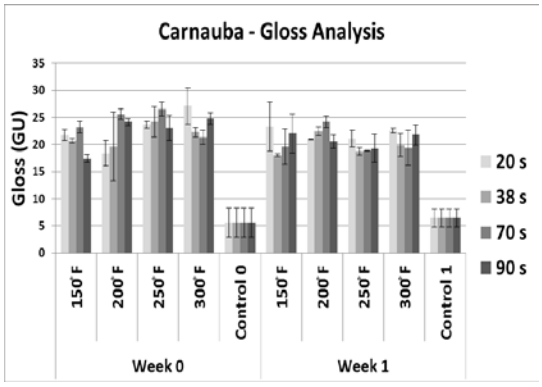

Fig 11. Glossiness data of Apples coated with Carnauba and dried by hot air impingement dryer.

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

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

Plant Trial Results:

Further during the initial part of the year 2015, another study was conducted to assess the impact of impingement drying on the glossiness of the apples through the storage period of 5 weeks. Weks 1 through 4 were in cold storage conditions and week 5 was when the apples were kept in the room conditions.

Golden delicious variety of apples was selected for testing, based on the availability in the plant at the time. The apples were coated with carnauba wax and dried in the impingement dryer at 4 different temperature settings of 150, 200, 250 and 300°F at their respective optimum drying times. The optimum drying time was determined by making sure the wax was fully dried and the apples were shiny visually without any damage to the typical wax quality.

Table 1. Gloss data of the impingement dried "golden delicious" apples over the storage period of 5 weeks.

Wax Type	Drying Temp (°F)	Drying Time (sec)	Gloss Data (GU)							
			Week 1	Week 1	Week 3	Week 3	Week 4	Week 4	Week 5	Week 5
			AVG	Std. Dev.	AVG	Std. Dev.	AVG	Std. Dev.	AVG	Std. Dev.
Un-Waxed	N/A	N/A	6.78	1.19	N/A	N/A	N/A	N/A	N/A	N/A
Carnauba	150	20	23.30	4.56	18.62	1.75	19.56	3.36	16.61	1.31
Carnauba	150	38	18.01	0.25	12.95	0.46	12.00	0.14	11.05	1.20
Carnauba	150	70	19.61	3.32	17.38	0.81	15.65	2.75	14.00	4.69
Carnauba	150	90	22.06	3.59	17.00	1.46	16.53	0.20	14.82	1.38
Carnauba	200	20	20.96	0.10	13.63	0.66	14.54	3.51	10.72	1.80
Carnauba	200	38	22.46	0.81	14.06	0.24	14.88	2.66	13.99	3.25
Carnauba	200	70	24.20	1.05	15.45	0.05	16.04	3.37	14.05	1.17
Carnauba	200	90	20.57	1.27	14.82	0.18	15.84	0.57	14.46	0.18
Carnauba	250	20	21.06	1.58	16.44	0.78	15.98	0.41	14.80	3.56
Carnauba	250	38	18.76	0.67	15.66	0.23	16.46	1.92	13.81	1.25
Carnauba	250	70	18.88	0.06	17.08	0.95	16.25	2.08	15.11	2.10
Carnauba	250	90	19.24	2.64	DNR	DNR	DNR	DNR	DNR	DNR
Carnauba	300	20	22.56	0.40	16.37	0.29	16.69	3.05	14.63	2.56
Carnauba	300	38	19.87	2.13	17.19	1.85	16.61	1.81	16.53	0.62
Carnauba	300	70	19.41	3.18	DNR	DNR	DNR	DNR	DNR	DNR
Carnauba	300	90	21.77	1.87	DNR	DNR	DNR	DNR	DNR	DNR

(Note for the table: N/A means not applicable; DNR means did not run, as the apple got heat damage)

From the Table 1, we can observe that the drying temperatures up to 300ºF, did not lead to any loss in the glossiness. Although, it should be noticed that the glossiness of the apples dried at 300ºF and drying time of greater than 38 sec, led to decrease in the glossiness. Further, this high temperature and increased drying time, led to loss of the overall quality of the apple. Thus these samples were discarded.

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

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

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

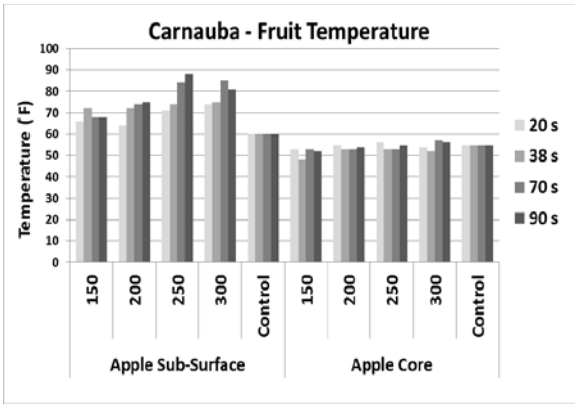

Fig 12. Temperature data of apples coated with Carnauba and dried by hot air impingement dryer.

SUMMARY:

The high temperature drying has shown the potential to dry the waxes (Carnauba and Shellac) effectively. Typically, it has been found that the higher temperature enhances the glossiness of the wax. The impingement drying method, in which the hot air is forced on to the apples, helps to dry the apples faster. With our experiments we consistently found that with the higher temperatures the drying time reduces very significantly. For example with 300ºF drying temperature, the drying time reduced to 30 seconds or less and at 250ºF, the drying time reduced to 60 seconds or less.

The next steps are to evaluate the effectiveness of the higher drying temperatures in reducing the microbiological loads of the fresh apples. Our hypothesis is that this high temperature on the surface of the fruit with the moisture present in the wax can help reduce the microbial loads. This can be shown with the help of the Fig 13a and 13b.

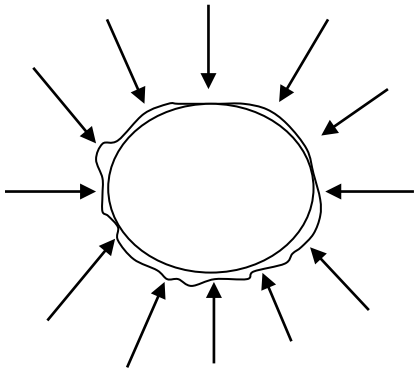

Fig 13a. Schematic of the apple before heating the surface

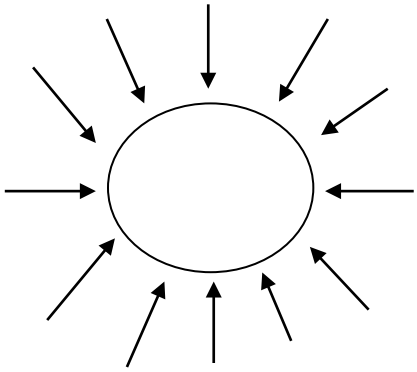

Fig 13b. Schematic of the apple after heating the surface

In the Fig 13a, we have shown the apple in the center with a thin layer of the wax solution on the surface. The wax is formulated with about 60 to 70% moisture (this is what we learnt from our conversations with the industry experts) which will evaporate during the hot air drying. In the Fig 13b, we show the layer of wax solution disappeared, suggesting that the water vapor in the wax has evaporated by creating a micro steam environment. This micro steam environment would help in reducing the microbial load.

Although, it is possible that when the apples are prepped for the waxing process the surface becomes fairly dry and the moisture present in the wax may not be enough to provide the micro steam environment that is needed to help reduce the microbial load.

Thus it may be necessary to test this hot air drying, while the excess moisture is present on the apple surface. We do know from the literature that the microorganisms are more resistant to heat in dry conditions than in moist conditions.

Because of the above reasoning and as a result of our preliminary microbiology studies that were conducted during this year (see the continuing project report); we are planning to modify the approaches for the microbiological experiments during this year's proposed work. Mainly, we will conduct inoculation studies in the laboratory with excess moisture on the apple surface and with the wax, to evaluate the effectiveness of the hot air drying on the microbial load reduction.

As it is we can confidently conclude that this drying technique if implemented in the packing lines can help with,

1. Reducing the footprint of the current dryer and thus providing more space for additional food safety interventions, such as additional chemical spray bars.
2. With the aid of the impingement drying process, the wax quality can be improved without sacrificing the fruit quality.
3. This drying method can help reduce the current drying times to less than a minute, if higher drying temperatures are used. This can help increase the product throughput.

OTHER OUTPUTS:

Two manuscripts as detailed below have been prepared from this research work and submitted to the peer reviewed journals for review.

1. Thapa BB, Behnam S, Wijesekara I, Aluwi NA, Kallu S, and Ganjyal GM. 2016. Impacts of convection drying temperatures and times on the postharvest quality of fresh waxed Fuji apples. *Postharvest Biology and Technology*. (In review).

2. Gu BJ, Behnam S, Wijesekara I, and Ganjyal GM. 2016. Characterization of glossiness of shellac and carnauba waxes under different drying conditions. *Postharvest Biology and Technology*. (In review).

We will be more than happy to share these manuscripts and all the data we have from this project with the WTFRC and all its members.

Executive Summary

The major goal of this project was to understand the impacts of the higher drying temperatures on the quality of the wax and the fruit over the standard storage period. Along with this, we had proposed to develop a pilot scale impingement drying unit that can be used to test the feasibility of this drying technique.

Tests were conducted in the laboratory using a convection drying oven to assess the impacts of the higher drying temperature on the wax and fruit quality. Temperatures of 100, 150, and 200^{o}F were tested at different drying times of 1, 2 and 3 min. The wax quality (glossiness) and the fruit quality (weight loss, soluble solids and pH) were tested on a weekly basis for three weeks of cold storage.

It was found that the higher temperatures resulted in higher gloss values. In other words, higher drying temperature led to more shiny apples for both Carnauba and Shellac waxes. Although, for the Shellac wax in specific, the wax quality was negatively impacted when the temperature of the drying was increased from 100 to 150^{o}F. But, at 200^{o}F the wax quality got better with increased glossiness compared to 100^{o}F drying temperature. But for the Carnauba wax, the glossiness increased with any increase in drying temperature. In general the apple quality was not negatively impacted by the increase in the temperature of the drying. There was a slight increase in the weight loss for the shellac coated apples compared to the carnauba coated as well as the control samples. But all other quality parameters were not significantly different among the treatments and the control samples.

This helps us conclude that higher temperatures up to 200^{o}F can be used for drying of the waxed apples. Based on this, we do recommend that further studies need to be conduct to assess the impact of the higher drying temperatures with the current plant dryers on the microbiological loading of the apples.

Further, to test the concept of the impingement drying process, an off the shelf dryer was purchased and modified. The dryer was modified to increase the clearance of the drying zone to fit wide range of the apple sizes. With the assistance of one of the co-operators, we also replaced the mesh conveyor to the roller belt conveyor. This enabled us to have an impingement dryer as close as possible to the dryers currently used in most of the packing houses.

The key benefit of this impingement drying method is the fact that the air is forced at a high velocity on to the fruit which leads to drying of the surface only. Our hypothesis was that this uniqueness of the drying method will help to raise the drying temperature and reduce the drying time. This would help to increase the production capacity and potentially benefit the fruit quality and safety.

The goal for this project was to see if the impingement drying will help increase the drying temperature without compromising the wax and fruit quality. We conducted a few plant trials with the dryer to determine the highest temperature that we can dry the fruit without compromising the wax and fruit quality. From this work, we found that depending on the variety of the fruit the drying temperature can be raised up to a maximum of 300^{o}F. With the drying temperature at 300^{o}F, the drying time was reduced to less than 30 seconds. We did find from our studies that some fruits cannot withstand this high temperature. For example, the red delicious variety, can withstand only up to 250^{o}F, compared to the Fuji variety that can withstand up to 300^{o}F.

As a results of this 1 year project work, we were funded a 3 year project to further evaluate the feasibility of this drying method to effectively dry the fruit and increase the food safety of the fresh packed apples.

We have completed the first year of the 3 year project where we have tested this dryer on few varieties and completed the studies on the wax and apple quality over the standard storage periods. Now we have started the work to evaluate the impacts of the impingement drying on the microbiological loads on the apples.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-15-103

PROJECT TITLE: Improving food safety of fresh apples by hot air impingement drying

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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:** Carrie Johnston/Ben Weller
Telephone: 335-4564 509-335-0052 **Email address:** carriej@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.
    - *This objective was planned to be completed during the first year of the project.*
    - It was proposed that we test the drying system in at least couple different plants and with few different varieties.

2) Study the effectiveness of impingent drying in reducing the microbial levels in apples.
    - *The objective has been planned to be completed in the 2nd year of the project.*

3) Develop scale-up strategies for commercial packing lines and complete the energy efficiency analysis.
    - *This objective was planned to be executed during the 3rd year of the project, provided the results are favorable during from the first and second years of the project work.*

PRELIMINARY RESULTS

In the year 2015, 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.

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

- Different varieties of apples were tested, which included, red delicious, gala, golden delicious, fuji, honey crisp and few others.
- For most of the varieties, we observed that at higher temperature, shorter drying time was better for maintaining gloss.
- There is an optimum drying temperature for each variety. Beyond this temperature, the apples incur heat damage.
- 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.
- 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.
- We now have the dryer in Pullman for conducting more microbiological studies to determine if the drying at high temperatures will help reduce the microbes with inoculated studies.

<u>Overall, we had very encouraging results from the first year of the project with respect to the apple quality. In the second year of the project we will exclusively focus on the microbiological testing. We will select one or two varieties and study the impacts of the drying on the microbiological loads with standard inoculation studies as described in the next steps on page #6.</u>

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, by continuing the work conducted from the last year. This part of the study 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^{o}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 will include the total soluble solids, pH and titratable acidity. The protocols were set based on the initial review and discussions with the co-operators.

For this objective the major piece of the equipment that was used is the "Modified Hot Air Impingement Dryer". The original dryer we bought is the Hot Air Impingement Oven (PS628E, WOW^{2}, Middleby Marshall, Elgin, IL) as shown in Fig. 1. The oven was modified to for drying of the range in apple size. The modified oven/dryer is shown in Fig. 2, during the testing process in a packing house.

Fig 1. The original oven

Fig 2. The modified oven/dryer in action

RESULTS & DISCUSSION

i) Impingement drying studies of different varieties of apples:

Series of studies were conducted in the plant settings to determine the impacts of the impingement drying conditions on the quality of the apples over the standard storage periods. The dryer was transferred into the respective packing house, before the trials. We greatly appreciate the support that each of the plants provided us with for these trials. We set our dryer next to the plant dryer. All experiments were conducted when the packing line was running with the fresh apples.

Washed apples were handpicked from the line after the wax was applied on them and before they entered into the plant dryer. The waxed apples were dried in the impingement dryer under different temperature settings. After drying, the apples were transferred into the boxes and transferred to Pullman in a car. All apples were tested for Gloss, ^{o}Brix, Titratable Acidity and pH. Further, the apples were stored for five weeks in a walk-in cooler in the pilot plant facilities. Apples were pulled out every week and the quality parameters were tested.

During the impingement drying testing, the temperature of the apple surface and core were recorded. Fig 3, shows the surface and core temperatures of the apples during drying. Please note that the surface temperature was measured using a handheld IR sensor and the core temperature was measure by inserting a thermocouple into the apple, immediately after the apple came out of the dryer. The data recorded for the surface temperature may not be accurate, as the temperature may drop significantly as the apples come out of the dryer.

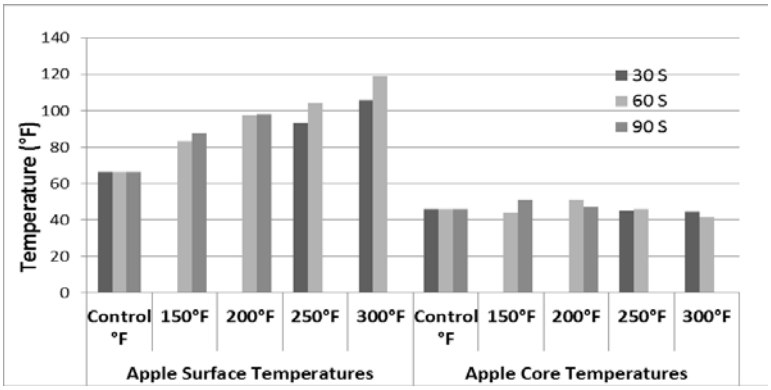

Fig 3. Apple surface and core temperatures during impingement drying

From the Fig. 3, we can clearly see that even at the dryer temperature of 300^{o}F, the apple core temperature is not rising. This suggests that the impingement drying, heats the surface only.

Data from plant trial #2:

In the plant trial #2, "red delicious" variety of the apple was used for the studies. On this day, the plant was using carnauba wax. The data of different quality parameters measured over the storage period of 5 weeks is shown in Fig 4. Fig 4(a) shows the glossiness data; Fig 4(b) shows the pH data, Fig 4(c) shows the soluble solids data and Fig 4(d) shows the titratable acidity data. After the 4th week of storage the apples were stored under room conditions for a week and the quality parameters were measured and recorded as the 5th week data.

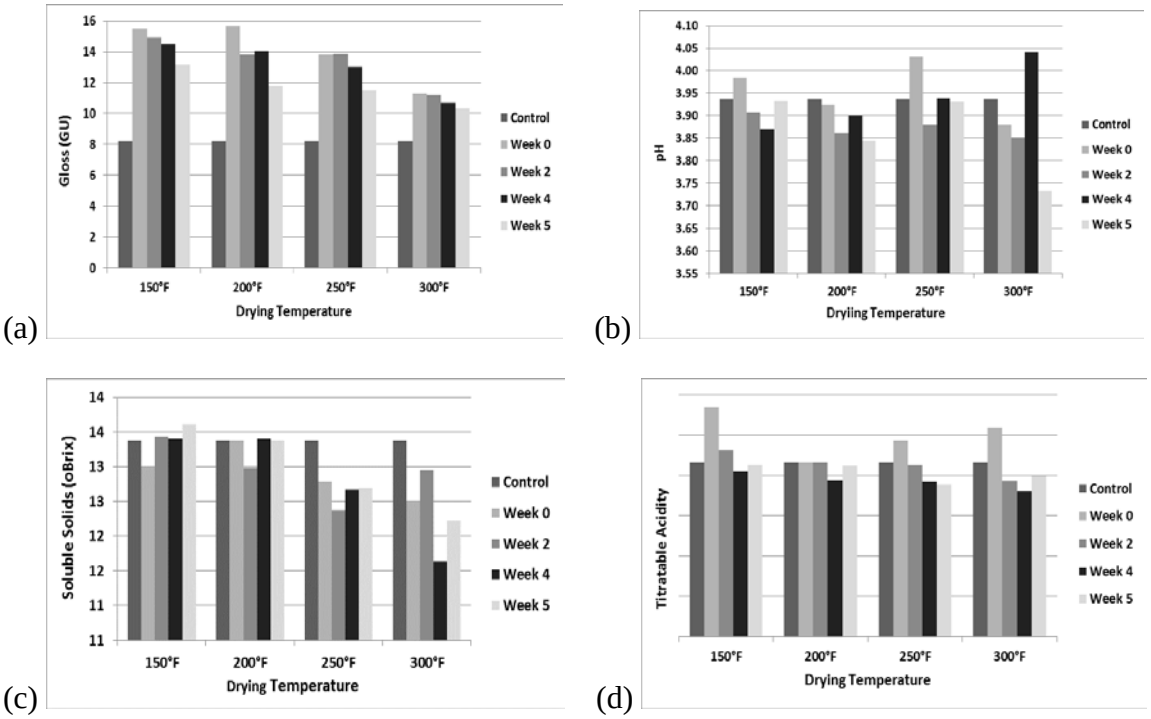

Fig 4. Apple quality data for different drying treatments over the storage period of 5 weeks from plant trial #2 with all drying treatments shown here for 60 seconds of drying only; (a) Gloss; (b) pH; (c) Soluble Solids; (d) Titrtable Acidity. (Note: the control data point presented here is only for the first week, so it shows constant throughout. Please refer to the table below for all the control data).

Table 1. Apple quality data for the control samples of both unwaxed (not dried) and waxed (dried in the plant dryer) from plant trial #2.

		Week 0	Week 0	Week 2	Week 2	Week 4	Week 4	Week 5	Week 5
	Control	Avg	Std Dev	Avg	Std Dev	Avg	Std Dev	Avg	Std Dev
Gloss	Unwaxed	8.23	2.83	8.65	3.09	8.76	3.08	7.66	2.86
	Waxed Dried	13.93	4.70	14.85	4.60	13.82	3.72	10.50	4.06
Soluble Solids	Unwaxed	13.38	1.53	13.59	0.87	12.56	0.43	13.56	0.77
	Waxed Dried	13.95	1.35	13.75	0.56	14.06	1.10	13.78	0.43
pH	Unwaxed	3.94	0.05	3.94	0.05	3.90	0.05	3.86	0.08
	Waxed Dried	3.92	0.06	3.98	0.06	3.90	0.06	3.87	0.07
Titratable Acidity	Unwaxed	0.22	0.05	0.24	0.02	0.21	0.02	0.20	0.03
	Waxed Dried	0.22	0.04	0.23	0.01	0.19	0.02	0.21	0.03

By looking a the Fig 4(a), we can clearly see that the glossiness data shows a decreasing trend with the increase in temperature. This was not the case in our observations for the "Fuji" and "Gala" varieties form last year's work. Previously, we had observed that the glossiness increases with the increase in the drying temperstures. By visual observations, it was clear that this variety of apple was sensitive to higher tempeatures. The apples at 250°F and 300°F, had parts of skin damaged because of the heat. The heat damage was prominent at 300°F.

Looking at the other quality parameters (pH and soluble solids) we can see clearly that the apples dried at 300°F, show difference in their quality. Only the titratable acidity did not show any major change. This suggests that the apple quality was negatively impacted at the higher tempeature of 300°F.

During this particular trial, the apples for all the treatments were stored at the packing house. The packing house R&D/Quality staff, also measure the quality of the apples over the storage period. They following the procedures they typically follow in their plant. Their observations were very similar to ours. We have not include their dat into this report, because of space limiataions. We will be happy to share this data upon request by any of the WTFRC members.

From this we concluded that the best tempeartures for the red delicious apple variety is 250°F or lower. This further suggests that the optimum drying temperature will vary based on the apple variety.

Data from plant trial #3:

In the plant trial #3, "golden delicious" variety of the apple was used for the studies. On this day, the plant was using carnauba wax. The data of different quality parameters measured over the storage period of 5 weeks is shown in Fig 5. Fig 5(a) shows the glossiness data; Fig 5(b) shows the pH data, Fig 5(c) shows the soluble solids data and Fig 5(d) shows the titratable acidity data. After the 4th week of storage the apples were stored under room conditions for a week and the quality parameters were measured and recorded as the 5th week data.

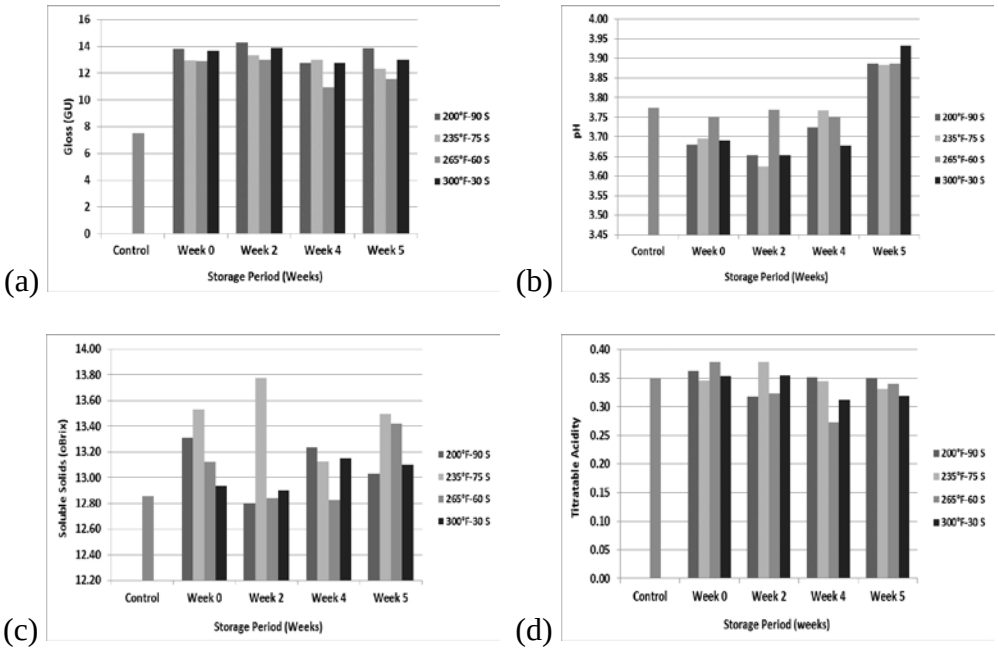

Fig 5. Apple quality data for different drying treatments over the storage period of 5 weeks from plant trial #3; (a) Gloss; (b) pH; (c) Soluble Solids; (d) Titrtable Acidity. (Note: the control data point presented here is only for the first week).

From the Fig 5(a), we can clearly see that the glossiness of these apples did not decrease with the increase in the dryign temperature, as we saw in case of the red delicious variety. Further, we can also see that the glossiness did not decrease during the storage time. The glossiness of the apples dried at 300°F, was more stable than the lower tempeatures. With respect to the other quality parameters of the apples, the soluble solids (Fig 5c) and titratable acidity (Fig 5d) were not impacted by the drying tempeatures. Altough there was a slight increase in the pH of the apples for all the drying temperatures.

In overall, we concluded that the "golden delicious" variety of apples are not negatively impacted by high temperatures (up to 300 ℉) of drying.

CONCLUSIONS FROM THE QUALITY STUDIES

- 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.
- Overall, this drying technique can provide economic benefits to the packing houses.

PRELIMINARY MICROBIOLOGICAL TESTING AND NEXT STEPS

We conducted few preliminary tests during the year 2015, to assess the impact of the high temperature drying on the reduction of the natural microbial loads on the apples.

We found that the settings tested did not show any effectiveness in reducing the total microflora loading. The testing was done with the waxed apples in the packing houses, where the incoming load of microorganisms was not controlled.

Based on these preliminary results, we will work on modifying drying parameters and dryer design to do extensive testing in the second year.

From our preliminary testing, we believe that the testing should include the drying of the apples soon after the washing step when the moisture is still present on the apples. It is well known that the heat is more effective in the presence of excess moisture to reduce the microorganism levels. The microbial resistance to heat, increases with the decrease in the moisture.

Initially, we had anticipated that the moisture present in the wax would be enough to make the heat more effective. But, from the preliminary results, it does look like we need to consider testing soon after the apples are washed and before prepping them for wax application. The excess moisture that is available at this step would potentially help to make the heat more effective in reudicng the microbial loads.

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 will be conducted in the pilot plant by utilizing surrogate microorganisms (non-pathogenic *E. coli* and *Listeria* strains). Three strains will be selected for both non-pathogenic *E. coli* and *Listeria* strains for the pilot-plant experiments. It is anticipated that three replications will be conducted for, surface moisture (no wax) and carnauba wax treatments at selected temperature and time combination. Two apple varieties (Fuji and Gala) will be selected for the fully replicated experiment with surface moisture (no wax) and with the carnauba wax treatments.

Apples will be obtained from cooperating packing facilities. Apples will be washed prior to inoculation. After inoculation, efforts will be made to achieve similar temperatures as apples in the packing facilities, before testing. We will use an immersion inoculation method to inoculate apples. Treatments will include: uninoculated, inoculated apple with or without wax coating and inoculated with or without surface moisture application, at specific temperature and time combinations.

Apples will be examined for microbial levels after drying. Enumeration will be performed using appropriate media for selected strains and a pre-enrichment for detection of stressed cells will be performed for samples identified to have low microbial recovery.

FINAL PROJECT REPORT

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

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Cooperators:

Joan Rosen, Ph.D., JC Rosen Resources
Five WA apple packing facilities
Biologics Resources, LLC
Pulse Instruments

Other funding sources: None

Total Project Funding: $94,000

Budget History:

Item	Year 1: 2014	Year 2: 2015
Salaries	$28,000	$33,750
Benefits	$5,600	$5,600
Wages		
Benefits		
Equipment		$6,279.44
Supplies		
Travel	$142.36	$3,031.56
Plot Fees		
Miscellaneous		$11,596.64
Total	$33,742.36	$60,257.64

As part of the Food Safety Modernization Act (FSMA), the recently enacted Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls (HARPC) for Human Food (the Preventive Controls Rule) focuses on preventive standards for the manufacturing and processing of food for human consumption. Data on food safety practices for the produce industry were last recorded by the USDA's National Agricultural Statistics Service (NASS) in 1999. Without data documenting current practices, it may be challenging for industry to implement FSMA rules that require scientific evidence for food safety practices (Perez, 2015). To meet the Preventive Controls Rule's validation requirements, Washington tree fruit packinghouses will be required to demonstrate that their preventive practices (i.e., sanitizer use in dump tank water) are effectively controlling for microbial contamination. Facilities that use water in their packing systems will be required to prepare and implement preventive controls using a food safety plan (HARPC) that includes hazard identification and evaluation, preventive control implementation for identified hazards, preventive control monitoring, verification of monitoring including process validation, corrective action procedures if preventive controls are not properly implemented, and a recall plan if contaminated food is released into the supply chain. To date, there has not been a study conducted for the tree fruit industry correlating microbial water testing data to preventive controls for dump tank water.

OBJECTIVES

1. **Collect industry data: As part of their previous work, IDS has collected individual company water, environmental and product test data from third-party laboratories for apples, pears and cherries. In addition IDS has worked with individual companies to obtain and analyze actual packing line monitoring data. In 2014 IDS proposes extending the data collection efforts to other packinghouses.**
2. **Data Analysis: Correlate dump tank water monitoring parameters (e.g. temperatures, pH, ORP readings, sanitizer levels and exposure by fruit and variety) to microbial levels. To the extent possible, examine how various sanitizers perform given the fruit and environmental conditions. Examine critical limits for critical control points, how they were determined, and how frequently they are exceeded.**
3. **Review HACCP plans: Based on the data analysis, develop a dump tank decision tree for packinghouse use. Recommend appropriate parameters for dump tank water in tree fruit packinghouse HACCP plans.**

SIGNIFICANT FINDINGS

- Over the two-year project, 368 total coliform (TC) tests and 74 generic *E. coli* tests were conducted (36 samples for TC testing and 8 samples for generic *E. coli* testing were lost by FedEx). Total coliform was present in 19% of dump tank water samples containing chlorine and in 35% of water samples containing PAA. In dump tank water containing either chlorine or PAA, generic *E. coli* was present 17% of the time.

- In dump tank water containing chlorine, ORP was the only predictor found to be significantly correlated to TC detection ($r^2 = 0.3924$; $p < 0.0001$); as ORP increased, TC concentration decreased. These findings suggest that maintaining a higher ORP would reduce TC concentration. Interestingly, chlorine concentration (measured using either strips or a ChlordioX) was not found to be a significant predictor for TC concentration. ORP, pH, water use duration, and conductivity were collectively strong predictors for generic *E. coli*.

- For facilities using chlorine as a dump tank water sanitizer, pH levels were maintained largely within the ideal range of 6.5 and 7.0.

- At facilities using chlorine as the water sanitizer, ORP, pH, and temperature were collectively found to be significantly correlated with free chlorine measurements ($r^2 = 0.475$; $p < 0.0001$). Individually ORP was not found to be a strong predictor of free chlorine concentration. When pH was within the optimal range for free chlorine formation (6.5 - 7.0), individually ORP was still not a strong predictor for chlorine levels ($r^2 = 0.2740$; $p < 0.0001$; $\beta = 0.096$).

- In PAA-containing dump tank water, PAA concentration, pH, conductivity, turbidity, water use duration, and ORP collectively were found to be significant predictors of TC concentration ($r^2 = 0.384$; $p < 0.0001$). High turbidity and conductivity measurements were positively associated with increases in microbial concentration. Only ORP was found to be a significant predictor for generic *E. coli* ($r^2 = 0.323$; $p = 0.0011$) with decreases in ORP associated with increases in generic *E. coli* concentration.

- At facilities using PAA, increases in turbidity and conductivity were found to be associated with an increase in PAA, probably due to more sanitizer typically being added as debris increase in the tank. Increases in water use duration, pH, and ORP were associated with a decrease in PAA concentration.

- Among all four facilities using PAA, levels ranged between 0 and 120 ppm during the sampling period. Sixty samples (17.9%) were measured at levels greater than the FDA's 80 ppm limit for wash water; 18.3% of these had levels greater than 100 ppm. ORP was observed between 246 and 685 mV while PAA was in use.

- All companies reported having HACCP plans; however, in response to the question of whether the dump tank was considered a critical control point (CCP) in their plan, one company said it was, one company said it was did not, and the other three did not answer the question. Based on the data analysis, sanitizer concentration, ORP, water pH and temperature, and microbial levels need to be measured for an entire dump tank water cycle (i.e., when tank is filled with clean water prior to fruit entry until water is drained from tank) when validating the HARPC/HACCP plan for a dump tank system. A diagram (Figure 1) illustrating operational areas to be considered when developing and validating a HARPC/HACCP plan are provided at the end of the report.

RESULTS & DISCUSSION

Sampling was conducted in six visits at five Washington apple packing facilities for a total of 177.5 operational hours. Prior to each site visit, companies were asked to fill out a 70-question questionnaire covering their HACCP plan, equipment, dump tank cleaning and sanitation, cleaning frequency, cleaning verification, unloading mechanism, apple variety and harvest information, chemical exposure, dump tank water, dump tank operations and monitoring, instruments used for measurements, instrument calibration, and microbial testing.

IDS tested physicochemical parameters on site. ORP, pH, temperature, and conductivity were measured using an Ultrameter II (Myron L Company, Model 6PFC). Turbidity was measured using a Compact Turbimeter (Palintest, Model CT12). Free chlorine was measured using a ChlordioX Plus (Palintest) and colorimetric strips (Water-Works, Free Chlorine High). PAA was measured using a hydrogen peroxide and peracetic titration kit (LaMotte) and colorimetric strips (Lamotte Insta-Test Analytic, Peracetic Acid).

Water samples were tested for total coliform and generic *E. coli*. Total coliform testing measures biologic pollution in water, includes all coliform species found in animal's intestines, soil, water and vegetation, and is usually associated with fecal polluted water. Generic *E. coli* tests specifically for bacterial species found in intestinal tract and are an indicator for contamination due to enteric pathogens (EPA, 2002).

For both total coliform and generic *E. coli* concentration, 100 millimeter (mL) water samples were collected and shipped overnight on ice to Biologic Resources, LLC in Portland, Oregon. TC

samples were tested according to Method 9221B and generic *E. coli* samples were tested according to Method 9221F. Water samples for generic *E. coli* tests were collected at the beginning and nearest to the end of each operating day as possible. Water samples collected for TC tests were collected every hour.

PAA and chlorine in the form of calcium hypochlorite were the sanitizers used in the sampled facilities (Table 1). In three of the facilities, either chlorine or PAA was used based on the fruit variety and/or the condition of the fruit arriving from the orchard. Two of the facilities use PAA or chlorine in the dump tank water, regardless of fruit variety and condition. Due to a limited number of project participants packing pears and cherries, data included in this report is from dump tank water used in apple packing only.

At each facility, dump tank water parameters were measured during normal operations throughout two dump tank water cycles. The first sample was taken in clean water before product entered the dump tank. Thereafter, sampling/measuring (in duplicates) continued at 30 minute intervals until water was discharged from the tank. The number of days the dump tank water was used prior to discharge varied by facility and was dependent on factors such as apple variety, appearance of the water, and fruit inventory. Data is summarized in Table 1.

Pearson correlations and step-wise multiple regressions were completed using Stata 12. One step-wise regression was completed for each variable. If the regression for the variable was individually found to be statistically significant (significance for inclusion in the model was set at $p < 0.015$), it was included in the regression model.

Table 1. Facilities visited and dump tank use.

Packinghouse	Line type	Tank ID	Days of use	Total water use time (hrs/tank)	Sanitizer	From Storage (S) or Orchard (O)
A	Commit to pack	A1	1	6.9	Chlorine	S
	Commit to pack	A2	1	7.7	Chlorine	S
B	Commit to pack	B1	3	33.1	Chlorine	S
	Commit to pack	B2	3	32.1	Chlorine & PAA	O
C	Commit to pack	C1	1	8.6	PAA	O
	Commit to pack	C2	2	17.5	PAA	S
D	Commit to pack	D1	2	20.7	Chlorine	O
	Commit to pack	D2	2	20.8	PAA	O
E	Commit to pack	E1	2	18.8	PAA	S
	Pre-size	E2	1.5	11.3	Chlorine	S

Each facility had separate measures and targets for maintaining their system (Table 2). Three of the four companies that used chlorine as a water sanitizer use ORP readings for monitoring

sanitizer efficacy. ORP target level ranged from 800 to 1,000 mV and acceptable readings varied among companies. Companies using PAA directly measured sanitizer concentration and did not rely on ORP readings for monitoring sanitizer efficacy. Target concentrations for PAA ranged between 50 to 80 ppm (80 ppm is the maximum concentration allowed by the FDA).

Table 2. Dump tank controls as reported in the questionnaire and visits.

Packinghouse	A	B	C	D	E
Parameters measured for sanitizer target level	Cl: FC ppm	Cl: ORP	PAA: ppm	Cl: ORP PAA: ppm	Cl: ORP PAA: ppm
Target sanitizer concentration	Total Cl: 40-60 ppm FC: 20-30 ppm	NA	PAA: 60-80 ppm	PAA: 50-70ppm	FC: 50-100 ppm PAA: 0-80 ppm
ORP target for Cl	900-1000 mV	800 mV	-	800 mV	920 mV
ORP acceptable readings for Cl	>750 mV	800 mV	-	675-900 mV	755-920 mV

Cl = Chlorine sanitizer; FC = Free chlorine; PAA = Peracetic acid; ORP = Oxidation Reduction Potential; T = Temperature; NA = not answered

Table 3 displays violations for each company when measurements were outside the company's acceptable readings. Packinghouse A had only 2 measurements outside of their ORP acceptable readings and did not have any positive tests. Of the 18 measurements outside of Packinghouse B's acceptable readings, all but one was below the minimum of 750 mV. Packinghouse C, using PAA, had a number of measurements exceeding their target maximum concentration of 80 ppm. No positives for either TC or generic *E. coli* were found when PAA was above 80 ppm; however when the concentration was below the target concentration range (60-80 ppm), 33% of the water samples tested for TC were positive. While using PAA as the sanitizer, Packinghouse D never reached their target concentration (50-70 ppm). When using chlorine as the sanitizer, Packinghouse D's readings never exceeded the upper limit (900 mV) of their acceptable ORP readings. However, when ORP was below the minimum acceptable reading of 675 mV, 100% of TC samples were positive. Packinghouse E had no ORP violations when chlorine was in use and 34 violations when PAA was in use, 33 of which were higher than the maximum acceptable reading (920 mV). However, no samples tested positive for TC or generic *E. coli*.

Table 3. Dump tank controls performance.

Packinghouse	A	B*	C	D	E
<u>Chlorine – ORP (mV)</u>					
Total samples† outside of acceptable ORP readings	2	18	N/A	12	0
No. of samples tested for TC outside of acceptable ORP readings (percent TC-positive)	2 (0)	12 (75%)	N/A	6 (100%)	0 (0)
No. of samples tested for GE	2 (0)	4 (50%)	N/A	4 (50%)	0 (0)

Packinghouse	A	B*	C	D	E
outside of acceptable ORP readings (percent GE-positive)					
PAA concentration (ppm)					
Total samples outside of target concentration	N/A	No range provided	65	86	34
No. of samples tested for TC outside of target concentration (percent TC-positive)	N/A	No range provided	21 (10%)	48 (90%)	18 (0)
No. of samples tested for GE outside of target concentration (percent GE-positive)	N/A	No range provided	6 (0)	8 (75%)	2 (0)

†Includes readings at time points when TC and GE were not measured.

*Packinghouse B – Target and acceptable ORP range was reported as 800 mV, assumed acceptable range was 750-850 mV; N/A = not applicable, GE = generic *E. coli*

Calcium hypochlorite (chlorine)

In dump tanks using calcium hypochlorite (chlorine) as the sanitizer (Table 4), all water samples that tested positive for generic *E. coli* also tested positive for TC. Generic *E. coli* was detected in six samples (3 duplicates), at an average concentration of 89 MPN/100mL (range: 4-170 MPN/100mL). For these generic *E. coli*-positive samples, the average TC concentration was 1,334 MPN/100mL (range: 4->1,600 MPN/100mL). Two of the six samples were at the maximum detection limit of 1,600 MPN/100mL total coliforms and three exceeded it. When samples tested positive for generic *E. coli*, the average ORP, pH, and free chlorine readings were 486 mV, 6.9, and <0.02 ppm, respectively.

Table 4. Microbial testing results when chlorine sanitizer was in use.

Packinghouse	Tank ID	Total Coliform			Generic *E. coli*		
		Number of tests	Positive tests	Percent positive	Number of tests	Positive tests	Percent positive
A	A1*	16	-	-	4	-	-
	A2	16	0	0	4	0	0
B	B1	58	5	9%	12	4	33%
	B2	32	12	38%	4	0	0
D	D1	40	16	40%	8	2	25%
E	E2	28	0	0	8	0	0
Total		174	33	19%	36	6	17%

* Tank A1 microbial results are not included as the microbial samples were lost by FedEx.

Thirty-three samples contained detectable levels of TC with an average concentration of 534 MPN/100mL (range: 2->1,600MPN/100mL). The average ORP, pH, and chlorine measurements for TC-positive samples were 608 mV, 7.1, and 12 ppm, respectively. Of the 33 TC-positive samples, five contained levels exceeding the 1,600 MPN/100mL detection limit. The average ORP, pH, and chlorine for TC-positive samples were 358 mV, 6.8, and <0.02 ppm, respectively.

Dump tank parameter measurements varied among facilities. Variations were also seen within each packinghouse data set with the greatest fluctuations in chlorine levels (the intra-packinghouse spread for free chlorine measurements averaged 53 ppm). In general, pH levels, controlled by adding various pH stabilizers (e.g., citric acid, sulfuric acid, or sodium hydroxide) were maintained largely within the ideal range of 6.5 and 7.0. However, two facilities (Packinghouse A and D) had mean pH readings (7.2 and 7.7, respectively) above ideal conditions for sanitizer efficacy (pH 6.5 − 7.0). Of the 84 water samples taken from tanks with a pH within the 6.5 − 7.0 optimal range, 11% had detectable TC levels versus 27% of the 90 water samples collected when pH was outside of the optimal range.

Pearson correlations were completed for all measured parameters. Measurements of free chlorine using chlorine strips and the ChlordioX were significantly correlated ($r = 0.881$, $p < 0.001$) however, differences between each of the two chlorine measurement methods and additional dump tank water parameters were found (see Table 5).

Table 5. Pearson correlations of each parameter for chlorine-containing dump tank water.

	†FC (C)	**†FC (S)**	**TC**	**G. *E. coli***	**ORP**	**pH**	**Turb.**	**Cond.**	**Temp.**	**WU**
FC(C)	1.000									
FC (S)	0.881**	1.000								
TC	-0.264*	-0.267*	1.000							
G. *E. coli*	-0.372*	-0.406	0.713**	1.000						
ORP	0.336**	0.385**	-0.626**	-0.384*	1.000					
pH	0.549**	0.674**	-0.015	-0.170	-0.108*	1.000				
Turb.	-0.061	0.086	-0.031	-0.192	0.223**	-0.352**	1.000			
Cond.	0.452**	0.588**	-0.004	-0.345*	0.047	0.543**	0.389**	1.000		
Temp.	0.377**	0.486**	0.161*	0.011	-0.278**	0.438**	0.254**	0.589**	1.000	
WU	-0.105	-0.078	-0.004	-0.292	0.166*	-0.370**	0.914**	0.438**	0.301**	1.000

FC (C) = Free chlorine measured using the Chlordiox; FC (S) = Free chlorine measured using strips; TC = Total coliform; G. *E. coli* = Generic *E. coli*; ORP = Oxidation Reduction Potential; Turb. = Turbidity; Cond. = Conductivity; Temp. = Temperature; WU = Water use duration in hours

† When calcium hypochlorite is added to dump tank water, the chlorine dissolves and takes multiple forms. In solution, chlorine (or total chlorine) can take either of two paths that determine its effectiveness: it can form the antimicrobial agent, free chlorine or it can lose its antimicrobial properties by reacting with organic compounds to form combined chlorine. Dump tank conditions that favor the formation of free chlorine are essential for minimizing pathogens.

*Significance of $p < 0.05$

**Significance of $p < 0.001$

In order to estimate the free chlorine levels collected with the ChlordioX, a stepwise multiple regression was conducted using the other water physicochemical properties. ORP, pH, and temperature were collectively found to be significantly correlated with free chlorine measurements ($r^2 = 0.475$; $p < 0.0001$). When modeled individually, ORP was not found to be a strong predictor of free chlorine concentration despite current industry use of ORP as a surrogate measurement for sanitizer efficacy. Even when pH was within the optimal range for free chlorine formation (6.5 and 7.0),

individually ORP was still not a strong predictor for chlorine levels ($r^2 = 0.2740$; $p < 0.0001$; $\beta = 0.096$).

A second stepwise multiple regression was completed in order to estimate free chlorine levels using measurements collected with colorimetric strips (commonly used by industry). ORP, conductivity, water use duration, and turbidity explained up to 75% of the variability observed in chlorine concentration measured with strips ($r^2 = 0.752$; $p < 0.0001$). Colorimetric strips are not considered to be very accurate measurers of free chlorine due to oxidizing agents in water that may alter readings. In addition there is limited variability in chlorine readings using colorimetric strips due to set increments in concentration. However, if used in conjunction with pH measurements, colorimetric strips may be a useful method for verifying free chlorine measurements taken with handheld instruments or inline probes.

An additional stepwise regression was completed in order to assess the relationship between dump tank water parameters and microbial content. ORP was the only parameter found to be a significant predictor of TC levels ($r^2 = 0.3924$; $p < 0.0001$); the analysis showed that as ORP increases, TC levels would be expected to decrease. These findings suggest that maintaining a higher ORP would reduce TC concentration. Interestingly, chlorine concentration (measured using either strips or a ChlordioX) was not found to be a significant predictor for TC concentration.

Peracetic acid

IDS used two methods to measure PAA: colorimetric strips and a titration kit. The colorimetric strips and titration kit tests are significantly correlated ($r^2 = 0.792$; $p < 0.001$; Table 7). However, the PAA strip method and titration kit method were not as highly correlated as one would expect. The difference may be due to the objectivity of reading the tester identifying colors and then determining where the strip falls on scale. The same color (or result) may be interpreted differently by different testers or the same tester at a different time. In addition, the scale provided is incremental and therefore the PAA level may match a color but theoretically the real value is lower or higher. The titration method, although requiring more technical expertise, is less likely to vary between users.

In dump tanks using PAA as a sanitizer, all water samples that tested positive for generic *E. coli* were also positive for TC (Table 6). All five samples containing detectable levels of generic *E. coli* were collected from Tank D2 and had an average concentration of 1,340 MPN/100mL TC. Four of the five *E. coli*-positive samples exceeded the TC detection limit of 1,600 MPN/100mL. Forty-three dump tank water samples contained detectable TC. When samples were TC-positive, the average ORP and pH readings were 319 mV and 6.3 and when samples were TC-negative 460 mV and 3.9, respectively. Tank D2 accounted for the majority (77%) of TC-positive tests.

Table 6. Microbial testing results when PAA sanitizer was in use.

Packinghouse	Tank ID	Total Coliform			Generic *E. coli*		
		Number of tests	Positive tests	Percent positive	Number of tests	Positive tests	Percent positive
B	B2	28	10	36%	6	0	0
C	C1	20	1	5%	4	0	0
	C2	20	2	10%	4	0	0
D	D2	48	43	90%	8	5	63%
E	E1	42	0	0	8	0	0
Total		158	56	35%	30	5	17%

Among all four facilities using PAA, levels ranged between 0 and 120 ppm during the sampling period. Sixty samples (17.9%) were measured at levels greater than the FDA's 80 ppm limit for wash water; 18.3% of these had levels greater than 100 ppm. ORP was observed between 246 and 685 mV while PAA was in use.

Table 7. Pearson correlations of each parameter for PAA-containing dump tank water.

	PAA tit.	**PAA strips**	**TC**	**G. *E. coli***	**ORP**	**pH**	**Turb.**	**Cond.**	**Temp**	**TU**
PAA tit.	1.000									
PAA strips	0.792**	1.000								
TC	-0.497**	-0.547**	1.000							
G. *E. coli*	-0.383*	-0.428*	0.707**	1.0000						
ORP	0.687**	0.451**	-0.507**	-0.568*	1.000					
pH	-0.704**	-0.572**	0.532**	0.505*	-0.930**	1.000				
Turbidity	0.121*	0.020	0.020	0.163	0.143*	-0.116*	1.000			
Conductivity	0.532**	0.213**	-0.218*	-0.177	0.686**	-0.644**	0.187**	1.000		
Temp	-0.195**	-0.172*	0.260**	0.424*	-0.352**	0.246**	0.083	-0.167*	1.000	
WU	-0.429**	-0.342**	0.164*	0.262	-0.263**	0.118*	0.479**	-0.121*	0.364**	1.000

PAA tit. = PAA measured using a titration kit, PAA strips = PAA measured using strips, TC = Total coliform, G. *E. coli* = Generic *E. coli*, ORP = Oxidation Reduction Potential, Turb. = Turbidity, Cond. = Conductivity, Temp. = Temperature, WU = Water use duration in hours
*Significance of $p < 0.05$
**Significance of $p < 0.001$

A multiple step-wise regression was completed in order to predict PAA concentration. It was found that water use duration, turbidity, pH, conductivity, and ORP are collectively significant predictors for PAA concentration ($r^2 = 0.715$; $p < 0.0001$) and were thus included in the model. Increases in turbidity and conductivity were found to be associated with an increase in PAA, probably due to more sanitizer typically being added as debris increase in the tank. Increases in water use duration, pH, and ORP were associated with a decrease in PAA concentration.

Additional step-wise regressions were completed in order to assess the relationship between microbial content (both TC and generic *E. coli)* and observed dump tank water parameters. PAA concentration, pH, conductivity, turbidity, water use duration, and ORP were found to be significant predictors of TC concentration ($r^2 = 0.384$; $p < 0.0001$). Increases in turbidity and conductivity were found to be associated with an increase in TC concentration and with increases in PAA and ORP, one would expect there to be a decrease in TC. Only ORP was found to be a significant predictor for generic *E. coli* ($r^2 = 0.323$; $p = 0.0011$) with decreases in ORP associated with increases in generic *E. coli* concentration.

Observations:

While testing, several observations were made regarding operating practices. They are:

Bin washing - All five participating facilities used bin-immersion dump tanks. Three of the five facilities used wood bins in the line sampled by IDS; one facility only used plastic bins and another used a combination of wood and plastic bins. Some bins (both wood and plastic) were visibly muddy. Wooden bins were occasionally lined with a plastic liner that was manually pulled out of the tank as the apples submerged. One facility used a hose to wash mud off bins as they were exiting the dump tank resulting in a large amount of mud being transferred from the bin back into the dump tank. In addition, water spray from the hose and subsequent splashing had contact with the fruit as it exited the dump tank. Bin condition was also variable: plastic bins were usually visually cleaner than

wooden bins. However, bins were not tested for bacterial content, and the amount of visible mud seemed to depend on where the produce was harvested and the harvesting practices utilized in the orchard and not bin type.

Bin labeling - Bins were labeled with stickers for tracking purposes. Prior to entering the dump tank, stickers were removed from the bins by hand. At most facilities, stickers were removed using a metal spatula. At one facility, an employee climbed up each stack of bins by stepping on the lower bins in order to remove the sticker by hand, potentially introducing contamination to each bin before it entered the dump tank.

Debris - At each facility, employees are responsible for removing plant debris from the dump tank water and putting it in designated trash bins. At some facilities, trash bins containing plant debris were removed immediately while other facilities frequently allowed debris to accumulate in trash bins. Excessive debris accumulation in trash bins may provide a place for pest harborage and increase the contamination risk in the facility.

Employee practices - Employee clothing practices varied among facilities. Hair nets and gloves were not required at all facilities. Most facilities used tools to avoid animal intrusion (i.e. mouse traps near doors and/or electrical discharge insect control systems).

Employee Training - All five facilities verified the dump tank water sanitizer concentration by manually testing at varying time increments. However, lack of employee training for performing measurements was observed on at least one occasion. An employee measuring PAA sanitizer level using a titration kit did not properly follow the manufacturer's instructions. As a result of adding multiple reagent drops at once, the employee detected 40 ppm when IDS measurements using two different methods showed zero PAA was present. It is critical that employees are trained in using verification test equipment and methods, and supervisors/other employees should review employee measurements on a routine basis.

Food contact surfaces – Some tools (e.g., items used to move apples) used in the dump tank were not treated as food contact surfaces.

Pest control – Pest control is a challenge for facilities. Fruit fly swarms and rodents were observed on several occasions.

Probes - Each facility's dump tank water system is equipped with probes for automated readings (typically for pH and ORP). Based on the results, adjustments are made by adding water or chemicals in order to maintain sanitizer efficacy. If the probes are not maintained properly or are located in a region where the sanitizer is unevenly mixed, inaccurate readings may occur. At several facilities, the probes were located in the debris filter and often were covered in leaves and dirt. Line employees sporadically shook the probes to remove debris, which also may result in inaccurate ORP and pH readings.

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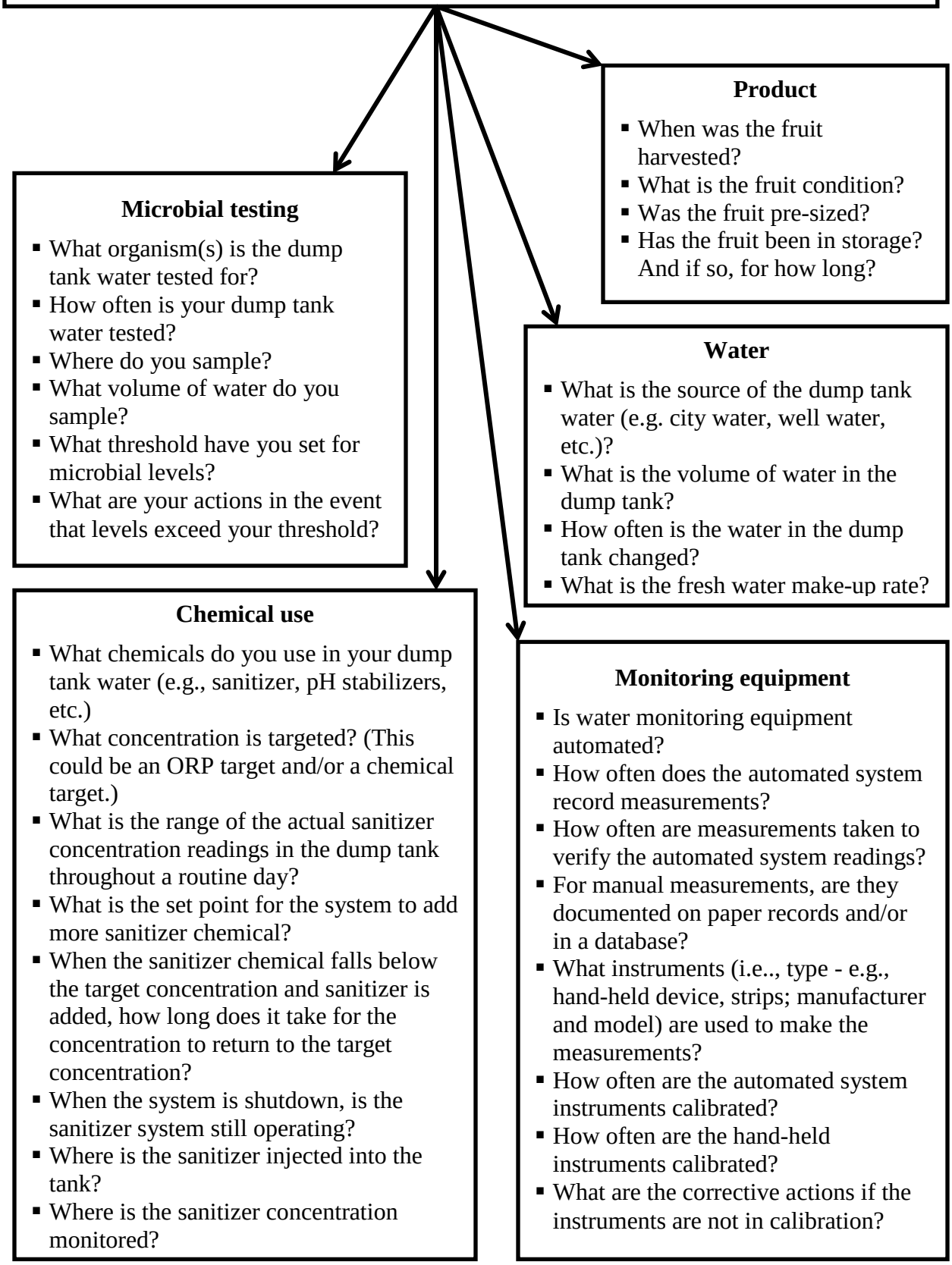
United States EPA. 2002. Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium). *U.S. Environmental Protection Agency Office of Water, Method 1604.* Accessed from: http://nepis.epa.gov/Exe/ZyPDF.cgi/P1002D57.PDF?Dockey=P1002D57.PDF

Figure 1. Questions to Consider for Dump Tank Water HARPC/HACCP Plan



EXECUTIVE SUMMARY

The study objectives were to collect dump tank monitoring data, correlate monitoring parameters (e.g. temperatures, pH, ORP readings, sanitizer levels and exposure by fruit and variety) to microbial levels, and use the findings to refine or inform development of dump tank water preventive controls as part of HARPC requirements or HACCP plans.

Sampling was conducted in six visits at five Washington apple packing facilities for a total of 177.5 operational hours. IDS tested the water's physicochemical parameters – sanitizer concentration (chlorine and peracetic acid (PAA)), ORP, pH, temperature, turbidity and conductivity on site. PAA and chlorine in the form of calcium hypochlorite were the sanitizers used in the sampled facilities. At each facility, dump tank water parameters were measured during normal operations throughout two dump tank water cycles. The first sample was taken in clean water before product entered the dump tank. Thereafter, sampling/measuring (in duplicates) continued at 30 minute intervals until water was discharged from the tank. For microbial testing (total coliform (TC) and generic *E. coli*), 100 millimeter (mL) water samples were collected and shipped overnight on ice to Biologic Resources, LLC in Portland, Oregon. Water samples were collected for TC tests every hour and for generic *E. coli* tests at the beginning and nearest to the end of each operating day as possible.

Over the two-year project, 386 TC tests and 74 generic *E. coli* tests were conducted. TC was present in 19% of dump tank water samples containing chlorine and in 35% of water samples containing PAA. In dump tank water containing either chlorine or PAA, generic *E. coli* was present 17% of the time.

In dump tank water containing chlorine, ORP was the only predictor found to be significantly correlated to TC detection ($r^2 = 0.3924$; $p < 0.0001$); as ORP increased, TC concentration decreased. These findings suggest that maintaining a higher ORP would reduce TC concentration. Interestingly, chlorine concentration was not found to be a significant predictor for TC concentration. ORP, pH, water use duration, and conductivity were collectively strong predictors for generic *E. coli*. ORP, pH, and temperature were collectively found to be significantly correlated with free chlorine measurements ($r^2 = 0.475$; $p < 0.0001$). Individually ORP was not found to be a strong predictor of free chlorine concentration. When pH was within the optimal range for free chlorine formation (6.5 - 7.0), individually ORP was still not a strong predictor for chlorine levels ($r^2 = 0.2740$; $p < 0.0001$; $\beta = 0.096$). Dump tank water pH levels were maintained largely within the ideal range of 6.5 and 7.0.

In PAA-containing dump tank water, PAA concentration, pH, conductivity, turbidity, water use duration, and ORP collectively were found to be significant predictors of TC concentration ($r^2 = 0.384$; $p < 0.0001$). Only ORP was found to be a significant predictor for generic *E. coli* ($r^2 = 0.323$; $p = 0.0011$) with decreases in ORP associated with increases in generic *E. coli* concentration. High turbidity and conductivity measurements were positively associated with increases in microbial concentration. Increases in turbidity and conductivity were found to be associated with an increase in PAA, probably due to more sanitizer typically being added as debris increase in the tank. Increases in water use duration, pH, and ORP were associated with a decrease in PAA concentration.

Among the four facilities using PAA, levels ranged between 0 and 120 ppm during the sampling period. Sixty samples (17.9%) were measured at levels greater than the FDA's 80 ppm limit for wash water; 18.3% of these had levels greater than 100 ppm. ORP was observed between 246 and 685 mV while PAA was in use.

All companies reported having HACCP plans; however, in response to the question of whether the dump tank was considered a critical control point (CCP) in their plan, one company said it was, one company said it was did not, and the other three did not answer the question. Based on the data analysis, sanitizer concentration, ORP, water pH and temperature, and microbial levels need to be measured for an entire dump tank water cycle (i.e., when tank is filled with clean water prior to fruit entry until water is drained from tank) when validating the HARPC/HACCP plan for a dump tank system.

FINAL PROJECT REPORT **YEAR**: 2 of 2

WTFRC Project Number: AP14107-202

Project Title: Updating the apple map, trait and marker databases in GDR

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

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

Other funding sources

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

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

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

Agency Name: NSF DIBBS
Amt. *requested*: $1.48 M (Jan 2015–Dec 2017)

Notes: "Tripal Gateway, a platform for next-generation data analysis and sharing". PI: Ficklin. Co-PIs include Main and Jung.

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

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

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

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

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

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

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

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

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

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

[a] 0.25 FTE Dr. Sook Jung (Senior GDR data curator), 4% salary increase in year 2.
[b] Computational hardware housing cost (10%), disk backup storage supplies, minor equipment maintenance
[c] Twice annual in-person meetings for feedback and training and quarterly teleconferences (gotomeeting).

OBJECTIVES

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

Specific objectives:

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

SIGNIFICANT FINDINGS

1. Data from 17 publications including 20 genetic maps, 22,896 molecular markers, 192 QTL, 42, MTL (Mendelian Trait Loci), 5174 genetic loci, 49 germplasm, 45 trait terms have been curated, uploaded and integrated with other GDR data. The data includes 20K apple SNP data. The curation effort includes the association of QTLs with Trait Ontology. These data will be used to help introgress genes/loci for traits of interest in the breeding programs.
2. New apple genome assembly and annotation (Malus x domestica V3.0.a1 has been made available through genome page and genome browser.
3. QTL data from WA Apple breeding program have been used in the analysis of finding sugar related QTL that exists in the conserved syntenic regions across the Rosaceae (peach and strawberry). The information on the conserved genomic regions in apple and strawberry have been transferred to the colleagues who will develop markers for pear and blackberry.
4. First version of data editing functionality of the breeder's toolbox has been developed. We are in the process of testing and will soon provide training for the personnel in WA Apple Breeding Program.
5. WA Apple Breeding Program one of 3 breeding programs being used to develop a comprehensive Breeding Information Management System

METHODS

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

Ontology (TO) so that users can browse standardized TO to find all the associated trait loci. We are actively adding more tree fruit ontology terms to TO, originally developed to describe traits in grass species, to expand to other plant species.

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

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

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

RESULTS AND DISCUSSION

Publicly available trait and marker data that is relevant to the WABP has been added to GDR and more performance and genotypic data specific to the breeding program will continue to be added over the next four years using funding from the USDA SCRI award to PI Main (co-PI's Jung, Evans and Peace). These additions will continue to provide an up-to-date breeding information management system for the WABP to facilitate routine marker-assisted breeding and more efficient development of new cultivars for WA apple growers. The additions of the QTL data from other Rosaceae crops, as well as from apple, enabled us to perform analysis to find QTL in evolutionarily conserved genomic regions across the Rosaceae crops. Ties with the other breeding projects, such as RosBreed projects in

US and European FruitBreedomics project, are strong, with data-sharing strategies in place. Significant funding of over $10 M has been obtained from federal sources by PI Main to continue to develop crop resources (GDR) and tools (markers for traits of economic importance) that will help make apple tree fruit breeding in WA even more efficient, accurate, creative, and rapid and keep WA apple growers competitive on a global scale. This funding allows us further development of breeder's toolbox into a comprehensive Breeding Information Management System (BIMS) that will be incorporated within the Tripal generic database platform which has been led by Washington State University researchers. It will provide breeders full privilege for uploading, editing, management, analysis and archiving of various breeding data and will include access to statistical packages such as R.

EXECUTIVE SUMMARY

Tree fruit breeding programs generate copious amounts of data. Utilizing this data requires proper management plans and interrogation tools to enable breeders to efficiently mine their data and extract what they need to enable more efficient breeding. Concordant with this is the need to also access all relevant public information such as what's known about traits, markers for these traits, germplasm containing useful traits in the same and related crops. Within the Rosaceae community database, GDR (www.rosaceae.org), a private breeding database for the WA Apple Breeding Program exists, connecting the programs private breeding data with all publicly available, quality checked, genomic, genetics and breeding data for Malus crops. Searchable interfaces allow the data to be searched by trait, trait levels, location, marker, pedigree, germplasm, year, etc and tools enable download of data for upload to analysis programs. The Cross-Assist tool takes this concept one step further. Using component data from the breeding program it outputs the optimal parents to cross and numbers of seedlings needed to generate the desired offspring and eventually new cultivar(s) which meet producer and consumer needs.

The Apple Toolbox in GDR will continue to be expanded to a more comprehensive Breeding Information Management System developed using the Tripal Database Platform and will be updated with the WAABP breeding data following the end of this WTFRC award. Funds from this WTFRC project were leveraged to support federal crop database proposals that have generated over $5M in new funds over the next 4 years. Tools to allow direct upload of the data are developed, which enable breeders to be responsible for managing their own data. The Field Book App currently being evaluated for this purpose is looking very promising for this purpose and we are working with its developers to optimize for tree fruit breeding. We will continue to develop the GDR breeding tools into a comprehensive breeding management system that provides secure, one stop access to all the data management and analysis tools that Rosaceae breeders and allied scientists need to more efficiently develop new cultivars.

FINAL PROJECT REPORT
WTFRC Project Number: AP-13-104

Project Title: Glyphosate fate in inland pacific northwest apple orchards

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Cooperators: Tim Smith

Total Project Request: **Year 1:** $37,787 **Year 2:** $40,536 **Year 3:** $15,000

Other funding sources: None

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

Item	2013	2014	2015
Salaries	$25,587	$28,1496	
Benefits	$ 2,200	$ 2,387	
Wages			
Benefits			
Equipment			
Supplies	$10,000	$10,000	$20,000
Travel			
Plot Fees			
Miscellaneous			
Total	$37,787	$40,536	$20,000

Footnotes:

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

Experiment 1.1: Fate of glyphosate in an orchard without a recent glyphosate use history. Assessment of first and second year data from field experiment 1 (Sunrise), field experiment 2 (Quincy NE), and field experiment 3 (Quincy SW) are completed.

Experiment 1.2: Absorption and translocation of basal-applied and soil-applied glyphosate. The greenhouse experiment for the absorption and translocation experiment 1 is completed, providing glyphosate absorption and translocation data.

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

Experiment 2.1: Genetic analysis of microbial communities.
Knowledge of the fungal and bacterial community composition within the nontreated control and the plots treated with glyphosate at 1920 g ae/ha at Sunrise and Quincy SW has been obtained.

SIGNIFICANT FINDINGS:

- No visual injury has been observed following the applications of glyphosate at Sunrise, Quincy NE, and Quincy SW.
- Tree growth was similar among treatments at Sunrise, Quincy NE, or Quincy SW regardless of glyphosate treatment - 80 trees per treatment were measured over the course of two consecutive seasons.
- Glyphosate absorption by bark treatments to juvenile trees in absorption and translocation experiment 1 was surprisingly higher than glyphosate absorption by the leaf treatment.
- Translocation of absorbed glyphosate from a basal application appears to result in translocation to the roots.
- Translocation of absorbed glyphosate from a foliar application appears to result in comparable accumulation of glyphosate above and below treated section.
- Relative to the 2013 growing season samples, there appears to be a modest partitioning of both microbial communities among the two soil treatments at Sunrise.
- Analysis for shifts in microbial communities at Quincy SW from root/rhizosphere samples were comparable to Sunrise.

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

measurements of area. Quincy NE and Quincy SW were established on May 13, 2014 in two separate Fuji blocks planted in 2013. Initial trunk measurements were recorded for each tree and the two field experiments were established with a randomized complete block design with four replications. Plots are 2.1 m wide by 24 trees in length. Each study includes three treatments; 1) no post emergence glyphosate and maintained weed free with applications of paraquat at 140 g/ha and hand weeding, 2) glyphosate at 840 g ae/ha, and 3) glyphosate applied at 1920 g ae/ha.

Prior to each glyphosate application at Sunrise, the no vegetation split-plots were hand weeded and the low hanging branches along with any suckers were trimmed. Glyphosate applications were applied to the whole plot and directed at the base of the tree. Glyphosate was applied on May 16, 2013, July 11, 2013, May 22, 2014, and July 31, 2014. To supplement the soil residue analysis as well as eliminate any concerns of glyphosate drift into the canopy during application, spray targets were placed systematically throughout the tree canopy and on the ground to document were the spray droplets were landing. Prior to each glyphosate application at Quincy NE and Quincy SW, the low hanging branches as well as any suckers were trimmed and the plots were hand weeded. Glyphosate applications were applied to the whole plot and directed at the base of the tree. Glyphosate was applied on May 22, 2014, July 31, 2014, April 28, 2015, and August 5, 2015.

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

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

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

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

Experiment 2.1: Genetic analysis of microbial communities.
A composite apple root sample with adhering rhizosphere soil was collected from two trees in each treatment plot from a depth of 5-15 cm. DNA was extracted from duplicate sub-samples (5 g) for each plot using the MoBio PowerMax Soil DNA extraction kits and resulting DNA was pooled.

Initial examination of microbial communities utilized a genetic approach to identify quantitative shifts in populations. Bacteria were quantified from the duplicate soil extracts by real-time quantitative PCR (qPCR) by targeting the 16S gene with the primer set 338F and 518R, and fungi using the primer set NSI1 and 5.8S. Quantification was achieved using the StepOne Plus Real Time PCR thermocycler. All reactions were performed using three technical replicates. The standard curves for PCR quantification were generated by diluting DNA plasmid containing cloned amplification product. The plasmid used for the bacterial 16S standard curve was constructed with the 16S gene from *Methylobacterium* sp. amplified from soil using the primers 8F (50-AGA GTT TGA TCC TGG CTC AG-30) and 1406R (50-ACG GGC GGT GTG TRC-30). The fungal standard curve was prepared from the ITS region of *Mortierella alpina* amplified from soil using the qPCR primers in which the complete and correct plasmid insert was previously verified by DNA sequencing. Qualitative changes in microbial community structure were initially examined using a coarse genetic approach by employing terminal restriction fragment length polymorphism (T-RFLP) analysis. This method was used as a cost savings approach and served to identify the most appropriate community to target for examination by pyrosequencing. T-RFLP analysis of bacterial and fungal communities was conducted using methods previously described and commonly employed by the collaborators (Weerakoon et al., 2012). These data were utilized to determine what, if any, microbial populations should be targeted for analysis by pyrosequencing. The bacterial 16S gene was targeted for amplification using the universal primer pair 8f and 907R. The fungal intergenic transcribed spacer region was amplified using the universal fungal primer pair ITS1F and ITS4.

RESULTS AND DISCUSSION:

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

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

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

Although the glyphosate treatments at Sunrise, Quincy NE, and Quincy SW did not have a significant effect on tree growth, there was a trend present at Quincy SW in year 1. As the rate of glyphosate increased, the tree growth in year 1 decreased. The trees at Quincy NE and Quincy SW were planted in 2013 and the trees at Quincy SW are smaller caliper trees than the trees at Quincy NE. Therefore, in the smaller and less mature trees, absorption and translocation of glyphosate may have occurred and resulted in reduced tree growth. The decreasing tree growth with increasing glyphosate at Quincy SW was not observed in year 2. Glyphosate applied at 1920 g ae/ha did result in the lowest total growth over two consecutive years of two applications per season at Quincy SW.

Table 1. Glyphosate treatment effects on the growth of 80 trees per treatment from Experiment 1.1.

	Sunrise		
	Year 1 tree growth (mm^2 $tree^{-1}$)	**Year 2 tree growth (mm^2 $tree^{-1}$)**	**Total tree growth (mm^2 $tree^{-1}$)**
Treatment	**Mean**	**Mean**	**Mean**
Nontreated	5.6 ± 1.1	3.3 ± 0.7	8.8 ± 1.2
Glyphosate 840 g ae/ha	10.1 ± 0.8	4.2 ± 1.1	14.3 ± 1.4
Glyphosate 1920 g ae/ha	6.2 ± 1.3	8.6 ± 2.4	14.8 ± 2.7
Split-plot	**Mean**	**Mean**	**Mean**
Vegetation	6.7 ± 1.1	6.4 ± 1.3	13.0 ± 1.7
No Vegetation	8.0 ± 0.9	4.4 ± 1.5	12.4 ± 1.7
	Quincy NE		
	Year 1	**Year 2**	**Total**
Treatment	**Mean**	**Mean**	**Mean**
Nontreated	41.7 ± 4.6	33.9 ± 3.0	75.6 ± 4.3
Glyphosate 840 g ae/ha	49.6 ± 3.9	18.9 ± 3.2	68.4 ± 6.2
Glyphosate 1920 g ae/ha	43.7 ± 5.8	26.9 ± 5.4	70.6 ± 7.1
	Quincy SW		
	Year 1	**Year 2**	**Total**
Treatment	**Mean**	**Mean**	**Mean**
Nontreated	100.8 ± 5.8	63.9 ± 3.6	164.7 ± 4.7
Glyphosate 840 g ae/ha	97.2 ± 7.6	73.0 ± 7.4	170.2 ± 12.0
Glyphosate 1920 g ae/ha	83.9 ± 8.7	74.5 ± 15.3	158.4 ± 22.8

Experiment 1.2: Absorption and translocation of basal-applied and soil-applied glyphosate. The absorption of glyphosate by the bark of young gala/M9 trees compared to the leaf was not an expected result (Figure 1). Overall translocation of absorbed glyphosate was less than 2% for treatments made to above graft basal (AGB), below graft basal (BGB), and to a leaf (Foliar). The total amount translocated was ~0.0051 ug of glyphosate, an exceedingly small amount.

Absolute translocation of glyphosate after 28 DAT was similar among applications (Figure 2). Increasing glyphosate per gram of plant material vs time was observed below treated section in both the AGB (Figure 3) and BGB (Figure 4) applications, whereas, comparable glyphosate per gram of plant material was observed in both above and below treated sections in foliar applications (Figure 5). Interestingly, once all plant material above the graft was harvested, the rootstocks were allowed to continue to grow and produce suckers and glyphosate was detected in the suckers (Figure 6). The detection of glyphosate in the suckers indicates that glyphosate was translocated to the rootstock and then remobilized into the suckers.

In summary, absorption was observed following basal applications. Observations of absorption following basal application would corroborate that glyphosate should not be used as a 'desuckering' treatment, and care should be exercised when applying glyphosate to juvenile trees. Although translocation was a very low percentage of absorbed, translocation following basal and foliar treatments *was* observed. Most importantly, basal applications appear to result in translocation to the roots. Future work is needed to determine if absorption and translocation in field conditions is similar to what we have observed in the lab. If it is, then we need to know if glyphosate accumulates in the tree after repeated applications to better understand whether or not injury from basal applications of glyphosate is possible or occurring. Additionally, absorption and translocation of glyphosate may differ by variety, rootstock, or timing of application.

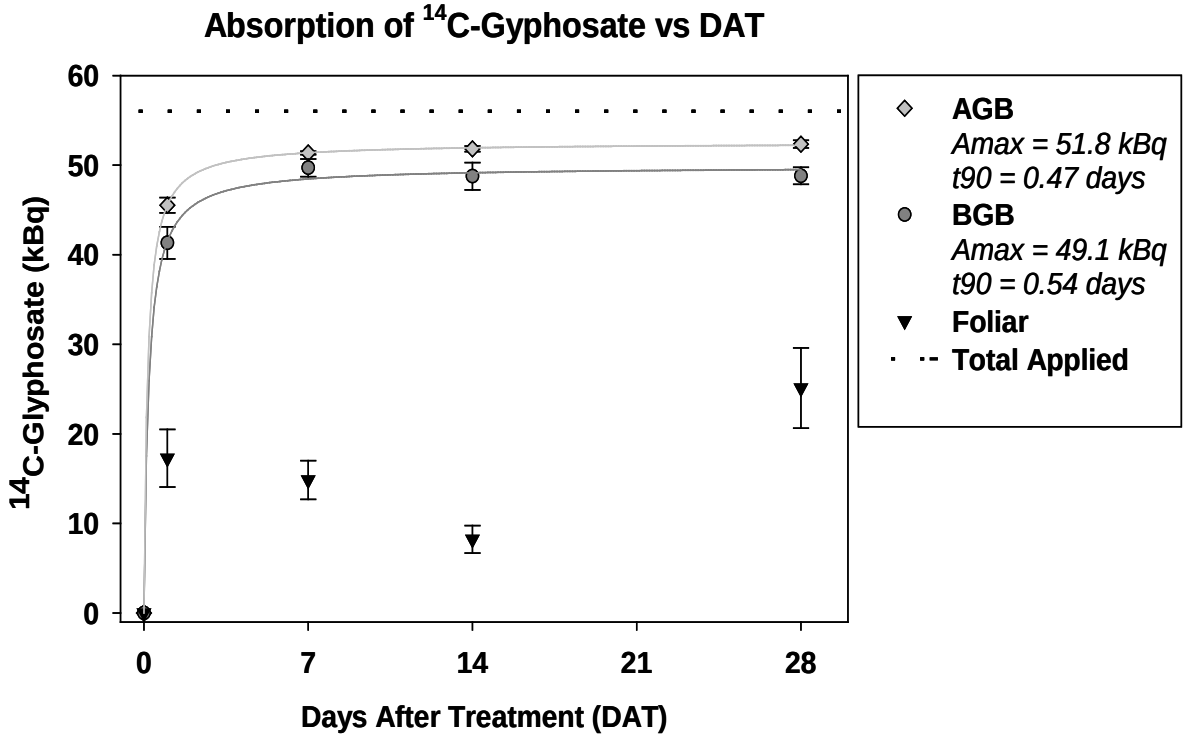

Figure 1. Percent of radiolabeled glyphosate absorbed by above graft basal, below graft basal, and foliar applications. Amax represents the maximum amount of glyphosate absorbed. The time for 90% of total glyphosate applied to absorb is represented by t90.

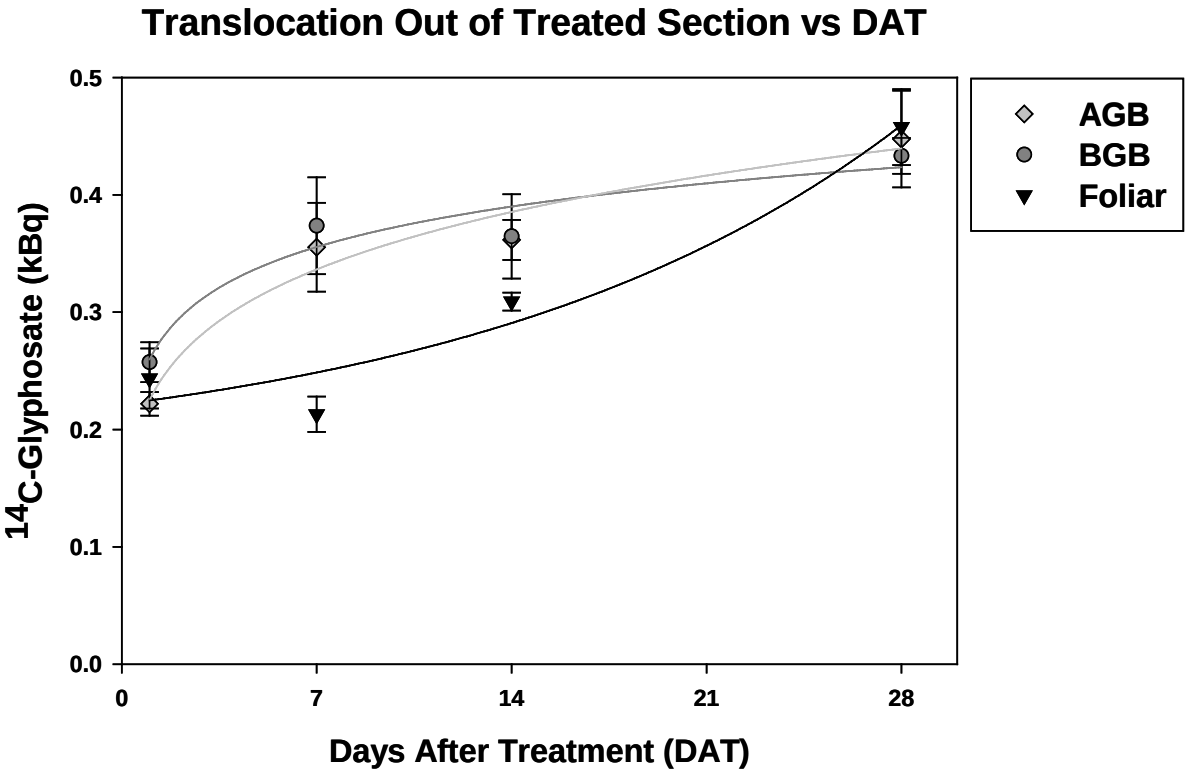

Figure 2. Translocation of glyphosate at 28 days after treatment.

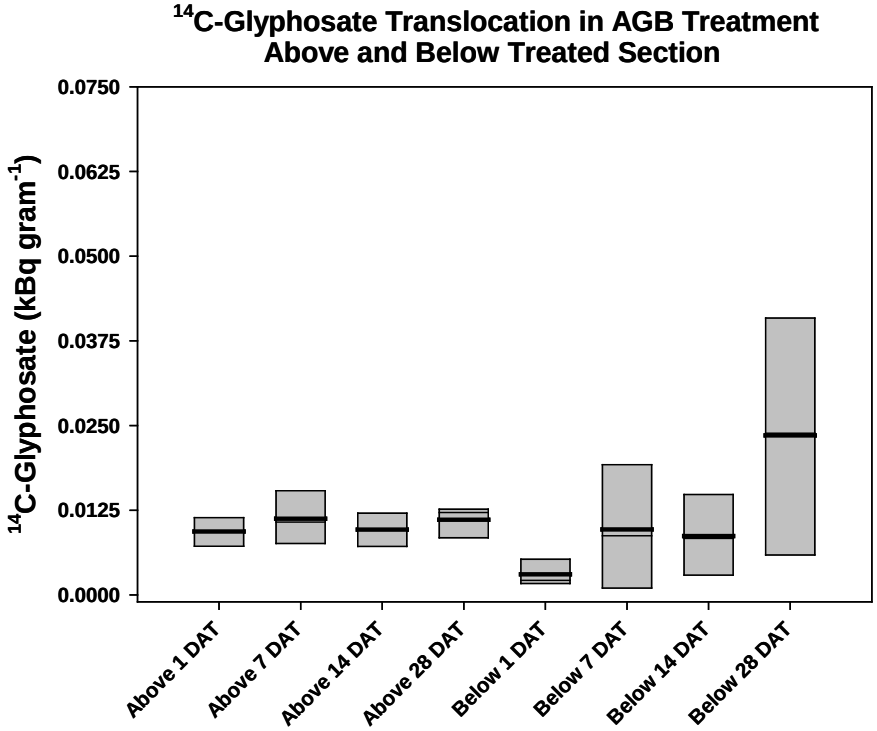

Figure 3. Glyphosate per gram of plant material vs time that was observed above treated section and below treated section in AGB treatment.

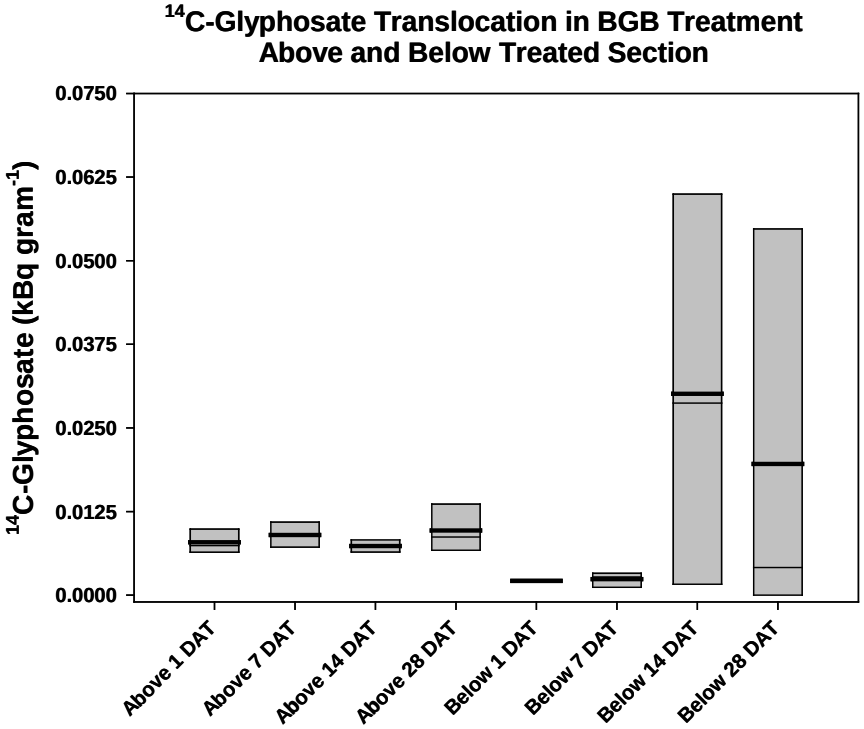

Figure 4. Glyphosate per gram of plant material vs time that was observed above treated section

and below treated section in BGB treatment.

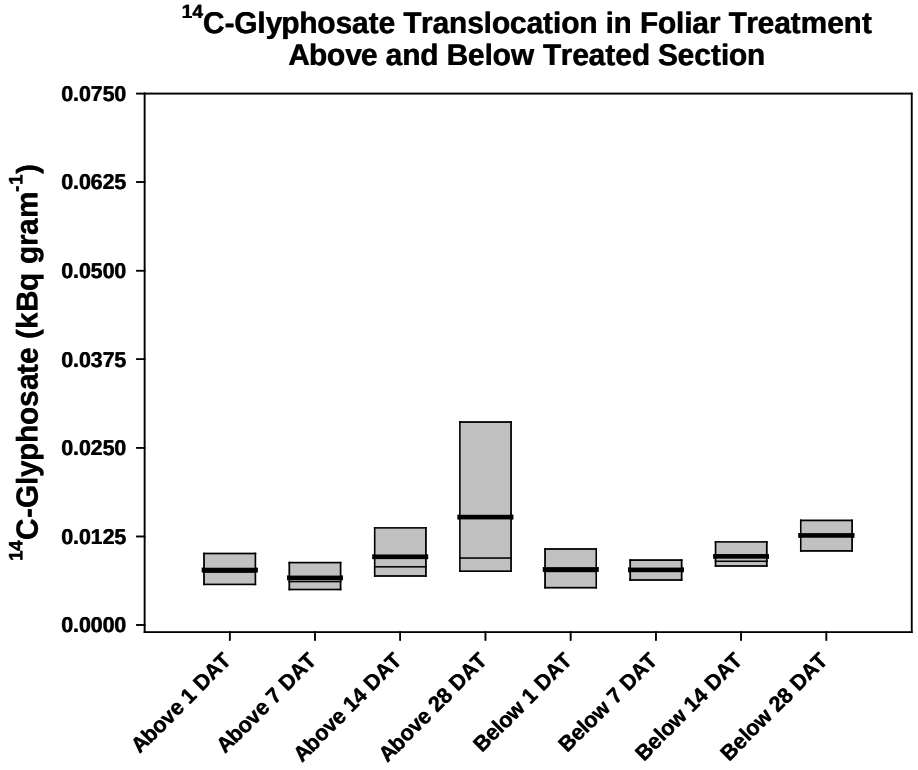

Figure 5. Glyphosate per gram of plant material vs time that was observed above treated section and below treated section in foliar treatment.

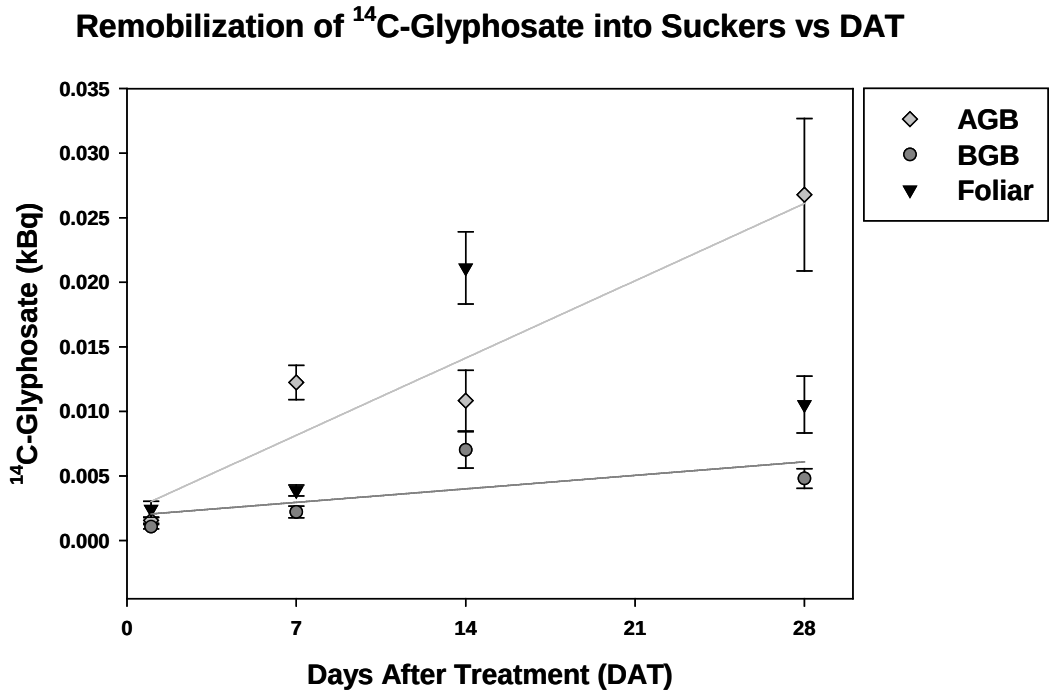

Figure 6. The detection of glyphosate in rootstock suckers vs time. More glyphosate was recovered from suckers arising from AGB treatments than from BGB or foliar treatments.

Experiment 2.1 Genetic analysis of microbial communities.
In year one, there were clearly no treatment effects of glyphosate on fungal or bacterial community composition between the nontreated plots and the plots treated with glyphosate at 1920 g ae/ha (2013 report). Principal coordinate analysis was conducted on bacterial and fungal community derived T-RFLP data for samples collected after the first glyphosate application at 1920 g ae/ha at Sunrise in May 2014. Statistically, there were no significant differences in composition of either the bacterial (Figure 7A) or fungal community (Figure 7B) between the nontreated and glyphosate treated plots. However, relative to the 2013 growing season samples, there appeared to be a modest partitioning of both microbial communities among the two soil treatments. In addition to principal coordinate analysis conducted on bacterial and fungal community at Sunrise, analysis was performed on root/rhizosphere samples at Quincy SW. Observations observed at Quincy SW (Figure 8) were comparable to previous year's results at Sunrise – modest clustering of treated and nontreated microbial communities.

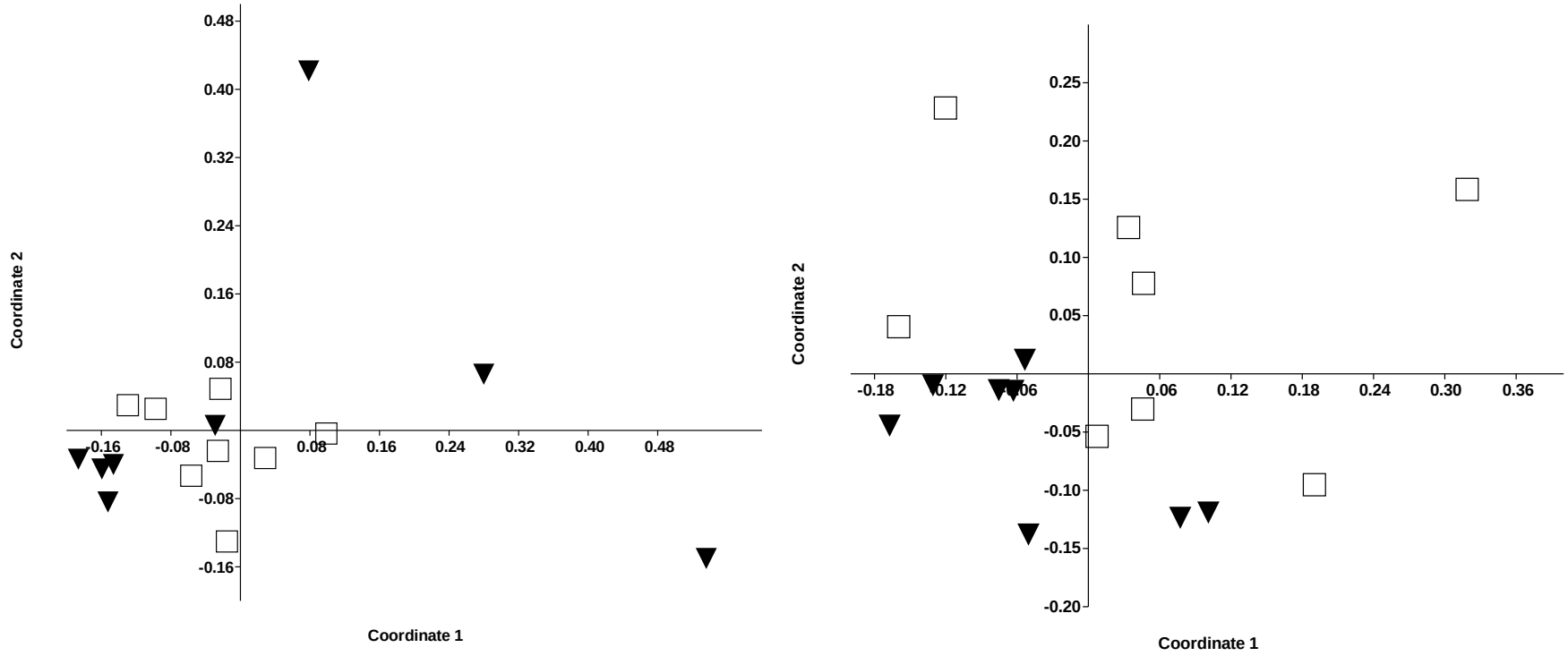

Figure 7. (A) The effect of glyphosate on bacterial community composition based upon principal coordinate analysis of T-RFLP data. Open squares represent nontreated control and inverted triangles represent data points from plots treated with glyphosate at 1920 g ae/ha. **(B)** The effect of glyphosate on fungal community composition based upon principal coordinate analysis of T-RFLP data. Open squares represent nontreated control and inverted triangles represent data points from plots treated with glyphosate at 1920 g ae/ha.

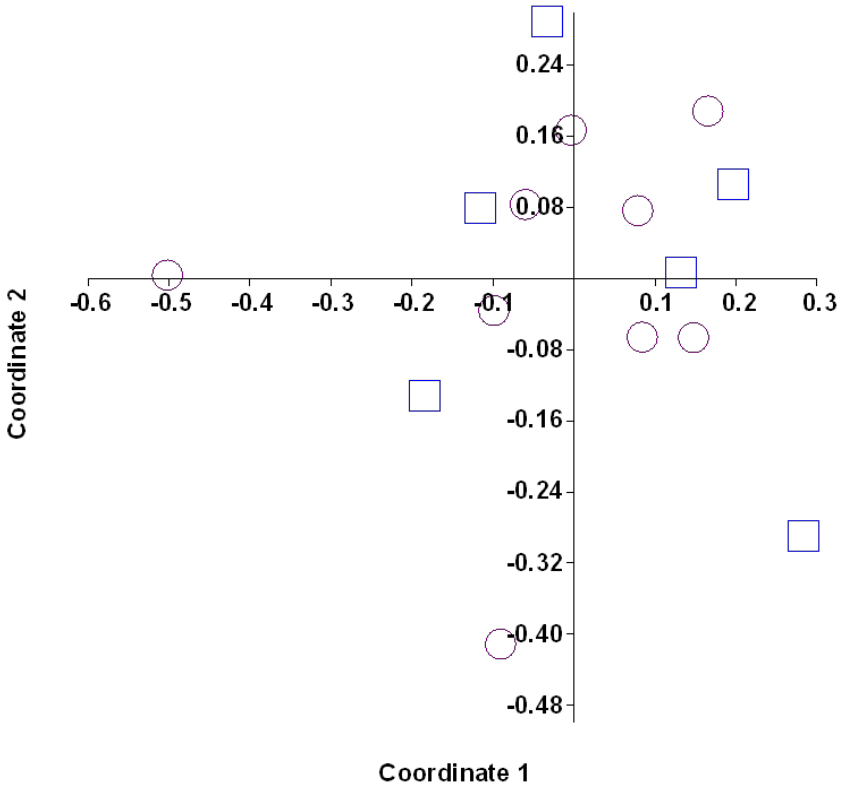

Figure 8. Nonmetric multidimensional scaling plot of the bacterial T-RFLP data from root/rhizosphere samples collected from Quincy SW in 2015 growing season. Open squares represent nontreated control and open circles represent plots treated with glyphosate at 1920 g ae/ha.

EXECUTIVE SUMMARY:

No visual injury has been observed in applications of glyphosate to apple orchards during the course of this research. Glyphosate did not cause injury following the applications at Sunrise, Quincy NE, and Quincy SW orchard experiments. Tree growth was similar among treatments at Sunrise, Quincy NE, or Quincy SW regardless of glyphosate treatment - 80 trees per treatment were measured over the course of two consecutive seasons per treatment. In a single year, 2014, there was a decrease in tree growth with increasing glyphosate rate at Quincy SW – tree growth was also the greatest that year at that location. The trees at Quincy SW were transplanted in 2013 as saplings, and thus were rapidly growing at the time of application.

Relative to the 2013 growing season samples, there appears to be a modest partitioning of both microbial communities among the two soil treatments at Sunrise. Analysis for shifts in microbial communities at Quincy SW from root/rhizosphere samples were comparable to Sunrise - statistically, there were no significant differences in composition of either the bacterial or fungal community between the nontreated and glyphosate treated plots. However, relative to the 2013 growing season samples, there appeared to be a modest partitioning of both microbial communities among the treated and nontreated soils. We do not know the functional consequence of the changes in microbial community composition as a consequence of glyphosate application.

In absorption and translocation greenhouse experiments, absorption was observed following basal applications – glyphosate entered trees in basal applications. In contrast to previous research, observations of absorption following basal application would suggest that glyphosate should not be used as a 'desuckering' treatment, and care should be exercised when applying glyphosate to juvenile trees. Although translocation was a very low percentage of absorbed (less than 2% of the applied material), translocation following basal and foliar treatments was observed, and more importantly, basal applications appear to result in translocation to the roots. Glyphosate was detected in suckers following basal treatments.

Future work is needed to determine if absorption and translocation in field conditions is similar to what we have observed in the greenhouse, and if glyphosate accumulation occurs following repeated applications. If accumulation occurs, then we need to know how much glyphosate can be applied basally before tree injury occurs. Additionally, absorption and translocation of glyphosate may differ by variety, rootstock, or timing of application. Finally, if glyphosate is accumulating in the roots, then it is likely leaking into the adjacent rhizosphere.

Glyphosate is an important labor saving tool for the tree fruit industry, and we encourage both continued research to understand the physiological and microbial consequences of its use as well as grower-focused training on minimizing glyphosate-bark contact in juvenile or injured trees.

FINAL PROJECT REPORT

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

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Cooperator: Tory Schmidt, WTFRC

Other funding sources: None

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
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

Footnotes: Salary, benefits for GS-6 technician

OBJECTIVES

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

SIGNIFICANT FINDINGS

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.

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)

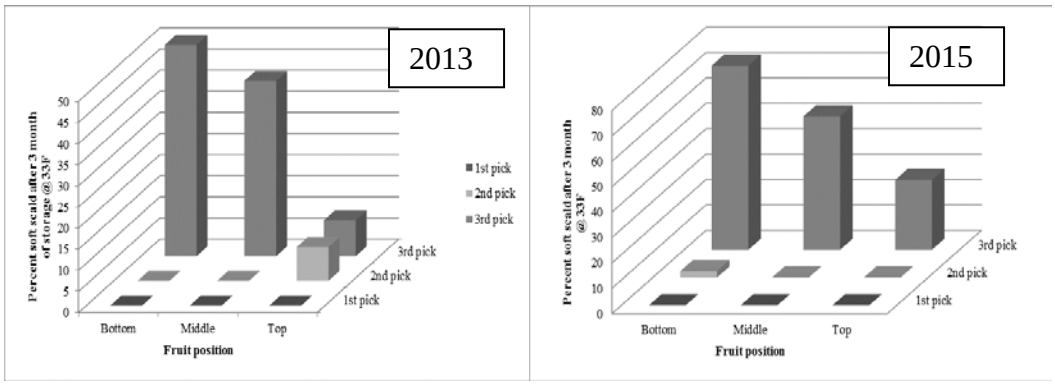

Figure 1: Development of soft scald in Honeycrisp apples stored for 12 weeks at 33°F. Fruit was harvested in 3 picks from three canopy positions from 2013 and 2015.

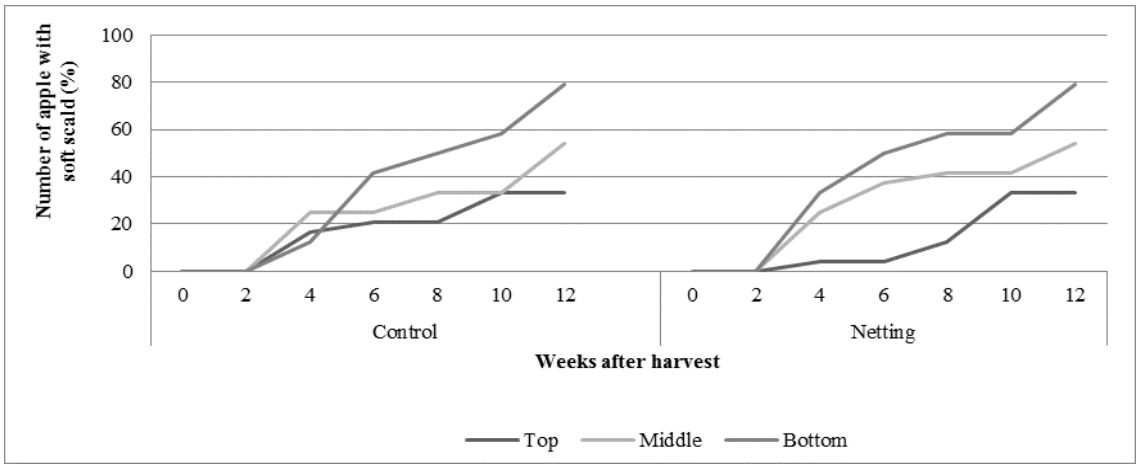

Figure 2: Time course of soft scald development in Honeycrisp apples stored for 12 weeks at 33°F in 2015. (Orchard 2, 3rd pick)

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).

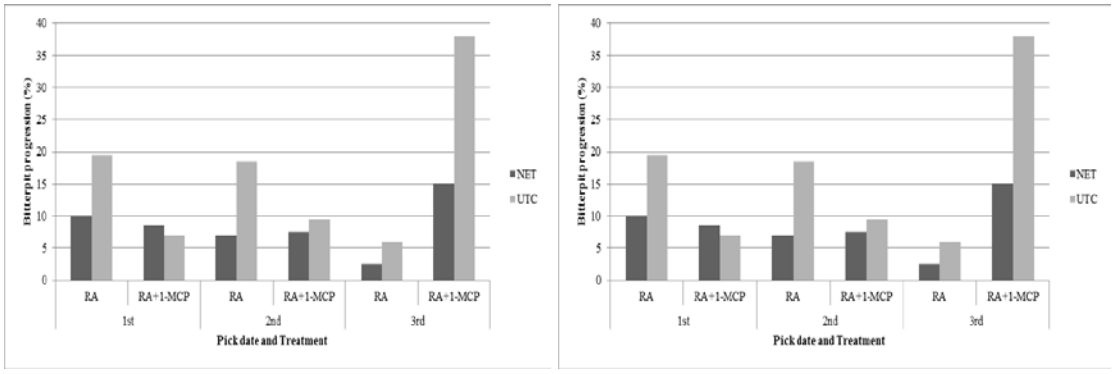

Figure 3: Bitter pit expression of Honeycrisp apples after three months of cold storage; fruit grown in two orchards near Gleed, WA in 2014 with and without shade netting

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 (2^{nd} and 3^{rd} pick) and higher DA meter values (2^{nd} 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.

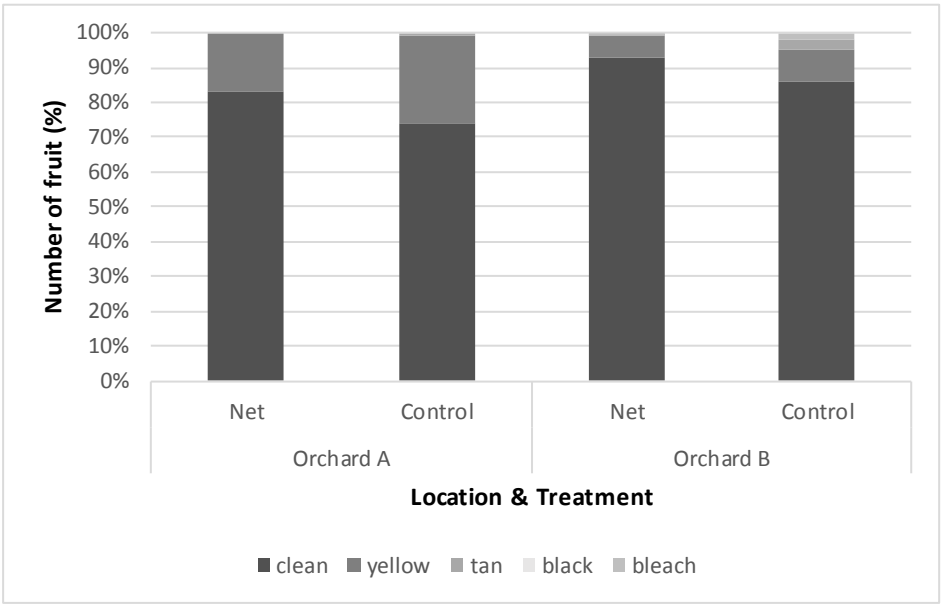

Figure 4: Sunburn incidence and severity in two Honeycrisp orchards at harvest in 2015.

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.

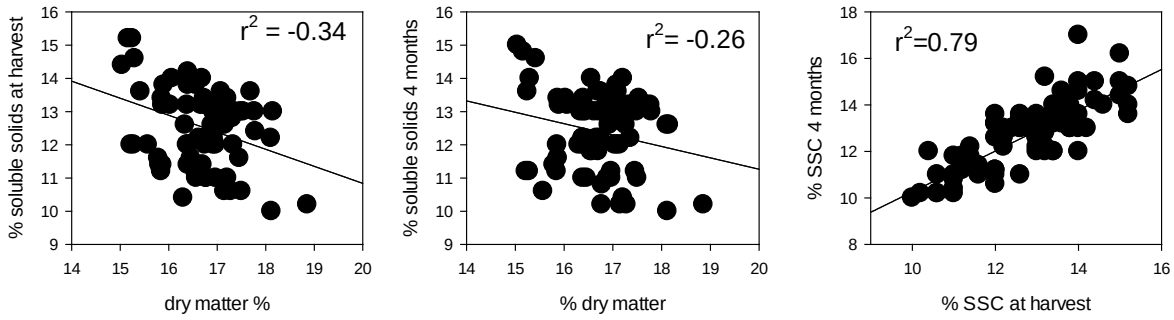

Figure 5. Relationship between fruit dry matter and soluble solids content at harvest and after storage. Fruit were stored 4 months in air then 7 days at 70 °F.

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.

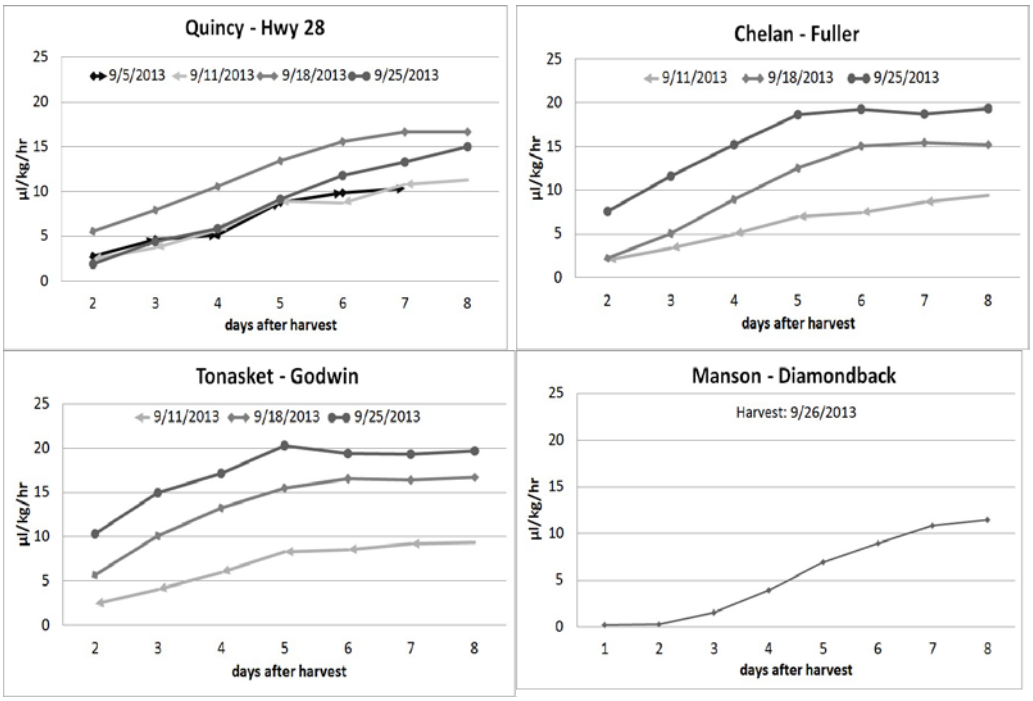

Figure 6. Ethylene production during 8 days at 70 °F following harvest.

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.

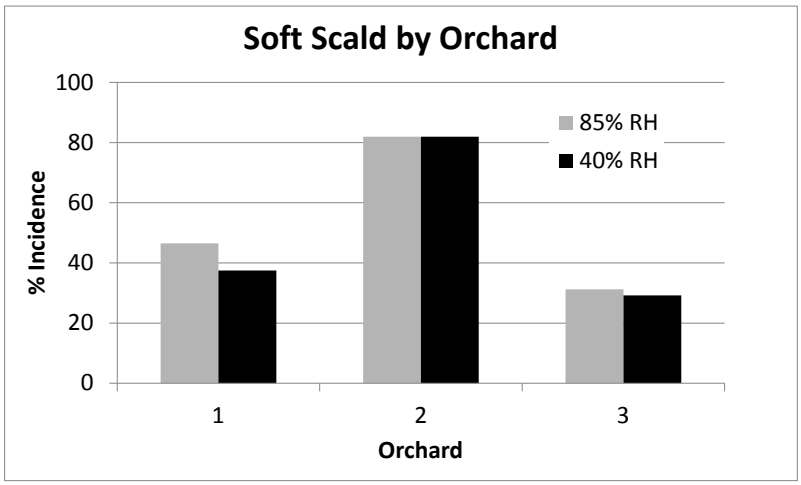

Figure 7. 'Honeycrisp' soft scald following 4 months cold storage in air.

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.

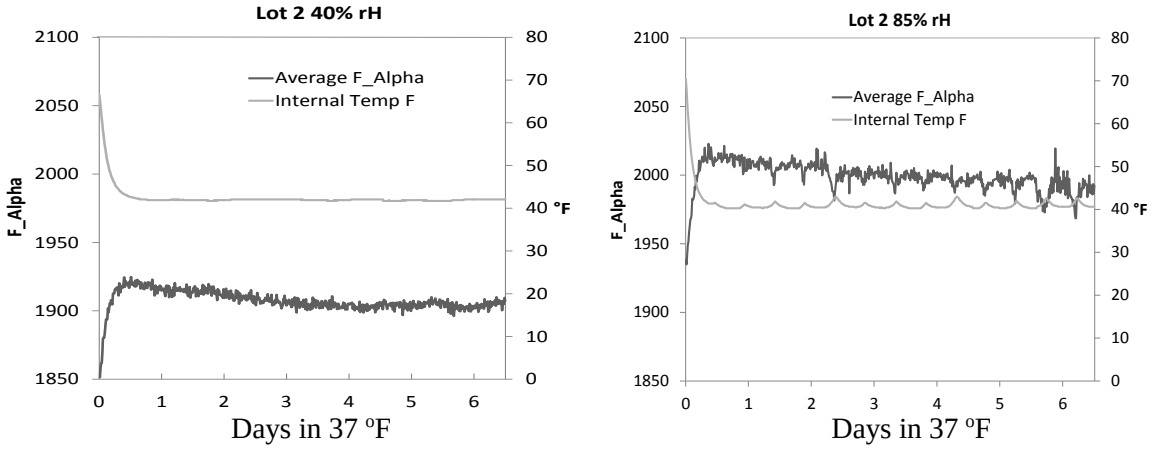

Figure 8. Honeycrisp chlorophyll fluorescence during cooling to 37 °F.

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 9). 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.

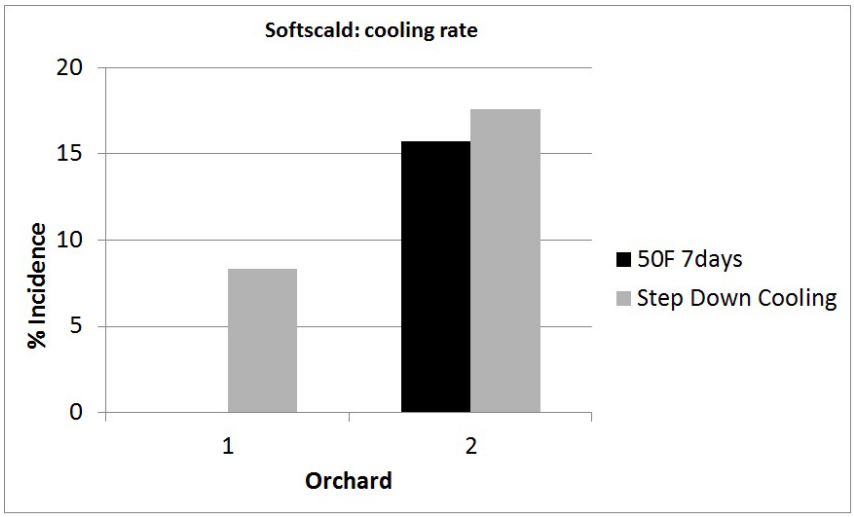

Figure 9. Soft scald incidence after 4 months cold storage in air.

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 ^{o}F.

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.

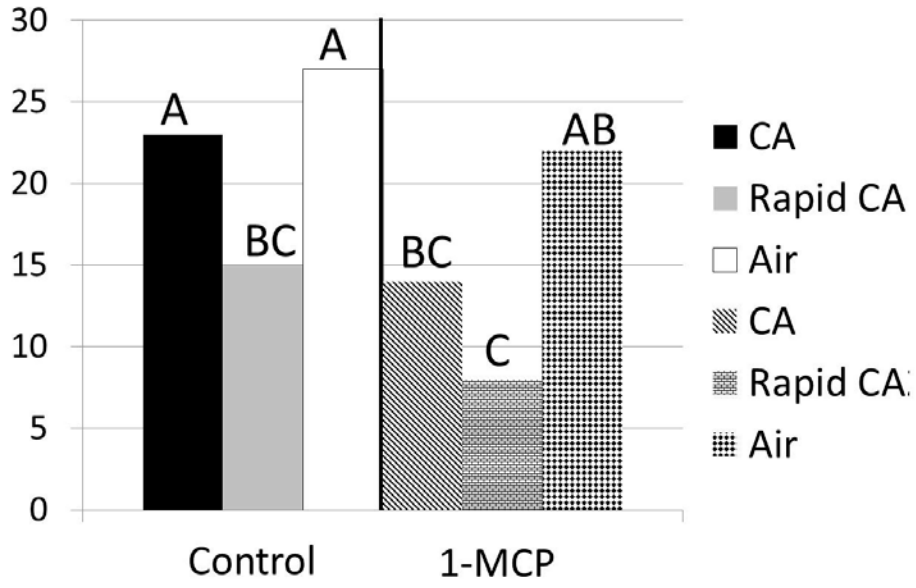

Figure 10. Honeycrisp bitter pit incidence following 4 months storage in air plus 7 days at 70 ^{o}F. Summary of 7orchard years (2 years 2 lots per year, 1 year 3 lots)

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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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. Monitoring scald risk assessment biomarker (SRAB) levels indicates which CA rooms 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. 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 have more impact on scald risk than orchard.
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 additional natural apple wax layer components that are also accurate SRABs.

RESULTS & DISCUSSION

Continuing work: Year 3 (In progress)
Granny Smith apples were harvested 2 weeks before, at, and 2 weeks after commercial maturity from 3 different locations (Basin and Wenatchee area). Scald incidence, SRAB levels, and peel samples are taken after 0, 0.5, 1, 2, 3, 4 and 6 months air storage. Peel samples will be evaluated for differences in peel chemistry associated with scald risk.

For validation of results from the previous season, Delicious apples from 4 orchards (per room) were acquired and sorted at receiving and placed alongside doorway samples in 4 rooms. SRAB levels are monitored monthly. Fruit were removed and placed in an RCA room at 3 months. Apples will be stored for 6 months and, then, moved to 36 °F air for up to 4 months. Scald will be monitored monthly. Peel will be stored for possible SRAB gene expression analysis.

Scald risk assessment for Delicious apples
In year 1, 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.

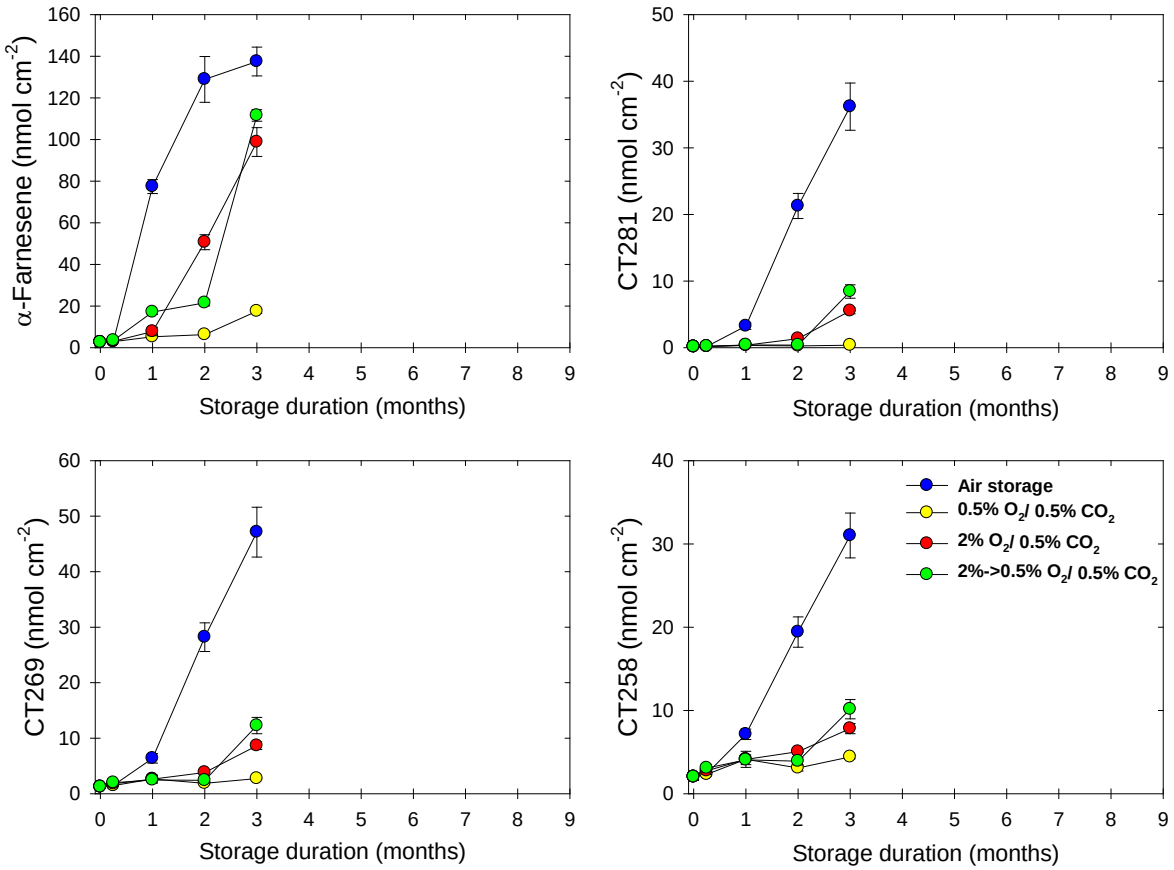
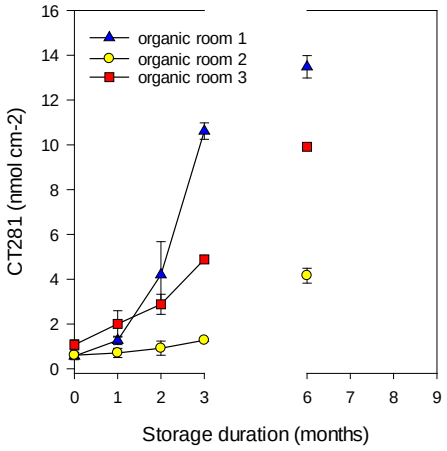
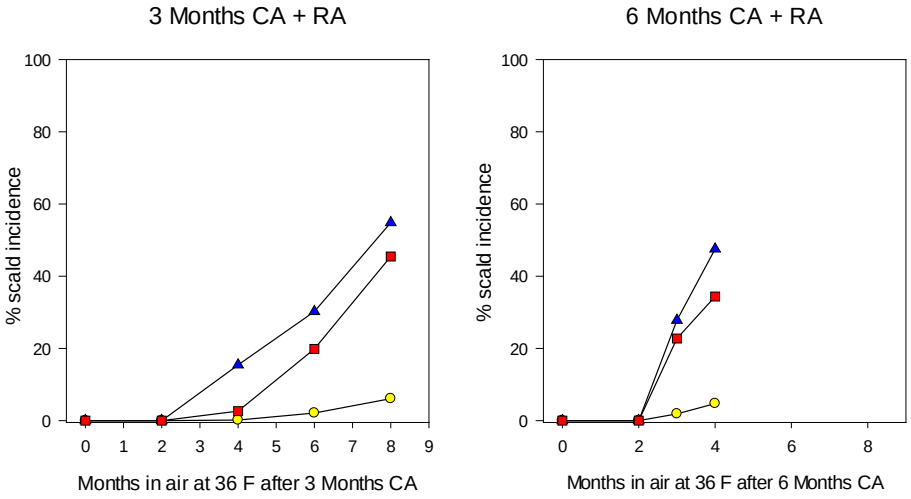


Fig 1. α-Farnesene (the "building block" of SRABs monitored in this study) and SRAB levels over the first 3 months in Delicious stored in test chambers at 33 °F in air or 0.5% or 2% O_2. The oxygen was reduced to from 2% to 0.5% O_2 when SRAB levels were found to be increasing. Scald began to develop 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 was 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 size. 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 °F RCA room (0.6% O_2, 0.5% CO_2), monitoring scald monthly. Fruit stored in the RCA room was removed to 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 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 °F 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.

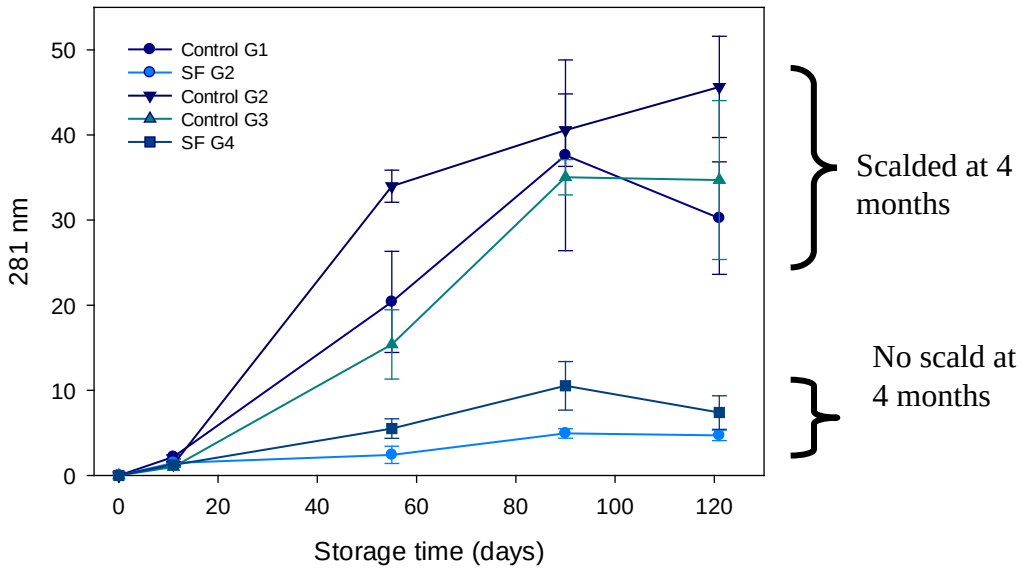


Fig. 3. Granny Smith SRAB levels and final scald incidence after 4 months commercial CA storage in an AgroFresh study using ARS methodology. Apples were chosen from a total of 4 growers treated with SmartFresh (SF) after harvest. Error bars represent standard error (n=3).

To improve upon the year 1 trials, year 2 (2014) trials employ 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 did not increase alongside IEC indicating other factors contribute to SRAB generation (not shown) and, therefore, IEC would not be a good 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.

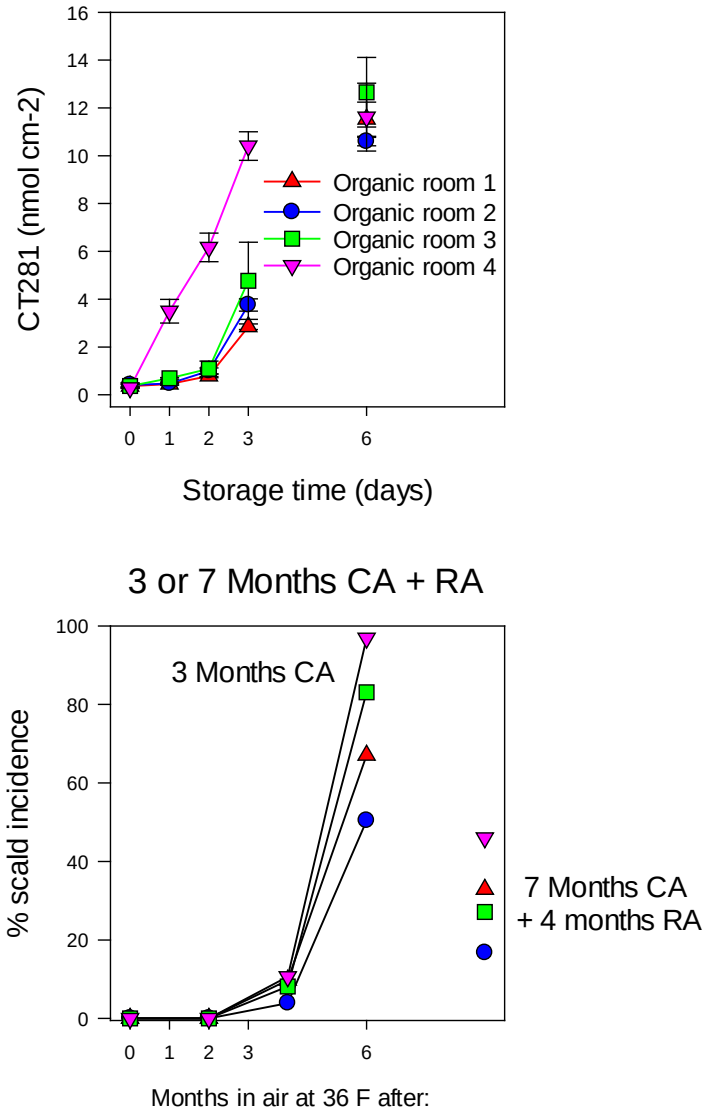


Fig 4. (top) Average SRAB levels for samples from 4 commercial organic Granny Smith storage rooms containing doorway samples from 3 orchards each. Fruit were moved at 3 months from commercial rooms into air at 36 °F or RCA room (0.6% O_2, 0.5% CO_2, 33 °F) and, then, to air at 7 months. Scald levels were evaluated periodically 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 °F air storage. As in year 1, SRAB and IEC were 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.

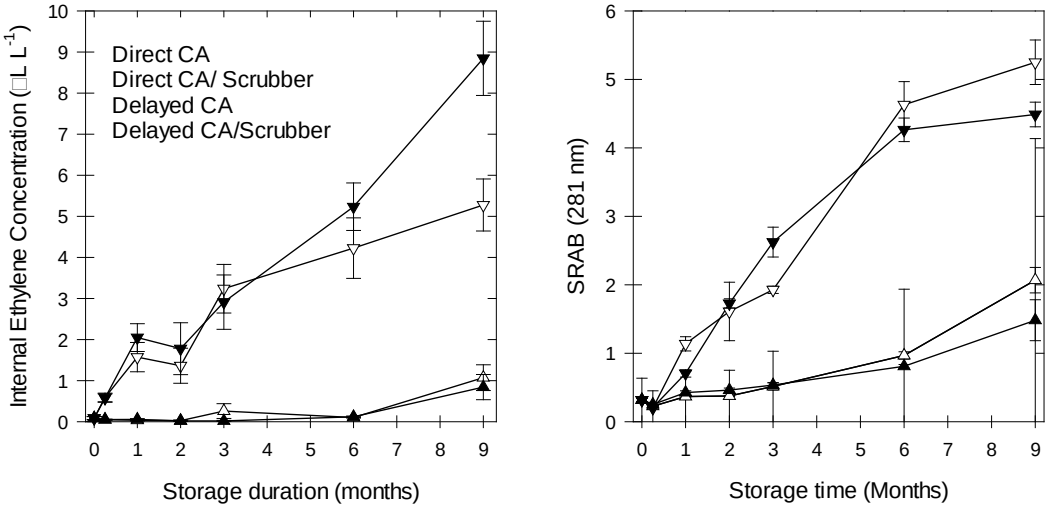


Fig. 5. (Year 1) Fruit internal ethylene concentration (left) and SRAB levels (right) over storage period in research CA rooms (30 bins, 1 orchard) set at 0.5% O_2/0.5% CO_2 immediately or following a 1 week delay. SRAB levels were higher in rooms with delayed CA imposition as were IEC values, although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

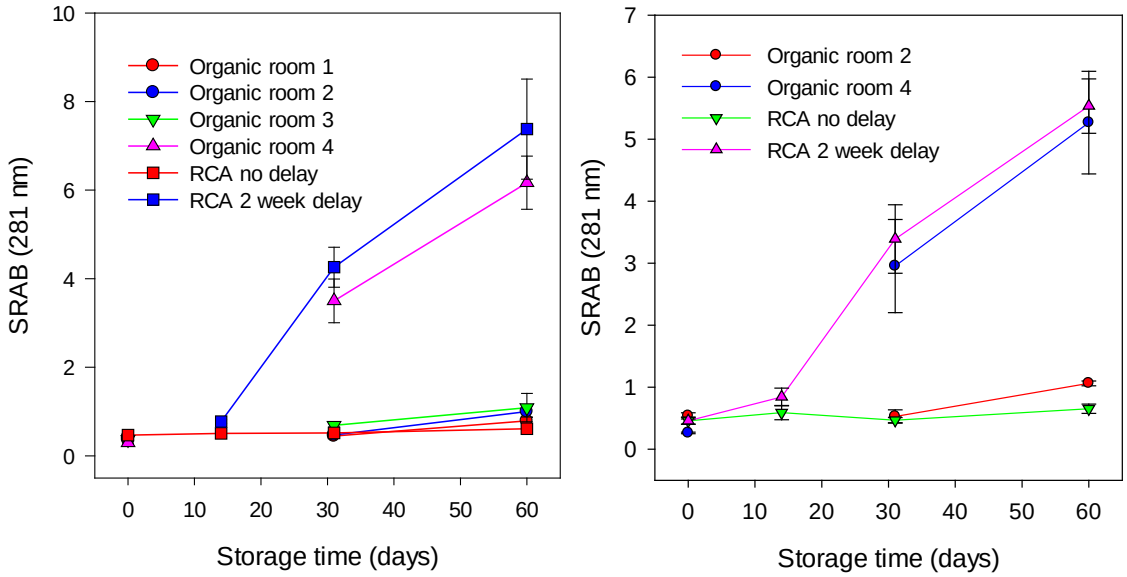


Fig. 6. (left) Average SRAB levels for samples from organic Granny Smith storage rooms and organic research CA room trials (Year 2). Averages represent samples from 3 orchards in each room. The low variability of SRAB levels among orchards in a particular room indicate factors evoked by the room have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

Identification of new SRABs as natural apple wax components

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 metabolic product where farnesene is enzymatically oxidized and then esterified. We have also found 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, 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 and refined a relatively inexpensive and easy means of monitoring a few of these SRABs correlated well with more our more costly and rigorous tests for the current tests. It was still 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. The final year's experiments continuing to validate the procedure on Delicious as well as revisiting the possibility of more natural peel chemical SRABs for at harvest risk assessment are ongoing.

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 scald risk assessment biomarker (SRAB) levels indicates which CA rooms 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. 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 have more impact on scald risk than orchard.
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 additional natural apple wax layer components that are also accurate SRABs.

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: Effect of crabapple pruning on Sphaeropsis and speck rot incidence

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Cooperators: Growers: Jeff Cleveringa (Oneonta/Starr Ranch Growers), Bob Bossen (Northern Fruit Company) , and Teah Smith (Zirkle Fruit Company)

Other funding sources: None

Total Project Funding: $63,191

WTFRC Collaborative Expenses:

Item	2013	2014	2015
Wages[1]	137	137	
Benefits	66	66	
RCA Room Rental[2]	2,100	2,100	
Travel[3]	190	190	
Total	$2,493	$2,493	

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

Budget History:
Organization Name: Pace International

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

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

RECAP ORIGINAL OBJECTIVES

The overall objective of this study was to generate practical information regarding the impact on commercial cultivars of removal of inoculum from infected 'Manchurian' crabapple pollinizers in commercial apple orchards as part of a postharvest decay IPM program. Specific objectives were to:

1. Understand the in-season incidence of fruit infection by *Sphaeropsis pyriputrescens* and *Phacidiopycnis washingtonensis* following winter pruning of adjacent crabapple trees

2. Evaluate the impact of crabapple pruning on the incidence of Sphaeropsis rot and speck rot in commercial cultivars following storage

SIGNIFICANT FINDINGS

- Crabapple twig and mummy samples collected from all orchards with a history of speck rot or Sphaeropsis rot harbored viable pycnidia of the fungi before and at the time of pruning, indicating that they are one of the primary sources of inoculum for fruit infections in apples.
- *P. washingtonensis* infections and pycnidial formations were more prevalent on mummified crabapple fruit than twigs with dieback and canker symptoms, whereas such differences were not observed in the orchard infected by *S. pyriputrescens*.
- Fruit infections monitored during the 2013 growing season were highly correlated to the development of speck rots in storage, whereas the correlation was not apparent in 2014 season.
- Regardless the treatments, no *Sphaeropsis* infection was detected by a conventional isolation method from stem and calyx-end tissues of apple fruit during the growing season while Sphaeropsis rot developed in stored fruit, indicating that in-season monitoring for fruit infection by the isolation method may not predict the incidence of Sphaeropsis rot in storage.
- Regardless of the degree of pruning (chainsaw or detailed), pruning crabapple trees significantly reduced the incidence of speck rot and Sphaeropsis rot in apples during cold storage compared to the unpruned control.
- Crabapple fruit infected by *P. washingtonensis* or *S. pyriputrescens* during the growing season produced pycnidia before harvest, which provide secondary inocula for fruit infections within the growing season.

RESULTS & DISCUSSION

Inoculum availability of the fungi on crabapple trees

In 2013, crabapple twigs with dieback or canker symptoms and fruit mummies that had not been harvested in the previous year were sampled at the time of pruning crabapple trees before bloom. In all three orchards, pycnidia were observed on the twigs and fruit mummies, indicating that these were one of the primary sources of inoculum for fruit infection in the orchard. Pycnidial spores from all samples with pycnidia were microscopically examined and identified as *P. washingtonensis* according to the descriptions of Xiao et al. (2005). The morphological characteristics on potato dextrose agar (PDA) also confirmed that the fungus grown out from the pycnidial spores were *P.*

washingtonensis. All sampled crabapple trees, except one tree from orchard A that was recently replaced with a young tree, harbored viable pycnidia of the fungus at the time of pruning (Table 1). Percent samples with viable pycnidia ranged from 53 to 57% of the sampled twigs and 77 to 83% of the fruit mummies in these orchards. The fungus was isolated from 73 to 87% of the diseased twigs and 90 to 100% of the mummified crabapple fruit. In general, mummified crabapple fruit appeared to have higher percentages of *P. washingtonensis* infections and pycnidial formations than diseased twigs of crabapple trees.

In 2014, the presence of *S. pyriputrescens* was identified by sampling crabapple twigs with dieback or canker symptoms and mummified crabapple fruit in two blocks of 'Red Delicious' orchard with a history of Sphaeropsis rot incidences. Viability of pycnidial spores from all samples was evaluated by culturing the fungus on PDA. The identification of the cultures isolated from diseased tissues was confirmed according to the descriptions of *S. pyriputrescens* (Xiao and Rogers, 2004). All crabapple samples harbored viable pycnidia of the fungus at the time of pruning before and after bloom (Data not shown). This result indicates that infected crabapple trees are the primary source of inoculum for *Sphaeropsis* infections in the orchard.

Table 1. Identification and inoculum availability of *Phacidiopycnis washingtonensis* on crabapple trees at the time of pruning before bloom in three 'Red Delicious' orchards in central Washington

Orchard	Type of sample[a]	% Trees with viable pycnidia[b]	% Samples with viable pycnidia	% Samples with *P. washingtonensis*[c]
A	Twig	90.0	53.4 ± 7.4[d]	86.7 ± 5.4
	Mummy	90.0	83.3 ± 10.3	90.0 ± 10.0
B	Twig	100.0	53.3 ± 5.5	80.0 ± 5.4
	Mummy	100.0	76.7 ± 7.1	100.0 ± 0.0
C	Twig	100.0	56.7 ± 5.1	73.4 ± 6.7
	Mummy	100.0	83.4 ± 5.6	100.0 ± 0.0

[a] Three samples each for twigs with dieback or canker symptoms and mummified fruit of crabapple per tree, ten trees per orchard were randomly collected.
[b] Viability of pycnidial spores was assessed by plating conidia on acidified potato dextrose agar and examined for the growth of *P. washingtonensis*.
[c] Isolation of *P. washingtonensis* was made from diseased crabapple twigs and mummified fruit.
[d] Values are mean and standard error based on the samples from 10 trees.

In-season monitoring of fungal infections on apples

In 2013, detailed pruning that removed all infected twigs, branches, and mummified fruit of crabapple trees significantly reduced the infections of *P. washingtonensis* on apple fruit compared with the unpruned control and chainsaw pruning in all three orchards (Table 2). Although chainsaw pruning of crabapple trees was not as effective as detailed pruning, it significantly reduced fruit infections of apples compared to the unpruned control in two of three orchards. It is apparent that removing infected crabapple tissues by *P. washingtonensis* in the apple orchard can reduce the fruit infections on apple fruit during the fruit growing season. However, pruning alone could not completely prevent fruit infection on apples.

Regardless of pruning treatments, fruit infections in orchard A were generally higher than those in orchard B or C (Table 2). This might be due to the different types of irrigation; orchard A was irrigated by overhead sprinklers, and orchards B and C were irrigated by under-tree sprinklers. However, our study was not designed to test the effect of different irrigation methods.

In 2014, the trend of fruit infection by *P. washingtonensis* in orchard A and C was similar to that of 2013 although percent infections were numerically lower than the 2013 growing season (Tables 2 & 3). All crabapple trees in these orchards that were not selected for the study were chainsaw-pruned by the growers after bloom in 2014. No or very few infections of *P. washingtonensis* were detected on apple fruit adjacent to crabapple trees that were detail-pruned in 2013 without additional pruning in 2014, while a limited infection (1.3%) was detected in the chainsaw-detailed treatment, of which crabapple trees were chainsaw-pruned in 2013 and detail-pruned in 2014 (Table 3). In 2014, an orchard with a history of Sphaeropsis rot was added and detail-pruned before and after bloom. However, the entire block including unpruned controls was accidentally pruned by the grower after bloom. Therefore, an additional block with similar crabapple planting in the orchard was added to compare unpruned vs. commercially pruned (chainsaw) crabapple trees. Even though viable pycnidia of *S. pyriputrescens* were detected in all crabapple trees randomly sampled in this orchard, fruit infections during the growing season could not be detected by an isolation method from stem and calyx tissues of apples (Table 3).

At harvest time, we observed many crabapple fruit that were already infected by either *P. washingtonensis* or *S. pyriputrescens* and formed pycnidia on the fruit surface, which indicate that secondary inocula of the fungi are commonly present in the orchard for fruit infections near harvest (Fig. 2A). In 2014, newly developed cankers from infected crabapple fruit were observed, even on the trees that had all infected twigs and branches removed in the previous year (Fig. 2B). Therefore, crabapple fruit should be removed or harvested to reduce major sources of inoculum on crabapple trees.

Fig. 1. Crabapple fruit infected and formed pycnidia (fruiting bodies of the fungus) by *Phacidiopycnis washingtonensis* at the time of apple harvest in 2014 (A) and a canker originated from infected crabapple fruit on the tree that was detail-pruned in 2013 (B).

Development of decays in storage

In a 2-year study, pruning crabapple trees that involved removing infected twigs and branches significantly and consistently reduced the incidence of speck rot in apples during cold storage compared to the unpruned control (Tables 2 & 3). Only a one-year trial was conducted in orchard B,

and no statistical difference among the treatments was observed, probably due to the non-uniformity of crabapple planting in this orchard (Table 2). Detailed pruning significantly reduced the fruit infections during growing season compared to the chainsaw pruning, but the difference was not significant in the development of speck rots in storage. It is apparent that removing infected tissues of crabapple trees by pruning reduces the incidence of speck rot in stored apples. As we observed in fruit infection monitoring during the season, we could not demonstrate a complete control of speck rot on apples by pruning crabapple trees. Similar to the fruit infection in the orchard, the incidence of speck rot was also higher in orchard A than orchard B or C, probably due to the use of overhead sprinklers in orchard A (Tables 2 & 3). Therefore, overhead irrigation should be avoided.

Table 2. Incidence of fruit infection in the orchard and speck rot in storage caused by *Phacidiopycnis washingtonensis* on apples adjacent to crabapple trees in three commercial 'Red Delicious' orchards in 2013-2014 season

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

[y] Eighty apples per replicate, 4 replications per treatment were harvested and stored for up to 9 months at 4°C.

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

To evaluate the effect of crabapple pruning on the incidence of Sphaeropsis rot in storage, apples were harvested from two blocks of 'Red Delicious' orchard (Orchard D1 and D2) after pruning crabapple trees before and after bloom. In orchard D1, all crabapple trees including the unpruned control were accidentally pruned; therefore no data was obtained for the unpruned control. All three pruned treatments showed low incidences of Sphaeropsis rot, although the incidence of detailed pruning after bloom was lower than those of either detailed pruning before bloom or chainsaw pruning after bloom (Table 3). In an additional block (D2) selected to replace the block D1, chainsaw pruning after bloom significantly reduced the incidence of Sphaeropsis rot in storage. This study clearly demonstrated that regardless of the pruning levels, crabapple pruning in commercial apple orchards resulted in consistently lower development of speck rot and Sphaeropsis rot in storage.

Correlation between fruit infection and speck rot development in storage

To analyze the relationship between the fruit infection monitored during the fruit growing season by isolating *P. washingtonensis* on stems and sepals of apple fruit and speck rot development during cold storage, Pearson correlation analysis was performed. There was significant correlation between the fruit infection by *P. washingtonensis* during the growing season and the decay development in storage

in all three orchards (P <0.05) in 2013-2014 season (Fig. 2), whereas a weak (orchard C) or no correlation (orchard A) was observed in 2014-2015 season.

Table 3. Incidence of fruit infection in the orchard and decays in storage caused by *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* on apple fruit adjacent to crabapple trees in commercial 'Red Delicious' orchards in 2014-2015 season

Orchard	Pathogen	Treatment[x]	Infection in-season (%)	Decay in storage (%)[z]
A	*P. washingtonensis*	Unpruned	6.3 a[y]	31.3 a
		Chainsaw-detailed	1.3 a	3.1 b
		Detailed-unpruned	0.0 a	0.6 b
C	*P. washingtonensis*	Unpruned	4.4 a	15.3 a
		Chainsaw-detailed	1.3 ab	3.0 b
		Detailed-unpruned	0.6 c	0.9 b
D1[w]	*S. pyriputrescens*	Unpruned-Chainsaw	0.0	2.0 a
		Detailed before bloom	0.0	1.7 a
		Detailed after bloom	0.0	0.2 b
D2	*S. pyriputrescens*	Unpruned	0.0	15.5 a
		Chainsaw	0.0	3.9 b

[w] All crabapple trees in orchard D1 were accidentally pruned by the grower after pruning treatments.
[x] Crabapple trees that were not selected for the study in all orchards, but D2 were chainsaw-pruned by the growers after bloom. Trees in chainsaw-detailed treatment were chainsaw-pruned in 2013 followed by detailed pruning in 2014. Trees in detailed-unpruned treatment were detailed pruned in 2013 and unpruned in 2014.
[y] Values within an orchard, when followed by a common letter, are not significantly different according to the analysis of variance and least significant difference (P = 0.05).
[z] One hundred sixty apples per replicate, 4 replications per treatment were harvested and stored for up to 9 months at 4°C.

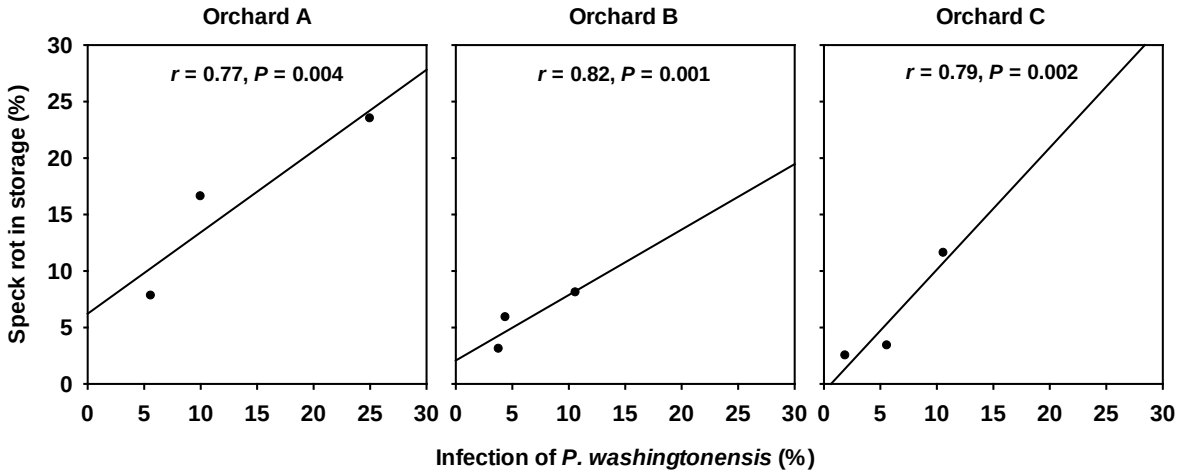

Fig. 2. Correlation between the infection of *Phacidiopycnis washingtonensis* on stems and sepals of apple fruit adjacent to crabapple trees during fruit growing season and speck rot development during cold storage harvested from three commercial 'Red Delicious' orchards in 2013-14 season.

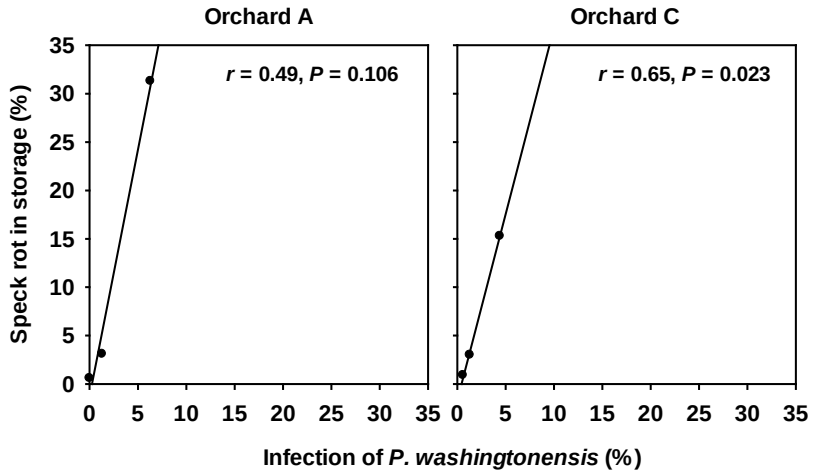

Fig. 3. Correlation between the infection of *Phacidiopycnis washingtonensis* on stems and sepals of apple fruit adjacent to crabapple trees during fruit growing season and speck rot development during cold storage harvested from two commercial 'Red Delicious' orchards in 2014-15 season.

REFERENCES CITED

Xiao, C. L., and Rogers, J. D. 2004. A postharvest fruit rot in d'Anjou pears caused by *Sphaeropsis pyriputrescens*. Plant Dis. 88:114-118.

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

EXECUTIVE SUMMARY

This study was designed to generate practical information regarding the impact of 'Manchurian' crabapple pruning in commercial apple orchards to reduce or control speck rot and Sphaeropsis rot of apple in storage. To demonstrate the importance of crabapple pruning, two specific objectives were addressed: 1) monitoring the incidence of fruit infections by *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* on apples adjacent to crabapple trees after various pruning treatments before or after bloom and 2) evaluating the development of speck rot and Sphaeropsis rot on apples in cold storage for up to 9 months.

In all orchards selected for this study, crabapple twigs and fruit mummies harbored viable pycnidia of the fungi before and at the time of pruning, indicating that they are the primary sources of inoculum for fruit infections in apples. In particular, fruit mummies left hanging on the trees formed more fruiting bodies (pycnidia) of the fungi containing millions of spores than twig diebacks and cankers. Moreover, infected crabapple fruit during growing season also produced pycnidia on the tree that serve as inoculum throughout the remainder of the season and caused twig cankers in the following season, further supporting the impotence that infected crabapples should be removed or harvested before the formation of pycnidia on the fruit.

The correlation between fruit infection monitored during fruit growing season and the development of speck rot in storage was relatively strong in one year, but not apparent in the following season. Therefore, monitoring fruit infections by isolating the fungus from stem and calyx tissues could not predict the incidence of speck rot on apples in storage. Similarly no *Sphaeropsis* infection was detected by a conventional isolation method from stem and calyx-end tissues of apple fruit during the growing season while Sphaeropsis rot developed in stored fruit.

In this study, pruning crabapple trees significantly reduced the incidence of speck rot and Sphaeropsis rot in apples during cold storage regardless of the degree of pruning (chainsaw or detailed). Although we could not demonstrate the difference between pruning before bloom and after bloom, it is clear that removal of diebacks and cankers of twigs and branches in the beginning of the season would help reduce the inoculum levels of the fungi in the orchard. Therefore, we clearly demonstrated that crabapple pruning should be implemented as part of a postharvest decay IPM program. As shown in this study, since pruning treatment alone could not completely prevent the development of speck rot and Sphaeropsis rot, additional treatments such as pre-harvest sprays with appropriate fungicides or/and postharvest fungicides are highly recommended.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP15-102A

Project Title: Apple scion breeding

PI: Kate Evans
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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: $3.6M (2015-2016 with 3 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 Konishi@ 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 proposed project plans to continue the existing WSU apple breeding program with the specific focus of producing new improved apple varieties for the Washington industry. This proposal addresses the highest priority of the WTFRC apple horticulture and post-harvest committee of 'Fruit quality pre and post-harvest'.

SIGNIFICANT FINDINGS

1. Twenty-two new crosses were made in 2015 with approximately 26,500 seeds produced in the WSU Apple Breeding Program (WABP).
2. Seedlings from approximately 18,000 seeds from 2014 crosses were grown in the greenhouse.
3. Approximately 7,000 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 along with another 1,100 seedlings that survived fire blight screening.
4. Seedlings at Willow Drive were propagated on M.9 rootstocks for future orchard evaluation. More than 4,500 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2016.
5. The final count of new Phase 1 trees planted in 2015 was approximately 3,880.
6. Promising selections already in Phase 2 trials (planted in 2007-2014) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
7. Three new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2015.
8. Nineteen promising selections made in 2014 were propagated in 2015 for planting in 2017 Phase 2 trials at three diverse sites in Central Washington.
9. One new promising Phase 2 selections was propagated onto Geneva 41 rootstock for advancement to Phase 3 initially at the Quincy site only.
10. A series of three WA 38 storage reporting & tasting sessions was initiated (March, May and July) were held in September.
11. 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.
12. CVT buds of WSU 46 were distributed to WA nurseries.

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.

a. Marker-assisted parent selection will be used to determine the most suitable combinations of parents for crossing to achieve our aim of a portfolio of new improved apple varieties. Using data from the SCRI-funded RosBREED project and the Peace lab, facilitated by the new Breeders Toolbox for breeding database interfacing developed by the Main lab, we will choose the optimum cross combinations from among available germplasm. As new parental germplasm is identified, samples will be genotyped with the full range of DNA tools

available. New parents with either resistance to mildew or resistance to fire blight will be incorporated into the crossing program from the previous WTFRC-funded projects lead by PI's Dhingra and Norelli.

b. Crosses will be made each spring, most likely aiming at annual production of around 20,000 seeds. Following vernalization, seedlings will be germinated and grown in the greenhouse at the TFREC. To optimize efficiency and accuracy of sample collection, leaf samples will be collected in the greenhouse from some of these seedling progenies and sent to the Peace lab for DNA testing. Genetic tests used will depend on the particular cross combination. Some progenies will be inoculated with fire blight to enable phenotypic selection for resistance. Only the un-culled seedlings will be planted in the nursery and then budded onto M.9 rootstock for further evaluation. If deemed appropriate, seedlings will be maintained in pots until sufficient propagating wood is available for propagation for Phase 2 (or Phase 1.5).
c. Budded trees will be planted at the TFREC Columbia View orchard for Phase 1 trials where their resulting fruit will be evaluated. Selection in the orchard will be initially based on fruit appearance (primarily color, uniformity, freedom from defects) followed by eating quality (primarily firmness, crispness, sugar/acid balance).
d. Promising selections will be propagated onto either M.9 or G.41 rootstock and placed in replicated Phase 2 trials (five trees/selection) at up to three diverse sites in central Washington. Data will be collected on fruit quality, productivity and tree health. DNA samples will be collected from all Phase 2 selections for screening with predictive markers to provide DNA-based information on genetic potential to enhance subsequent selection decisions.
e. Outstanding selections will be propagated as 'elites' for Phase 3 trialing with an aim of approximately 75 trees in up to three diverse grower sites in central Washington. Phase 3 is conducted in cooperation with the WTFRC, with trial sites managed by Tom Auvil. When appropriate, propagated selections may be planted in just one site (Phase 2.5 or 'staggered start') before propagation for full Phase 3. Harvested fruit will be subjected to a range of storage treatments managed by Ines Hanrahan. Budwood from all Phase 3 selections will be sent to the Clean Plant Center to establish certified, virus tested material ready for distribution to nurseries.
f. Outstanding selections will be proposed for commercialization, patent data will be collected and submitted and the nursery mother trees will be confirmed as true-to-type by genetic fingerprinting.

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

a. Using the labeling system initiated in the Phase 1 orchard, fruit samples collected will be bar-code labeled to correspond to the source tree, the pick number and the harvest date. These labels will then remain with the fruit as it is evaluated in the fruit laboratory thus minimizing mixing of samples and data-entry errors. Harvest date is determined using the starch-iodine test and the Cornell starch chart however identification of the optimum harvest maturity continues to be challenging with the seedling fruit.
b. Ten fruit samples will be divided into five fruit for instrumental evaluation and five for sensory evaluation. The fruit for instrumental evaluation will be tested for maturity using the Cornell starch chart. Texture, size and weight will be recorded with the Mohr® DigiTest and the remaining fruit will be juiced for soluble solids concentration and titratable acidity measurements.

c. Sensory analysis will usually be performed by a team of four, producing a detailed breakdown of appearance and eating quality attributes. The breeding team was trained in sensory profiling by the Ross lab in Pullman in 2010.
d. First-season seedling fruit will be stored in regular atmosphere storage at the TFREC at 34ºF for two months followed by one week at room temperature prior to evaluation. If a sample achieves the appropriate overall rating, the same seedling tree will be harvested at more than one pick date the following year (subject to fruit availability). Second- and third- season samples will be evaluated at harvest as well as after two months (plus one week) storage. If sufficient fruit is available, a four month stored sample with one week at room temperature will also be evaluated.
e. Fruit evaluation will continue as selections move forward through Phases 2 and 3, with samples taken at up to four pick dates and evaluated at harvest and, after two and four months of regular storage with one week at room temperature. Data from Phase 2 fruit evaluation will be analyzed using the R-based statistical software 'Elite Advance' developed by Craig Hardner which will rank the selections trait by trait based on genetic potential. This data together with accumulated knowledge of the specific character of each advanced selection guides the decision of which to move into Phase 3. Larger volumes of fruit from Phase 3 will be drenched with a post-harvest fungicide, tested with and without a 1-MCP treatment and stored in regular and controlled-atmosphere storage using the Stemilt facility. Fruit out of storage will be tested in the WTFRC lab as well as the TFREC lab.
f. Fruit from promising selections from Phase 3 will be sent to the Ross lab in Pullman for consumer evaluation as required.

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.

Breeding program priority traits were discussed with the Breeding Program Advisory Committee (BPAC) in November. The list of traits was updated with the addition of some possible new traits for consideration and can be seen in Table 1. If time allows during the WTFRC apple horticulture and post harvest review, a clicker survey to re-prioritize traits will be completed.

DNA test information was used was used to help design crosses for the 2015 season and approximately 7,000 seedlings from 2014 crosses were screened with DNA markers for fruit quality. Markers used were as follows: Ma-indel (acidity, crispness, bitter pit resistance), PG (firmness) and ACS (firmness/storage). Almost 60% of these seedlings were culled in the greenhouse as they were predicted to have less than favorable fruit quality.

Approximately 7,000 seedlings were inoculated with fire blight in the greenhouse to select for resistance. The resistant individuals were planted out in the field and will be re-inoculated in 2016 to confirm their resistance.

Pollen was collected from a mildew resistant *M. zumi* derivative ('Honeycrisp' × *M. zumi*) for crossing in 2016. Seedlings of 'WA 2' × 'White Angel' from the 2014 crossing season were germinated and provided to the Peace lab. 'White Angel' has a different gene for resistance to mildew than *M. zumi*. Fei Xiong Luo (a graduate student in the Peace lab) is developing a DNA test for this gene based on the genetic map data previously published by Evans and validated by the seedling population provided.

Seedling trees at Sunrise orchard recovered well from the previous season of water stress; final evaluation of seedlings harvested in 2015 is still on-going, however 186 were selected, harvested and evaluated in the 2014 cropping season.

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

Fruit from the 2015 season harvest is still being evaluated, however 320 seedling trees were harvested and evaluated during the 2014 season. In addition, fruit from 102 'keeper' selections was evaluated (300 samples in total), plus 63 Phase 2 selections and controls (1,148 samples) and two Phase 3 selections (30 samples).

All samples were routinely bar-coded at harvest and then tracked through post-harvest evaluation. 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. Nineteen promising seedling selections were propagated in fall 2015 for inclusion in Phase 2 plantings in 2017. One advanced selection from Phase 2 was propagated in fall 2015 for inclusion in Phase 3 plantings.

<u>WABP Publicity</u>
Fruit of WA 38 were delivered to numerous industry contacts and events throughout the year. Storage data and fruit of WA 38 were presented on March 6th and May 1st in Wenatchee and July 6th in Yakima. A field day at the Quincy Phase 3 site was open to the public on September 24th. WA 38 was featured as part of the 'Big Ideas' celebration event on Pullman campus (9.18.15) and in a NY Times article 'Beyond the Honeycrisp' (11.4.15).

<u>Talks and posters:</u>
April 2015 – Dr. Evans presented "The WSU apple breeding program" as part of the WSU IAREC Seminar series, Prosser, WA.
July 2015 – Julia Harshman (graduate student) presented the WABP and hosted a WA 38 tasting at the National Association of Plant Breeders annual meeting in Prosser and Pullman, WA.
August 2015 - Julia Harshman presented the results of her WABP efficiency studies (in collaboration with Craig Hardner) at the American Society for Horticultural Science meeting, New Orleans.
September 2015 – A SeedWorld interview with Dr. Evans "The challenges of apple breeding" was featured on line. (http://seedworld.com/kate-evans-associate-scientistassociate-professor-wsu-napb-annual-meeting-2015-giant-views/)
October 2015 - Jamie Coggins (graduate student) presented WABP products and testing protocols in a middle school STEM event in Yakima.

November 2015 – Dr Evans presented a talk 'Breeding pome fruit in Washington State' at the 'Advances in field-based high-throughput phenotyping and data management' meeting in Spokane, WA.
December 2015 – Fruit of advanced selections was available for tasting at the Washington State Horticultural Association meeting in Yakima, WA. Dr Evans also presented a talk 'Developing and implementing new technologies for and from the WSU pome fruit breeding program'.

Table 1: Breeding program priority traits (prioritized December 2014, Breeding Program Advisory Committee)

1	Firmness retention: stays firm vs. softens in storage
2	Crisp vs. not crisp
3	Consistency of flavor: retained after storage vs. not retained
4	Yield: At least commercial standard yield vs. low yield
5	Juicy vs. not juicy
6	Acidity: some (TA 0.4+) vs. bland (TA <0.4)
7	Russet: Low vs. high incidence
8	Susceptibility to bitter pit: Resistant vs. susceptible
9	Susceptibility to sunburn: Resistant vs. susceptible
10	Precocity: precocious/normal vs. slow-bearing
11	Sweetness: sweet (SSC 15%+) vs. not sweet (<15%)
12	Fruit size: normal (88+ box size) vs. small
13	Susceptibility to scald: Resistant vs. susceptible
14	Watercore: No or low susceptibility vs. high susceptibility
15	Skin thickness: not tough vs tough
16	Proportion of red skin cover: >20% vs. <20%
17	Powdery mildew resistance: No or low susceptibility vs. high susceptibility
19	Fire blight resistance: No or low susceptibility vs. high susceptibility
20	Yellow vs. green ground color
	Possible additional traits:
	Harvest timing: early – mid - late
	Stem length/ease of mechanical harvest
	Bearing habit
	Post-harvest disease resistance
	Self-thinning
	Non-browning flesh

CONTINUING PROJECT REPORT **YEAR**: 2015
WTFRC Project Number:

Project Title: Apple rootstock and scion evaluation

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WTFRC staff cooperators: Ines Hanrahan, Mano Mendoza, Tory Schmidt, Jim McFerson, Kyle Tynan

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

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

Total Project Request: **Year 1:** 83,965 **Year 2:** 57,900 **Year 3:** 112,165

WTFRC Expenses:

Item	(2015)	**(2016)**	(2017[3])
Salaries[2,3]	40,500	30,500	40,500
Benefits[2,3]	13,365	10,000	13,365
Crew Wages[3]	11,000	5,000	30,000
Crew Benefits[3]	2,200	1,000	9,900
Stemilt RCA room	8,400	8,400	8,400
Shipping			
Supplies			
Travel[1]	8,500	3,000	10,000
Miscellaneous			
Total	83,965	57,900	112,165

[1]Fuel and maintenance
[2]Salaries and benefits for Auvil, Hanrahan, Schmidt, and Mendoza apportioned to this project.
[3]75% of the effort is in rootstock trial work. 2016 will have less activity. 2017 will see large increase in fruit harvest, storage and lab activity.

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

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.
2. Evaluate new apple scion genotypes entering Phase 3 evaluation.

Scion evaluation activities and findings:

- 2 new Phase 3 (P3) genotypes (WSU 64, 65) with 100 trees or more were planted at Quincy and three genotypes grafted onto discontinued P3 genotypes at Prosser P3 site. A bin of fruit was picked in 2015 from 2014 grafts of WSU 68 in Quincy. No bitter pit or lenticel issues were apparent from these lightly cropped, vigorous trees.
- BPAC advised no additional storage samples be evaluated on WA 2 or WA 38, however Prosser displayed a potential for delayed sunburn, therefore an evaluation for delayed sunburn from the 2015 crop is underway.
- Held field days in mid-September in Quincy and Prosser. WA 38 sampling sessions were available in Yakima and Wenatchee to taste/examine fruit from three sequential harvests, see table 1.
- WA 38 grafts in Prosser and Quincy produced fruit on 3rd and 4th leaf trees with no bitter pit.
- WSU 46 does not maintain texture or acidity (flavor) through the storage season. Contemplating discontinuing evaluation activities.

Rootstock findings and activities:

- G.11, G.41, G.214, G.935, G.969, G.210, G.30 and G.890 were planted in 5 trials with standards of B.9, M.9 337 and M.9 Nic 29. M.106 is a standard rootstock in 2 trials. CG 5257 and CG 4011 are in trials in Wapato.
- Field days were held in October at all 2015 plantings plus a 2006 trial that was grafted in 2011.
- 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 much less union breakage. Some scions on G.41 such as Honeycrisp, Scilate and Scifresh seem to have more difficulty with the unions of large caliper tress > 5/8".
- 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.

Figure 1. Trunk cross sectional area Brewster Pazzaz

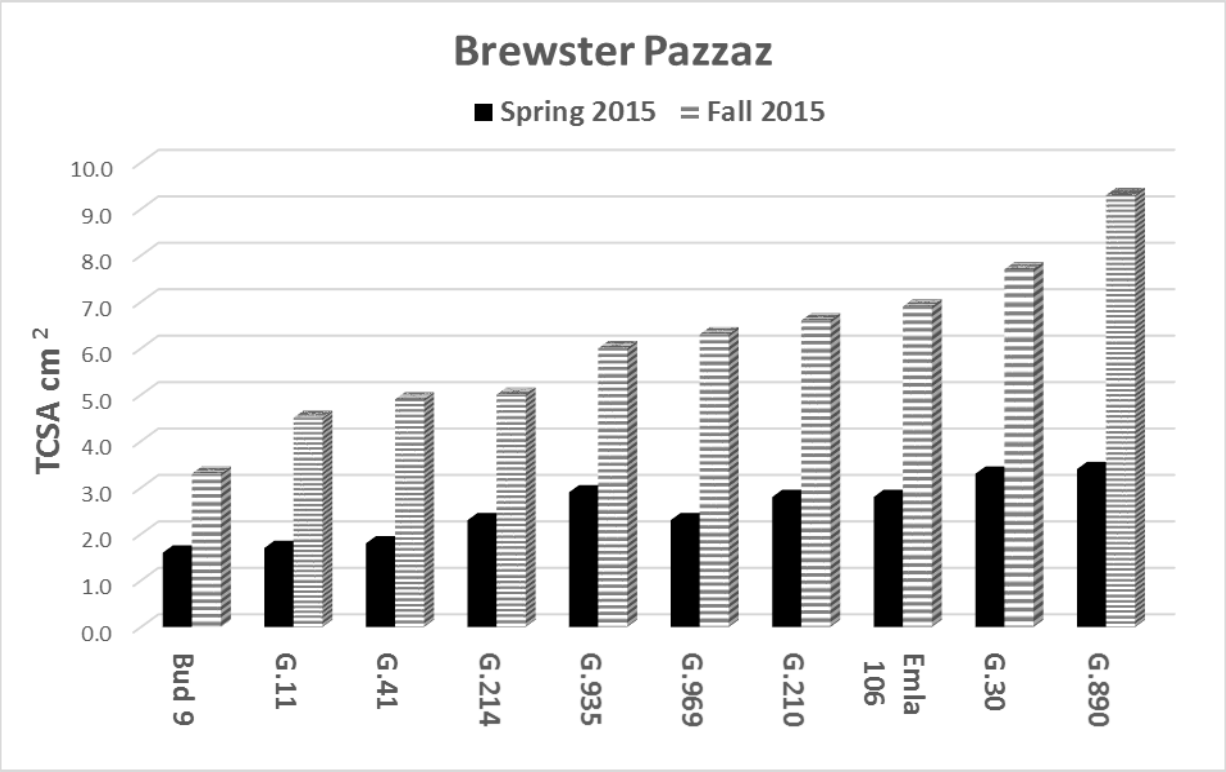


Figure 2: Oroville Honeycrisp trunk cross sectional area

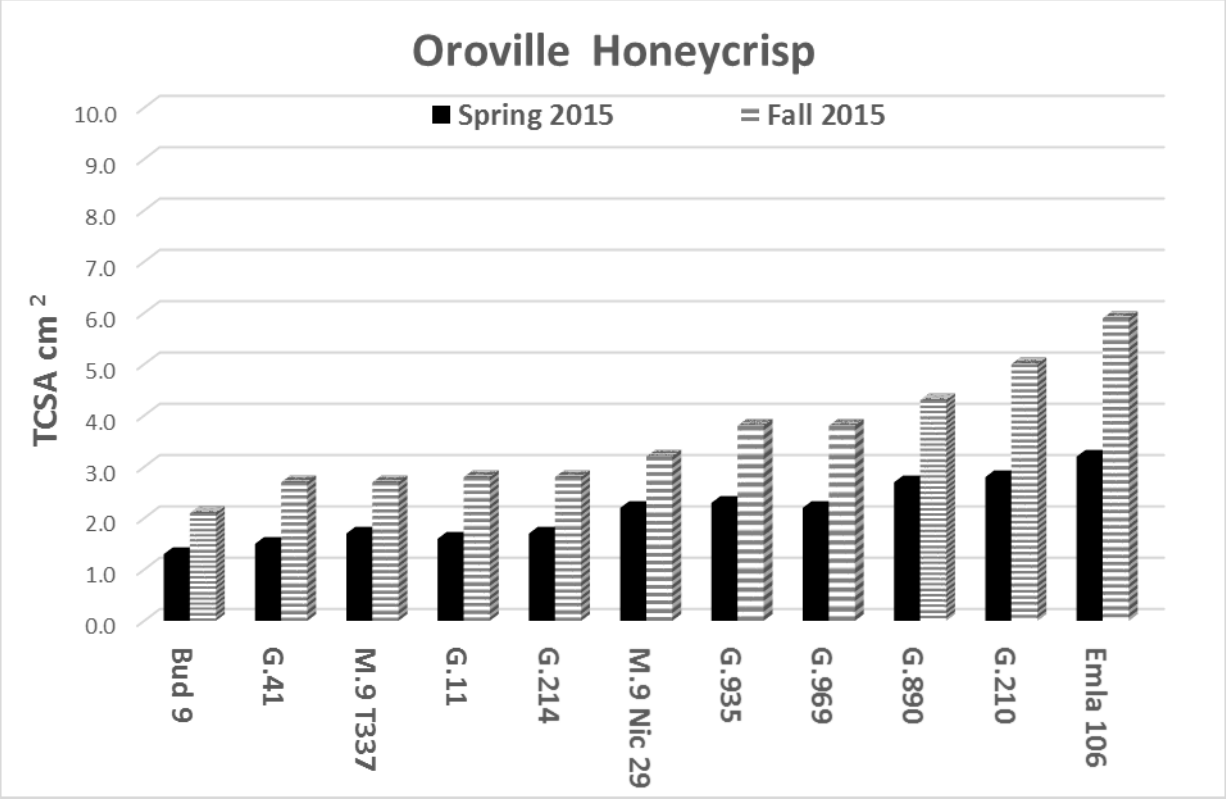


Figure 3: East Wenatchee Honeycrisp trunk cross sectional area

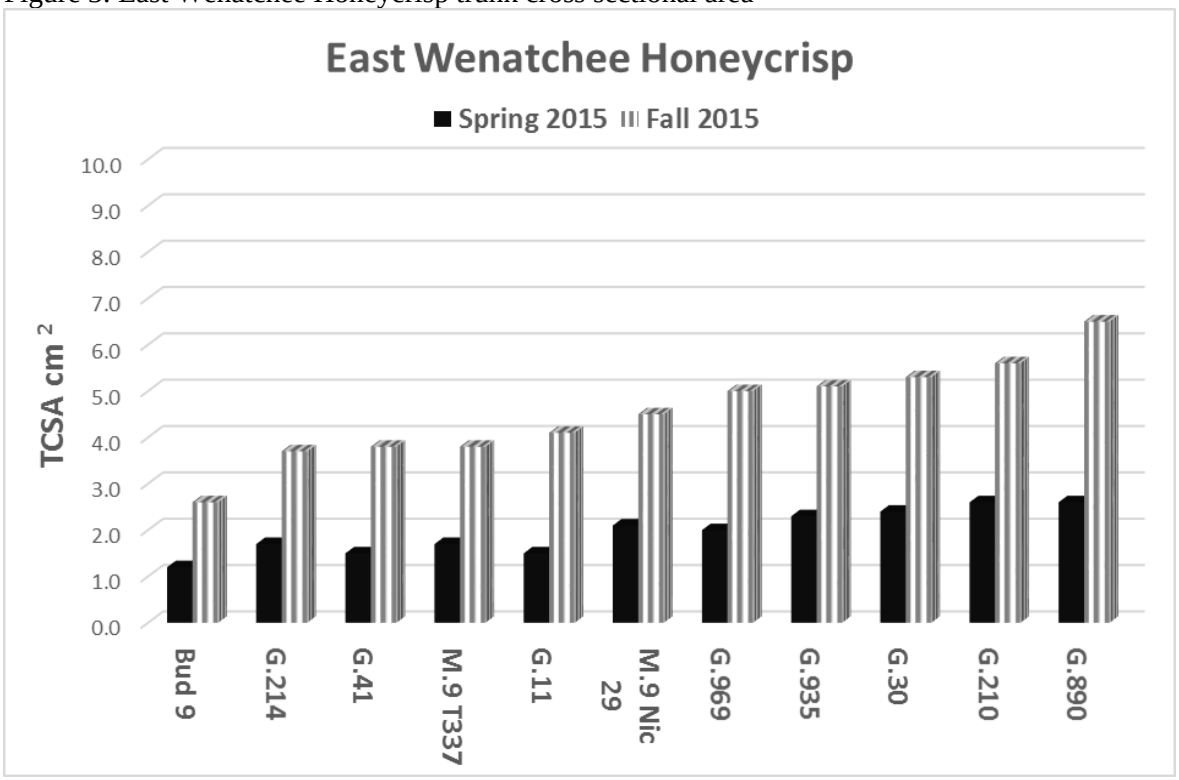


Figure 4: Wapato Red Delicious replacement tree trial trunk cross sectional area

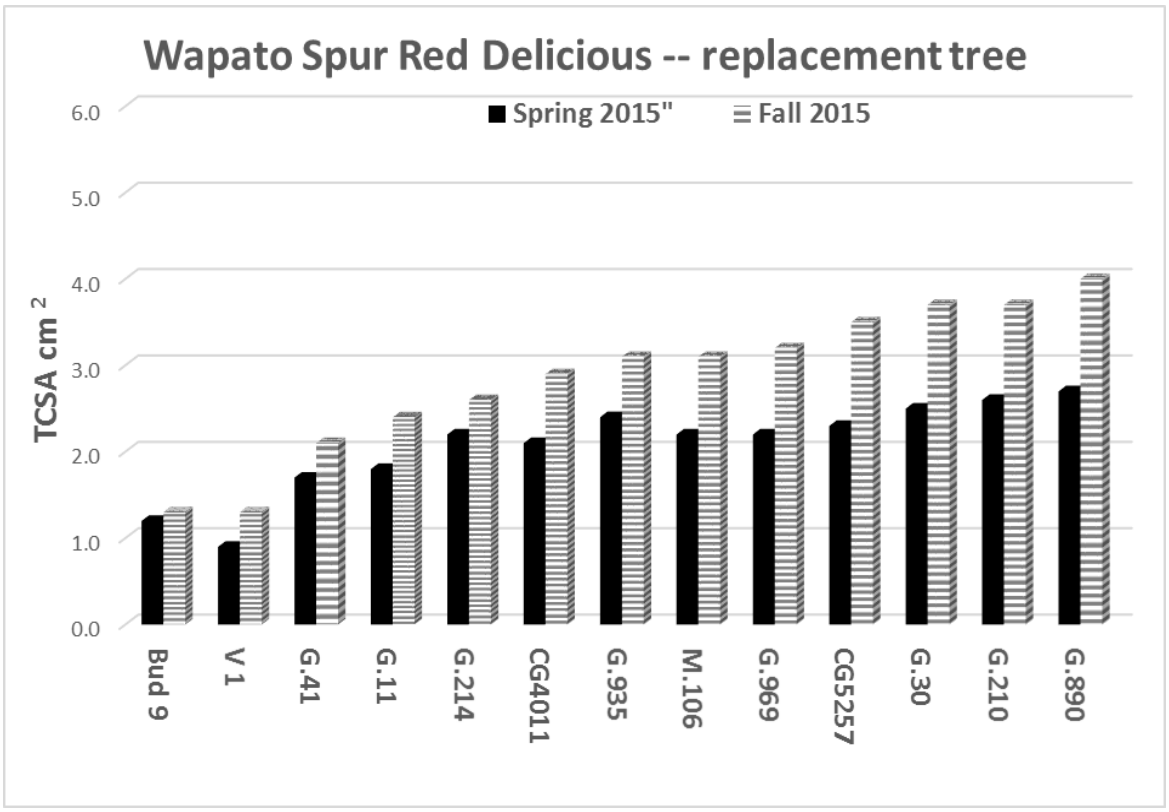
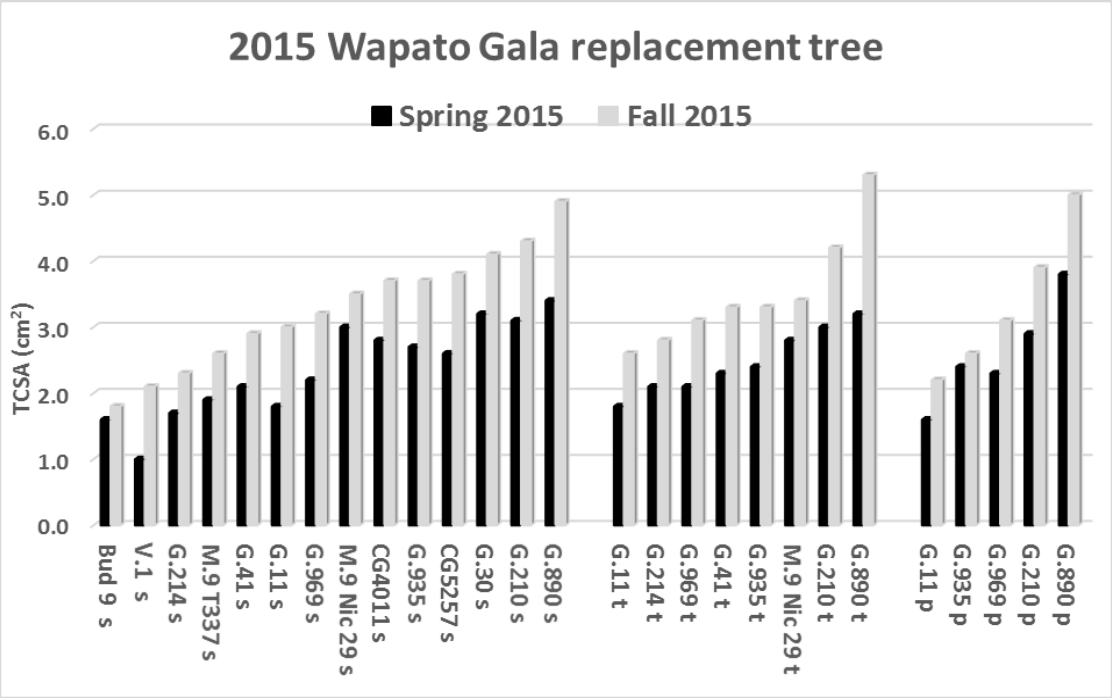


Figure 5: Trunk cross sectional area of Wapato Gala replacement tree trial.



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

- 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.
- 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 this trial is extremely high in the established M.9 trees. About 10% of the trees planted in 2010 have dead rootstocks. Another 30% or more have strikes.
- 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.

Table 1: WA 38 harvest timing and storage trial from 2014 harvest in Quincy

		Harvest				**8 month CA**			**8 month CA+MCP**		
Pick	date	SSC	TA	Starch[1]	Firm.	SSC	TA	Firm.	SSC	TA	Firm.
Old trees	10.2.14	12.0	0.500	4.3	20.6	12.6	0.408	18.4	12.1	0.374	17.4
	10.8.14	12.1	0.510	3.7	20.6	12.8	0.337	16.7	13.0	0.366	17.2
	10.14.14	12.2	0.440	5.9	15.4	12.8	0.373	16.9	12.2	0.350	16.6
Graft	10.2.14	11.9	0.640	2.9	22.4	13.2	0.384	18.3	13.8	0.457	19.7
	10.8.14	12.5	0.600	2.3	21.5	14.0	0.403	18.6	14.0	0.453	19.1
	10.14.14	13.5	0.630	2.9	18.5	13.7	0.420	18.8	14.4	0.460	18.9

[1]Starch values are from the Cornell 1 to 8 chart. A Cornel '3' has the starch cleared to the core ring, which is the same as the Washington 1 to 6 chart with a rating of '2'.

- After 8 months of storage, and a week of warm room, the October 14 harvested fruit from the 'older trees' lacked 'apple flavor'. MCP may provide some flavor benfits on fruit with very advanced maturity.
- There are two differences in the fruit from 'grafts' versus the 'old trees':
    - The old trees have a much higher crop load, nearing 100 bins per acre as compared to 40 bins per acre on the grafts.
    - The vigor level of the grafts is much higher, so the fruit to leaf ratio is much higher for the grafts as compared to the older trees.
        - The implication is there may be a relationship very high crop loads reducing fruit eating quality.

Anecdotal observations:

- In 2015, commercial harvest timing was completed September 24. A few trees were left for photographing on Oct 7 in Quincy. The color advanced to dark red, and stem bowl splits increased from negligible to 35%. The eating quality was superb. 'Best ever'.
- WA 38 does not appear as susceptible to decay as many other varieties. This anecdotal observation is compared to suseptible varietes such as Honeycrisp or Fuji. With Gala or other stem bowl cracking prone cultivars, decay in the stem or calyx crack is very evident after a few weeks in storage. While decay does form in the WA 38 open wound, it tends to not progress beyond the margins of the wound.

CONTINUING PROJECT REPORT **YEAR:** 2 of 3
WTFRC Project Number: AP14-103A

Protect title: WA 38 rootstocks and training systems

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Cooperators: Sara Serra (WSU-TFREC), Ryan Sheick (WSU-TFREC).

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

Other funding sources: none

WTFRC Collaborative expenses

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

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

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

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

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

OBJECTIVES

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

SIGNIFICANT FINDINGS

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

Vegetative parameters

Sunrise:

- The highest trunk cross-sectional area (TCSA) and annual trunk growth were reported for trees trained to Spindle (like in 2014).
- G41 rootstock had significantly higher TCSA and annual trunk growth than Nic29 (like in 2014).
- The “bending” pruning technique had higher TCSA than the “click” technique in Spindle, but the difference in TCSA between pruning techniques was not significant in V.
- The average number of rootsuckers per tree was higher in Spindle than V and in Nic29 than G41 (like in 2014).

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 2014).
- The “bending” pruning technique had higher TCSA than the “click” technique for both systems.
- The average number of rootsuckers per tree was higher in Nic29 than in G41 and for “click” in V, but not in Spindle.

Pruning

- In both locations, the V system pruning (in terms of total hours of pruning per acre per year) took significantly longer than the Spindle system.
- In Sunrise, trees on G41 rootstock required more pruning time than Nic29, while no differences in time required for pruning were reported in Roza (data confirmed in both years).
- The "click" pruning technique took significantly more time during winter for both Sunrise and Roza orchards, while the total time of pruning per year was significantly higher for “click” in Sunrise only.

Yield and fruit size

- Spindle trees had more fruit per tree and higher yield per tree in both locations (opposite than 2014), while yield per acre was higher in V system in Roza, and not significant in Sunrise.
- Trees on G41 rootstock had less fruit per tree and lower yield per tree and per acre than those on Nic29 (but with a higher fruit weight) in Sunrise, while in Roza there was no statistical difference between rootstock behaviors.
- “Click” technique induced larger fruits in Spindle in Roza and in V in Sunrise in comparison to “bending”.

Fruit quality and storage (fruit 2014- only Roza)

- Nic29 showed a higher percentage of red overcolor than G41.
- After six months of normal air storage, Spindle fruit had higher soluble solid content (SSC) than V with no differences in firmness.
- G41 fruit from Spindle had higher SSC than Nic29 in the same system.
- The highest drop in DA index and fruit weight was reported for G41 in V system, indicating a faster ripening.

- In general fruit had more than 8 viable seed in the seed pockets with no difference among treatments.
- Fruit belonging to Nic29 trained at V showed significantly lower titratable acidity and higher pH in comparison to G41-V.

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

- In Roza, WA38/Nic29 trained at bi-axis had significantly bigger fruit than on G41. Mechanical winter pruned + hand pruned treatment in bi-axis produced less than hand pruned (winter+summer) bi-axis trees.

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

- Total pruning cost in both Roza and Sunrise was the lowest with V system, NIC29, and click and the highest with spindle, G41, and click.
- Commercial 'Fuji' pruning cost (for both spindle and V) ranged between $0.10 and $0.13 per tree. The total pruning cost for the WA38 in Roza ranged between $0.19-$0.29 per tree and in Sunrise $0.12-$0.31 per tree, across all treatments.
- In Roza, the break-even price for WA38 was lower compared to 'Fuji' only under spindle. Break-even prices for angled (V) WA38 were higher compared to angled 'Fuji'. In Sunrise, the break-even price for WA38 was lower compare to spindle 'Fuji' only under WA38 spindle, NIC29, bending. For all other treatments the break-even price for WA38 was higher than 'Fuji'.

METHODS

Two thousand trees of the new WSU scion variety WA 38 (Cosmic Crisp) propagated on M9-Nic29 and G41 rootstocks were planted in June 2013 in two locations at the WSU Sunrise (Wenatchee) and WSU Roza (Prosser) orchards to compare vegetative and productive performance. In both sites, two main training systems are compared: spindle (3 ft x 10 ft) and V system (1.5 ft x 10 ft). Another trial with WA 38 bi-axis trees (1 year younger) on the same rootstocks has been set up to assess the possibility of mechanized thinning, pruning and harvest. Both orchards are irrigated with sprinklers and drippers; a weather station including soil moisture probes and an automatic irrigation timer (Sunrise) were also installed.

In 2015, trees were managed according to the training system in order to achieve the correct structure/shape; in both locations the trees are well developed, but more vigorous in Roza than in Sunrise. 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 and trying to develop more buds close to the stem to avoid the "blind wood" issue that characterizes the "type IV habitus" apple varieties.

Winter and summer pruning has been done by hand in both locations respectively in March 2015 and July 2015. Start of blooming was at the beginning of April; Roza bloomed a little earlier than Sunrise. Post-bloom thinning was not performed in any of the treatment groups at either location.

In June, rootsuckers counting was performed in both locations.

Harvest was done September 17-18th in Roza and September 21st in Sunrise. Fruit size and yield were measured for all treatments on selected trees and samples for quality analyses were collected for Spindle and V systems (T0 at harvest and T1 for 6 months of storage). External disorders like sunburn, green spots, lenticels breakdown/bitter pit and bird damage were assessed in the field on

picked fruit. In September, trunk diameter at 10 cm above the grafting point was measured in both location to assess trunk cross sectional area (TCSA) and annual trunk growth.

In March 2015, we pulled out the 6 months (6M) stored apples harvested in 2014 from Roza (only) and we assessed the quality analyses. Parameters analyzed: weight drop (6M), I_{AD} index drop, dry matter %, overcolor % and background color, internal ethylene concentration (IEC), firmness, number of viable seeds, starch (1 to 8 Cornell scale), soluble solids content (SSC), titratable acidity and pH. Similar assessments have been done for the new harvested fruit from both locations in 2015 at T0; T1 samples will be pulled out in March-April 2016.

For the economic analysis we followed two approaches. First, we compared -using least squares mean analyses- the pruning time and cost across three treatments: training system (spindle and V), rootstock (NIC29 and G41) and pruning technique (bending and click). We compared the main effects and interaction effects of these three treatments. Pruning was 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.. This enables to have an approximate idea of what would likely be the WA38 costs at a commercial scale. We developed a production cost study (establishment and full production) for commercial 'Fuji' under two different trellis systems spindle and V. In this report, we present results for the 3rd year of establishment for both 'Fuji' and WA38. The returns for WA38 were estimated using a weighted average FOB price of 'Honeycrisp' (price x size), which is $1,353.40 per 925-lb bin or $58.53 per 40-lb box, considering the different fruit sizes of WA38 apples that were harvested in 2015.

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 a V 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.

Vegetative parameters

At Sunrise, spindle had higher TCSA in comparison to V system (11.04 and 8.29 cm^2 respectively) and G41 rootstock had significantly higher TCSA than Nic29 (1.56 and 7.95 cm^2 respectively). The comparison between the two pruning techniques within Spindle resulted in "bending" showing significantly higher TCSA than the "click" technique (data not shown). Analyzing as combinations, Spindle-G41-bending was the treatment combination with the highest annual trunk growth (4.67 cm^2), while V-Nic29-bending and click reported the lowest annual growth (2.02 and 2.22 cm^2). The average number of rootsuckers per tree was higher in Spindle (almost 5) and Nic29 (7.8, data not shown). The highest TCSA at Roza was in trees trained to Spindle (13.86 cm^2, data not shown). Nic29 had higher TCSA than G41, contrary to Sunrise; this difference was statistically significant in Spindle but not in the V system. Also in Roza, the combination V-Nic29-click showed the lowest annual trunk growth (data not shown). The "bending" pruning techniques showed higher TCSA than "click" in both systems (data not shown). The average number of rootsuckers per tree was higher in Nic29 than in G41 and in "click" than in "bending" but only within the V system (data not shown).

Pruning

Pruning in the V system took significantly longer (more than 55 hr/acre) than spindle at the Roza site (30 hr/acre, Table 1). More wood and fruit was cut and removed per tree during winter and summer pruning from Spindle in comparison to the V system. G41 and Nic29 in Roza did not show any significant difference in winter or summer pruning time (45 and 42 hr/A, respectively; Table 1).

During winter pruning more wood was cut from Nic29 than G41. “Click” techniques required more time for pruning in winter than “bending” (27 and 23 h/A, respectively); while summer pruning and total pruning time per year were not found to be significantly different between pruning techniques. “Click” pruning within the Spindle system took significantly longer: almost 7 hr/A more than “bending” in the same system (data not shown). In summer pruning, the fruit removed with the cuts were similar in the two techniques (4.8 and 4.6) but “click” fruit were 15 g larger, this can suggest a positive effect of this kind of cut on fruit size (Table 1).

In Sunrise, the V system required more time to be pruned in winter, summer and as total hours/A/year. More fruit/tree have been removed in summer from Spindle than V. In this location a significant difference between rootstocks has been observed; G41 required 20 more hours/A/year than Nic29, showing a meaningful difference in terms of vigor between them in this location. Also in Sunrise, “click” techniques required more time for pruning in winter than “bending” (20 and 14 h/A respectively) and total pruning time per year was 4 hr/ A longer for “click”. Among all combinations, V-G41-click/bending were the two that required the longest time to be pruned (total hr/ A/year).

Yield and fruit size

In Roza, the yield per tree was higher in Spindle, while calculated yield per Acre (Mton/A) was significantly higher in V due to the higher planting density (71 bins versus 40). No significant differences in terms of yield were observed between the two rootstocks. “Click” pruning technique produced less fruit per tree in comparison to “bending”, but they were bigger (279 and 269 g respectively). This difference was more evident within the Spindle system. Fruit size distributions in fruit categories (mm) showed a higher percentage of V system apples in the bigger size categories (85-90 mm).

In Sunrise, Spindle produced more fruit per tree than V with a higher fruit weight (209 g and 200 g, respectively), while in terms of calculated yield per Acre there was no statistical difference. Contrary to Roza, in Sunrise we registered a meaningful difference in the behavior of the 2 rootstocks: Nic29 produced more fruit per tree, smaller in size than G41 but with a calculated yield of 16.5 and 13 Mton/A, respectively (41.5 and 32.5 bins, Table 2). In agreement with observations made during summer pruning, “click” fruits were found to have higher weight than “bending” fruits (212 g and 196 g, respectively). The significance of this comparison is stronger in V, but is not significant within Spindle (data not shown). The most productive combination in Sunrise, as calculated yield (Mton/A), was V-Nic29-click in 2015, while combinations with G41 performed worse. Fruit size distributions in fruit categories (mm) showed a higher percentage of Spindle apples in the bigger size categories (75 to 85 mm) in Sunrise while in Roza the V system showed more fruit in the 85-90 mm size classes.

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

Trees in Roza completely fill the space available and present two strong leaders. Due to the water stress during 2014 and the local soil type and environment, trees in Sunrise are less developed. In Roza, one bi-axis row has been mechanically pruned in winter 2015 to build up the comparison between mechanical versus hand pruning for the next year. The trees in Sunrise were not fully developed yet to apply mechanization.

In Roza, WA38/Nic29 trained at bi-axis had significantly bigger fruit than on G41, while yield and fruit per tree were not different. Mechanical winter pruned+ hand pruned treatment in bi-axis produced less than hand pruned (winter+summer) bi-axis trees, but no difference has been reported in term of fruit weight.

Objective 3: Conduct an economic analysis of WA 38 production.
Overall, total pruning cost in both Roza and Sunrise was the lowest with V, NIC29 and click, and the highest with spindle, G41 and click. Results from the field trial were compared to pruning time and costs for commercial 'Fuji'. Pruning one acre of 'Fuji' trees in the third year takes on average 12 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 between 0.011- 0.008 hour per tree, and the cost ranges between $0.10-$0.13 per tree. The total pruning cost for the WA38 in Roza ranged from $0.19 to $0.29 per tree and in Sunrise $0.12-$0.31 per tree (see table 3).

At the third year from plantingWA38 yields in Roza were higher than the third year 'Fuji' yields across all treatments. In Sunrise, WA38 yields were higher than 'Fuji' under the spindle system, and lower under the V system.

The WA38 total production costs for all treatments were higher than 'Fuji' in Roza, and were higher only for spindle and lower for angled in Sunrise. In Roza, the break-even price for WA38 was lower compared to 'Fuji' only under spindle; under angled WA38 break-even prices were higher compared to angled 'Fuji'. In Sunrise, the break-even price for WA38 was lower compared to spindle 'Fuji' only under WA38 spindle, M9-NIC29, bending. For all other treatments the break-even price for WA38 was higher than 'Fuji'.

Table 1. Pruning labor hours at Roza and Sunrise orchards and aterial removed from cut (wood and fruit), 2015. Labor time is presented as hours per acre.

	winter PRUNING (hours:min:sec/A)		summer PRUNING (hours:min:sec/A)		total annual pruning 2015 (hours:min:sec/A/year)		cut material (wood) in winter (kg/tree)		cut material in summer (kg wood and leaves/tree)		num fruit/tree removed with summer pruning		kg fruit/tree removed with summer pruning		avr fruit weight (g) removed	
ROZA	Mar-15		Jul-15													
Trainin systems																
SPINDLE	20:39:04	b	9:50:20	b	30:29:24	b	0.652	a	0.787		5.216	a	0.43		80.7	
V	30:24:47	a	25:30:03	a	55:54:50	a	0.537	b	0.717		4.161	b	0.34		82.7	
signif.	***		***		***		**		ns		*		ns		ns	
Rootstocks																
G41	26:27:45		18:35:20		45:03:05		0.516	b	0.701		5.497	a	0.45	a	80.4	
M9 NIC29	24:50:03		16:58:50		41:48:53		0.653	a	0.790		4.082	b	0.33	b	82.6	
signif.	ns		ns		ns		***		ns		*		*		ns	
Pruning treatment																
BENDING	23:13:52	b	20:12:48		43:26:39		0.581		0.729		4.771		0.35		74.4	b
CLICK	27:49:59	a	15:07:36		42:57:34		0.608		0.775		4.606		0.41		89.0	a
signif.	**		ns		ns		ns		ns		ns		ns		***	
SUNRISE																
Trainin systems	**Mar-15**		**Jul-15**													
SPINDLE	12:10:28	B	13:48:06	B	25:58:34	B	0.31		0.39		5.4	A	0.30		56.8	
V	22:30:52	A	24:16:31	A	46:47:23	A	0.32		0.45		3.3	B	0.19		59.1	
signif.	***		***		***		ns		ns		*		ns		ns	
Rootstocks																
G41	20:39:38	A	25:58:37	A	46:38:14	A	0.41	A	0.59	A	5.3	A	0.30	A	58.8	
M9 NIC29	14:01:43	B	12:06:00	B	26:07:43	B	0.21	B	0.25	B	3.3	B	0.19	B	57.2	
signif.	***		***		***		***		***		*		*		ns	
Pruning treatment																
BENDING	14:32:47	B	19:43:15		34:16:02	B	0.29		0.45		5.9	A	0.32	A	54.2	B
CLICK	20:08:33	A	18:21:21		38:29:55	A	0.33		0.39		2.8	B	0.17	B	61.8	A
signif.	***		ns		*		ns		ns		**		*		**	

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

Table 2. WA 38 fruit yield at the Roza and Sunrise harvest September 2015.

Training system	Rootstock	Pruning trt	total number fruit/ tree		kg fruit/tree		Average wt/fruit (g)		yield Mton/Acre		Yield Bin/A
ROZA											
SPINDLE			40.02	a	10.72	a	271		16.07	b	40.3
V			34.43	b	9.48	b	277		28.43	a	71.2
Sign.			**		*		ns		***		
	G41		38.22		10.21		271		22.19		55.6
	NIC29		36.48		10.02		276		22.30		55.9
	Sign.		ns		ns		ns		ns		
		BENDING	39.55	a	10.53		269	b	23.16		58.0
		CLICK	34.90	b	9.68		279	a	21.34		53.5
		Sign.	*		ns		*		ns		
SUNRISE											
SPINDLE			45.7	a	9.4	a	209	a	14.1		35.4
V			26.3	b	5.1	b	200	b	15.2		38.2
Sign.			***		***		*		ns		
	G41		30.7	b	6.4	b	210	a	13.0	b	32.5
	NIC29		40.5	a	8.0	a	198	b	16.5	a	41.5
	Sign.		***		**		*		***		
		BENDING	37.5		7.3		196	b	15.0		37.7
		CLICK	33.4		7.0		212	a	14.4		36.0
		Sign.	ns		ns		**		ns		

ns , not significant; *, p <0.05; **, p<0.01; ***, p <0.001 according to SNK *post hoc* test.

Table 3. Estimated pruning time (hr/tree) for WA38 during the third year (2015) given the interactions of various training systems, rootstocks and pruning techniques, and in comparison with the baseline ('Fuji').

Location	Treatments	Winter Pruning[1]		Summer Pruning[1]		Total	
		hour/tree		hour/tree		hour/tree	$/tree
Roza	Spindle, G41, Bending	0.0117	A[2]	0.0061	A	0.0178	$0.21
	Spindle, G41, Click	0.0168	A	0.0072	A	0.0241	$0.29
	Spindle, M9-NIC29, Bending	0.0118	A	0.0065	A	0.0182	$0.22
	Spindle, M9-NIC29, Click	0.0151	A	0.0065	A	0.0215	$0.26
	V, G41, Bending	0.0092	A	0.0101	A	0.0193	$0.23
	V, G41, Click	0.0119	A	0.008	A	0.0199	$0.24
	V, M9-NIC29, Bending	0.0124	A	0.0105	A	0.0229	$0.27
	V, M9-NIC29, Click	0.0098	A	0.0057	B	0.0155	$0.19
Sunrise	Spindle, G41, Bending	0.0088	A	0.0132	A	0.0221	$0.27
	Spindle, G41, Click	0.0138	B	0.0124	A	0.0262	$0.31
	Spindle, M9-NIC29, Bending	0.0064	A	0.0056	A	0.0119	$0.14
	Spindle, M9-NIC29, Click	0.0093	A	0.0056	A	0.0149	$0.18
	V, G41, Bending	0.0074	A	0.0114	A	0.0189	$0.23
	V, G41, Click	0.0104	A	0.0104	A	0.0208	$0.25
	V, M9-NIC29, Bending	0.0049	A	0.0055	A	0.0104	$0.12
	V, M9-NIC29, Click	0.0073	A	0.0051	A	0.0124	$0.15
Baseline	'Fuji', Spindle, M9					0.011	$0.13
	'Fuji', Angled (V), M9					0.0083	$0.10

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

CONTINUING PROJECT REPORT **YEAR:** 2 of 3
WTFRC Project Number: AP-14-105A

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

PI:	Cameron Peace	**Co-PI**:	Kate Evans
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Email:	cpeace@wsu.edu	**Email**:	kate_evans@wsu.edu
Co-PI:	Craig Hardner	**Co-PI**:	Dorrie Main
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Cooperators: Paul Sandefur, Sushan Ru, Luo Fei Xiong, and Ashley Powell (graduate students, WSU Pullman), Sook Jung (WSU Pullman), Fred Bliss (Davis, California)

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

Other funding sources

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

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

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

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

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

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

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

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

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

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

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

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

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

OBJECTIVES

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

Specific objectives

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

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

SIGNIFICANT FINDINGS

- **Powdery mildew resistance** DNA test developed and ready for deployment
- **Blue mold** DNA test developed and ready for deployment
- **Fire blight** candidate DNA test developed
- **Pink flesh** candidate DNA test developed
- DNA test deployment strategies comprehensively consider cost, time and genetic gain efficiencies, operational logistics, and essential vs. enhancing trait levels

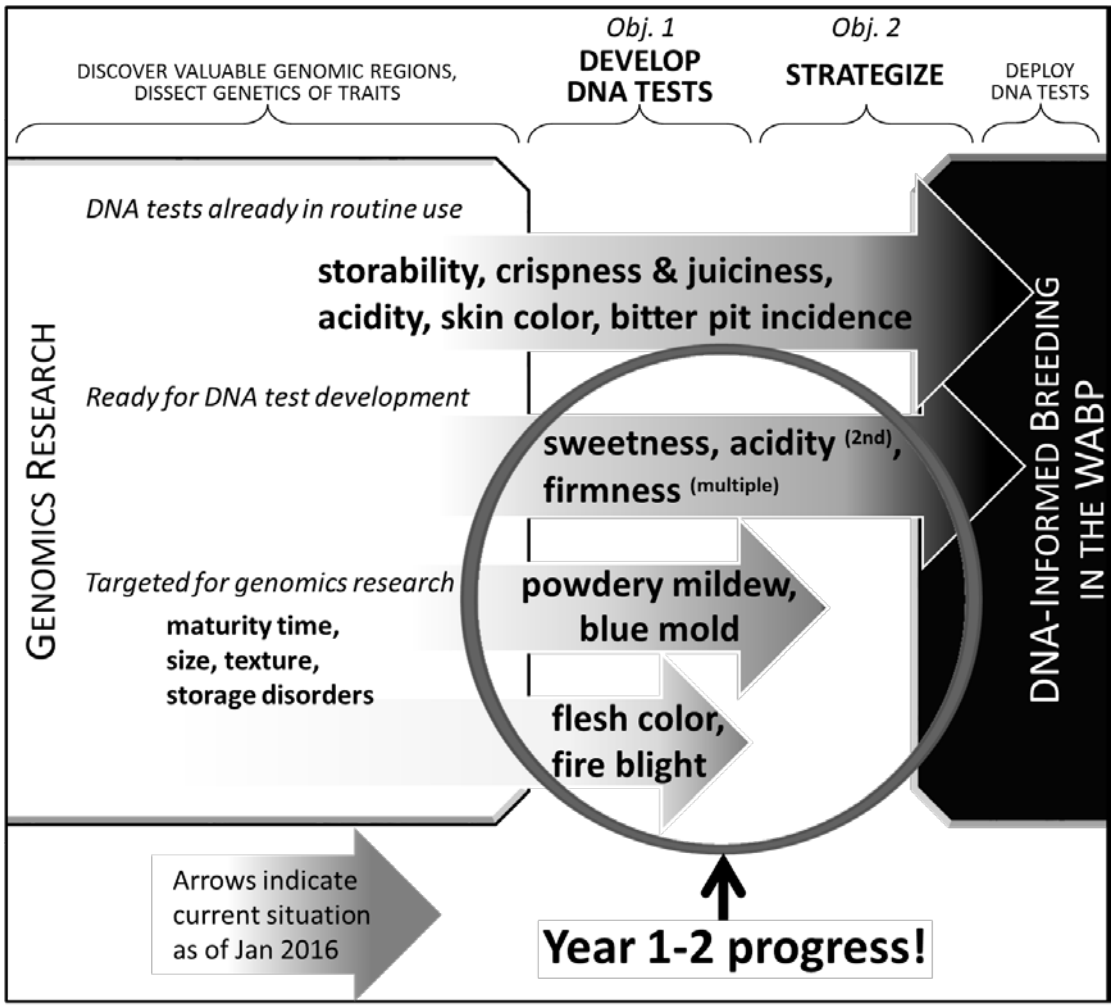

Figure 1: Progress made in years 1-2 to improve the efficiency, accuracy, creativity, and pace of the Washington Apple Breeding Program (WABP) via DNA-informed breeding.

METHODS

Activity 1: DNA test development

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

DNA test development process

A) *Establishment of functional genotype patterns:* Phenotypic data for a specific trait and genotypic data generated during the RosBREED project are combined, from which functional genotype patterns are developed. These genotype patterns are "functional" because they are associated with specific, breeding-relevant trait levels, and form the basis from which DNA tests can be developed.

B) *Candidate assay preparation:* Using Genome Database for Rosaceae tools, genomic sequences from the targeted regions are retrieved. For initial testing, five sites in each region are targeted with candidate assays.
C) *Candidate assay checking:* Each candidate assay is checked on a small set of individuals that represent functional genotype patterns. Checking includes amplification and visualization of the candidate assay product followed by examination of assay products. This same process is then repeated across a larger set of diverse, breeding relevant individuals (~50) to confirm initial results. If matching results are observed, the candidate assay becomes an official DNA test and is ready immediately for subsequent low-throughput genotyping needs involving few breeding individuals (parent material) and conversion to a high-throughput system for use on many breeding individuals (seedlings).
D) *High-throughput conversion:* The successfully developed DNA test is converted to a high-throughput platform (ABI 3730 DNA Analyzer) to maximize resource efficiency. After successful conversion, the DNA test is now named appropriately and is ready for full application.
E) *Effects calculations:* No additional confirmation of trait level predictions is required because only candidate assays with products that directly match the original functional genotype patterns are chosen to be DNA tests. However, as additional breeding material that was not part of the original association calculations is evaluated for commercial performance and examined with the new DNA tests, more accurate effect calculations are conducted using standard statistical procedures.

Activity 2: DNA test deployment strategies

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

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

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

historical data collected in the WABP to evaluate the efficiency of this weighted selection index approach. Based on results, the method will be refined and extended to all available DNA tests.

RESULTS AND DISCUSSION

Activity 1: DNA test development

Significant progress was made in 2015 in developing DNA tests for **foliar powdery mildew** and **blue mold resistance**. The new DNA tests will be ready for application in seedling selection beginning in early 2016. Progress was also made in developing a DNA tests for **fire blight resistance**, **fruit pink flesh color**, and for additional sources of **foliar powdery mildew** and **blue mold resistance**, with the candidate assay designs completed and trials initiated. DNA information for seven traits was used to guide 2015 crossing decisions: for storability, crispness, juiciness, bitter pit, skin color, acidity, and fruit fructose content.

Foliar powdery mildew resistance
"Md-Plw-SSR", a DNA test for prediction of foliar powdery mildew resistance from 'White Angel', was developed and is now ready for deployment in the WABP. This new DNA test appears to differentiate individuals with complete resistance from those susceptible to the disease. If this DNA test is confirmed to be robust, seedlings from crosses using 'White Angel' can be screened with Md-Plw-SSR and only powdery mildew resistant seedlings field-planted. Breeders do not consider genetic resistance from a single source to be durable in the long term. Therefore, DNA tests described in the literature for additional sources of powdery mildew resistance are currently being examined for their predictiveness in the WABP. We are aiming for a DNA test that can be used to identify individuals with broad resistance to powdery mildew from three different sources ready for WABP deployment in spring 2017.

Blue mold resistance
A new DNA test, "Md-BM3-SSR", was developed for resistance to the post-harvest disease blue mold. This DNA test targets a major genomic region associated with blue mold resistance located on apple chromosome 3, discovered by Dr. Jay Norelli (USDA-ARS West Virginia). The resistance source for this genomic region is the primary wild ancestor of cultivated apple, *M. sieversii*. Using the blue mold test, parents can be identified that have the genetic potential to produce offspring with some resistance to this costly disease. Additionally, offspring without resistance to blue mold can be identified and discarded prior to field planting. Candidate assays were also prepared that target another Norelli-discovered genomic region associated with resistance to blue mold on chromosome 10 (also *M. sieversii* source). Once checked and converted, the two blue mold DNA tests will be combined in a similar fashion to the DNA tests we use to target multiple genomic regions for apple texture and acidity – maximizing predictiveness for this important trait.

Fire blight resistance
A candidate assay was developed targeting a genomic region on apple chromosome 12 associated with a significant proportion of observed differences among WABP individuals for resistance to fire blight. This genomic region was reported in the literature in 2009 and the original source of the resistance allele is *Malus floribunda*. The predictiveness of the candidate assay is currently being checked and it is expected that a DNA test will be ready for deployment in spring 2016 seedling selection and to support accurate 2016 crossing decisions. Candidate assays for fire blight resistance from two additional sources are also currently being checked. An improved fire blight DNA test that combines the predictive power of all three genomic regions associated with

fire blight resistance is expected to be ready for deployment in spring 2017. Such a DNA test would provide a tool for incorporating durable resistance to fire blight into future WABP cultivars.

Pink flesh
A candidate assay was developed for pink flesh. Prior to use in the WABP, additional flesh color phenotypic data is needed to confirm prediction accuracy. We are seeking this data from international collaboration. A DNA test for prediction of pink flesh is expected to be ready for WABP deployment in spring 2017.

Storage disorders: scald, shrivel, and water core
Scald, shrivel, and water core, three storage disorders of importance to the Washington apple industry and the WABP, have been targeted for DNA test development. Although development of DNA tests for these traits awaits genomics discoveries, it is expected that a DNA test for at least one of these traits will be ready for deployment in spring 2017.

Activity 2: DNA test deployment strategies

Effectively deploying DNA information in the WABP requires an experience-based understanding of opportunities for time, cost, and genetic gain efficiency to allocate limited resources and on-the-ground logistic feasibility for planning each year's operations, collecting tissue samples, conducting DNA testing, delivering and interpreting the outcomes, and acting on that new DNA information. We are at an advanced stage in all of these areas, as described below.

Some opportunities for time efficiency are available to deliver superior new cultivars quicker. Time considerations are relatively simple for the WABP's single-generation breeding scheme. In the WABP, marker-assisted seedling selection currently does not improve time efficiency. This is because performance evaluation of field-grown grafted seedlings is still required in Phase 1 for all the productivity and fruit quality traits that the DNA tests do not (yet) evaluate. Several years could be saved if DNA testing was conducted for enough traits that some seedlings could skip Phase 1 field trials and be advanced directly to Phase 2 replicated field trials; however, the breeder would have to be confident in the genetic superiority of such seedlings to justify the expenditure of entering them directly into multi-location multi-tree trials. DNA test use on elite cultivar candidates can shave years off their presence in Phase 2 and Phase 3 field trials. By supporting performance results, elite selections can be removed from further consideration or advanced to the next phase, rather than decisions waiting on another year or two of trial data. For example, DNA test results indicating high acidity and fructose content could support trial results indicating that a selection might have consistently intense-flavored fruit. Finally, DNA information about cultivars available to growers can support their decisions about which cultivars to plant and to what extent. The additional information from DNA test results can thereby mitigate risks associated with new cultivars that have relatively unknown genetic potential. Therefore, DNA test use on young seedlings does not save time in the WABP, but use of DNA information in later phases can shave years off the time to cultivar release and the time to commercial impact.

We have more than six years of experience in evaluating and managing logistic feasibility and cost efficiency of DNA testing in the WABP. In prior years, we had conducted scientific research on these considerations in early seedling selection. That research – and its practical deployment – has been described in numerous reports of previous WTFRC projects of PIs Peace and Evans. A paper published this year (Edge-Garza et al., 2015: "Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding") described the theoretical underpinnings of our consideration of cost efficiency and presented our Excel-based spreadsheet for identifying cost-efficient situations. In short, it is cost-imperative to deploy one or more DNA tests that each removes genetically inferior seedlings prior to the large resource expenditures that arise in traditional operations. For the WABP, cost efficiency is maximized when the DNA tests are for fruit quality

characteristics and the testing is conducted prior to field planting. The most valuable DNA tests are those that identify genetic superiority for traits typically identified only after extensive trialing of elite cultivar candidates because the resource savings extend to costs of trialing inferior selections in Phase 2 and Phase 3. However, extension of these principles to the wider breeding scheme indicates that the most compelling DNA test deployment is for *parents*. Making cross combinations predicted to lead to superior outcomes (i.e., a large proportion of seedlings above minimum thresholds for all cultivar-relevant traits and/or at least some seedlings likely to have exceptional attributes) guided by DNA information is even more cost efficient than seedling selection – and is also simpler logistically.

Genetic gain efficiency has been recently integrated into our DNA deployment considerations. A computer simulation study of genetic gain efficiency from marker-assisted seedling selection was conducted and submitted to a peer-reviewed journal for publication (Ru et al., submitted). Results from this study were illuminating for DNA test deployment in the WABP. In particular, the study identified the two major features of each DNA test that determine whether and to what extent it should be used in seedling selection to improve on (or at least not go backward in) genetic improvement achievable with traditional phenotypic selection. The two features are (1) heritability, *H*, the degree to which observed performance variation among seedlings us due to underlying genetic differences, and (2) predictiveness, *P*, the proportion of heritability that is captured by a DNA test. The first feature is a function of the trait itself, which depends on which superior and inferior alleles have been captured in the germplasm used by the breeding program; low heritability limits genetic improvement using any type of selection including traditional. The second feature is a function of the genomic region(s) targeted by a DNA test, which depends again on the germplasm used by the breeding program and can be improved by expanding a DNA test to capture further influential genomic regions and their alleles. All DNA tests available for the WABP can be placed into a framework delimited by these two features, and their position determines their appropriate deployment. Prior to this objective framework, the range of subjective, intuitive breeder reactions to the use of early DNA-based seedling culling included apparently mutual incompatibilities (e.g., “I only want to cull the seedlings predicted to have the worst genetic potential” and “It is clearly advantageous to keep only the seedlings with the very best genetic potential”). Now, it is clear that each such reaction is fair – but depends on the features of each DNA test. In short, for DNA tests targeting trait levels essential in a new cultivar:

- High *P*, low *H*: Use DNA test as much as possible on seedlings to keep only the best genotypes – maximizes both genetic gain and cost efficiency
- High *P*, high *H*: Use DNA test as much as possible on seedlings to keep only the best genotypes – maximizes cost efficiency for similar genetic gain outcomes
- Low *P*, high *H*: Use DNA test only to cull the worst genotypes – maximizes cost efficiency for similar genetic gain outcomes
- Low *P*, low *H*: Treat as if targeting an trait level that is only enhancing

For DNA tests targeting trait levels that are only enhancing for a new cultivar, our studies lead to the conclusion that optimal seedling selection decisions use a selection index that simultaneously weighs all available information on genetic potential for multiple traits, rather than culling seedlings based on their genotype for a single DNA test as done for essential trait levels.

For completion in 2016, we are currently developing a streamlined statistical approach via software programming that accounts for all the above considerations to support breeding decisions about DNA test deployment across the breeding cycle.

References

Edge-Garza D, Luby J, Peace C. 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, Peace C. Modeling of genetic gain from marker-assisted seedling selection in clonally propagated crops. Theoretical and Applied Genetics (submitted)

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

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

PI: Keith Yoder
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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:** $56,591 **Year 2: $56,591**

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 158 automated weather stations.

WTFRC Collaborative expenses:

Item	2015	2016
Salaries	10,000	10,000
Wages	2,000	2,000
Supplies	500	500
Travel	500	500
Total	$13,000	**$13,000**

Budget 1
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	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
Supplies	1000	1,000
Contractual services & repairs	1,000	1,000
Total	$43,591	**$43,591**

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

OBJECTIVE:

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 (Yoder et al., 2009).

SIGNIFICANT FINDINGS 2015:

Low-temperature pollen tube growth rates from first year of low temperature testing were compared to present model parameters on all models. Hourly differences between presently used models predicted timing of first bloom spray vs predicted spray timing using 1st year preliminary growth rate for each model is shown in the following graphics. Also shown is the percent of hours that temperatures were below 55°F from start of model to application of first bloom thinning spray.

Golden Delicious – (Figure 1)

- **Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray ranged from 43% to 88%.**
- **Difference in application timing (hours) comparing present model versus first year low temperature test data range from 4 hours to 22 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.**

Gala – (Figure 2)

- **Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray ranged from 30% to 88%.**
- **Difference in application timing (hours) comparing present model versus first year low temperature test data range from 3 hours to 18 hours.**
- **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.**

Fuji – (Figure 3)

- **Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray ranged from 48% to 89%.**
- **Difference in application timing (hours) comparing present model versus first year low temperature test data range from 5 hours to 32 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.**

Cripps Pink – (Figure 4)

- **Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray ranged from 68% to 78%.**
- **Difference in application timing (hours) comparing present model versus first year low temperature test data range 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.**

Honeycrisp – (Figure 5)

- **Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray ranged from 37% to 64%.**
- **Difference in application timing (hours) comparing present model versus first year low temperature test data range from 1 hour to 30 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.**

Granny Smith – (Figure 6)

- **Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray ranged from 37% to 74%.**
- **Difference in application timing (hours) comparing present model versus first year low temperature test data range from 2 hours to 53 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.**

Red Delicious – (Figure 7)

- **Percent of hours below 55°F from start of model fertilization period to first bloom thinning spray ranged from 78% to 81%.**
- **Difference in application timing (hours) comparing present model versus first year low temperature test data range from 6 hour to 22 hours.**
- **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.**

METHODS:

Dormant trees growing in root bags were removed from the AHS-AREC orchard, placed in cold rooms to delay bud break, and then later moved to a heated greenhouse to force bloom. A designated number of flowers at the late balloon stage was be selected on each tree, tagged, and emasculated. The emasculated flowers will be pollinated with 'Snowdrift' crabapple pollen using a brush. The rest of the flowers on the trees were removed one day before hand pollination to prevent cross-contamination of pollen. Trees were then placed into temperature-controlled growth chambers at the selected temperatures. Duration of tests ranged from 12 to 48 hours depending upon the rate at which the pollen tubes were growing.

Flower samples were prepared by boiling for 15 minutes. Pistillate organs were 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 were stained for 24 hours before examination at 100X under fluorescent light using a Zeiss HBO-50 high-pressure mercury vapor light source and a Nikon Optiphot microscope. Collected data includes 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.

RESULTS AND DISCUSSION:

A random selection of four orchard blocks from different areas were used to compare predicted timing of bloom thinning sprays using pollen tube growth models presently available vs data from first year of low temperature testing. The difference in application timing is shown in the following figures. In addition the percent of hours accumulated below 55°F during that period is shown also. Examining the hours of growth the fertilization period accumulates during that period gives a better understanding of why and how much growth is happening at the lower end of the model temperature range. As is shown in Figure 1, application timing of first bloom thinning spray can range from 85 hours after start of model program at one orchard block to 210 hours at a different block in Golden Delicious. In the case of some blocks such as the Deer Mtn. block in Figure 1, the first bloom thinning was applied early because predicted pollen tube growth rate was extremely slow due to the fact that of the 139 hours from start of model, 88% of those hours were below 55°F. In comparison the Pinata1 block showed only 54% of its 145 pollen tube growth rate hours below 55°F. Average style length was 13.88 mm for Deer Mtn. block and 12.68 mm for Pinata1 block.

First year low temperature tests in Gala (Figure 2) and Red Delicious (Figure 7) dictated that bloom thinning application timing would have been later than predicted application timings using presently available models. In all other models bloom thinning application timing would have been earlier using first year low temperature tests data. The widest range of hours below 55°F occurred in Gala selected blocks ranging from 30% to 80%. The smallest range occurred in Red Delicious blocks with a range of 78% to 81% below 55°F. Difference in application timing (hours) comparing present model versus first year low temperature test data ranged from 1 hour to 30 hours in Honeycrisp (Figure 5) to 2 hours to 53 hours in Granny Smith (Figure 6). Results from first year low temperature data showed predicted application timing for bloom thinners would have been earlier in Golden Delicious, Fuji, Pink Lady, Honeycrisp, Granny Smith. Application timing for Gala and Red Delicious would have been later than predicted timing using present models. Second year testing is needed to verify the first year results. Average style length in Fuji blocks (Figure 3) showed one block (BVO-18) with 10.43 mm average style length and shortest amount of time predicted from end of pollination of desired crop load to application of first bloom thinning spray of 73 hours. Cripps Pink testing showed least amount of difference in application timing between the tests blocks of 7 hours (Figure 4). Difference in application timing between Granny Smith test blocks when compared to presently used model showed a range of 2 hours in BVO 214 block to 53 hours at Weasel block (Figure 6).

Figure 1 – Golden Delicious

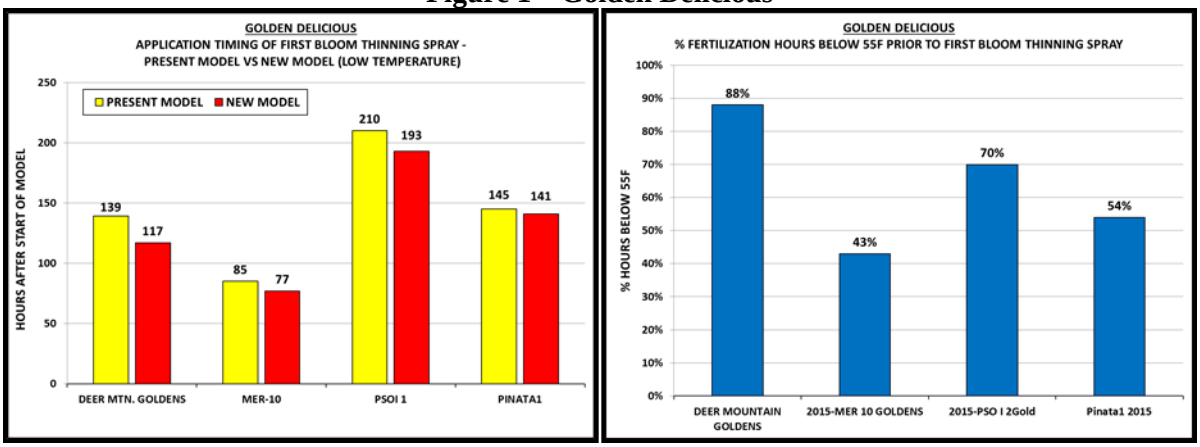


Figure 2 - Gala

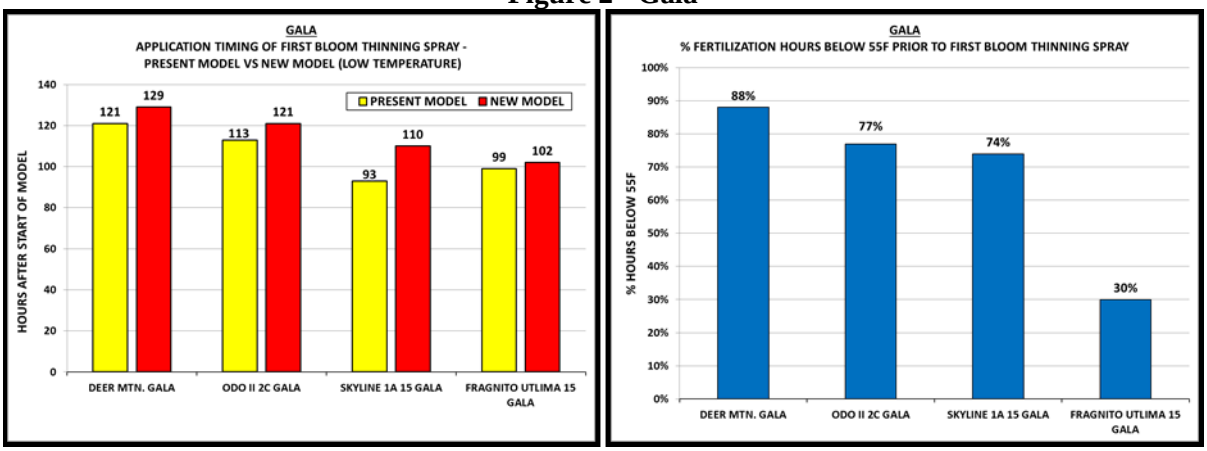


Figure 3 - Fuji

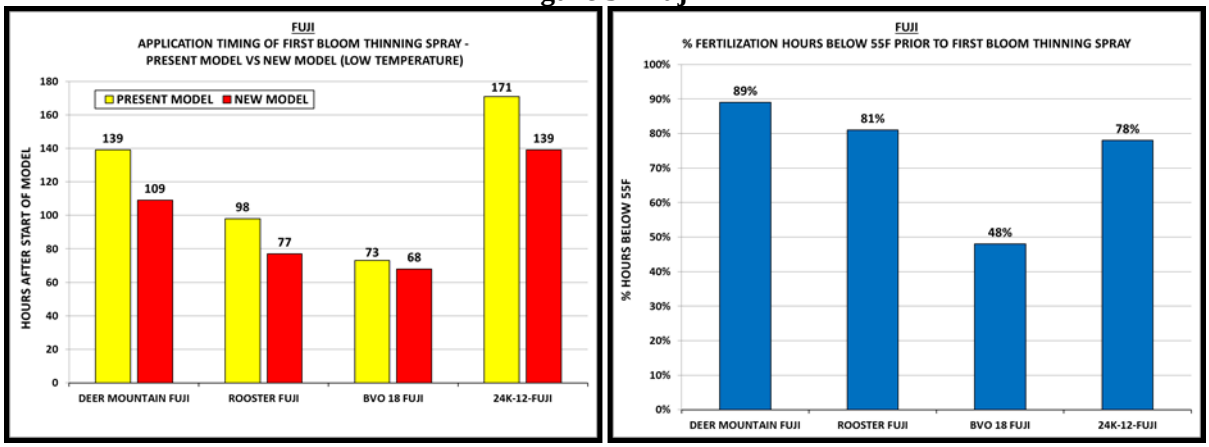
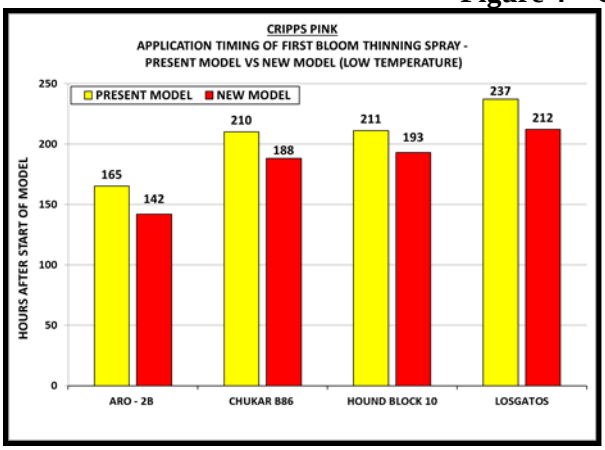


Figure 4 – Cripps Pink



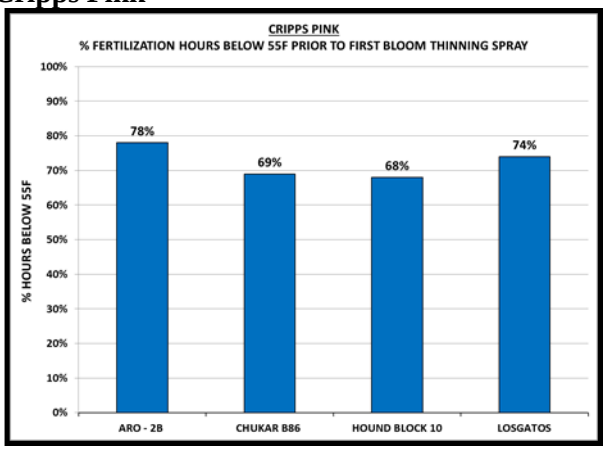
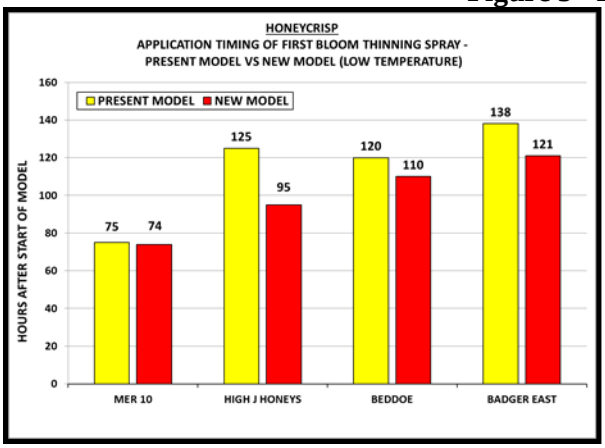


Figure 5 - Honeycrisp



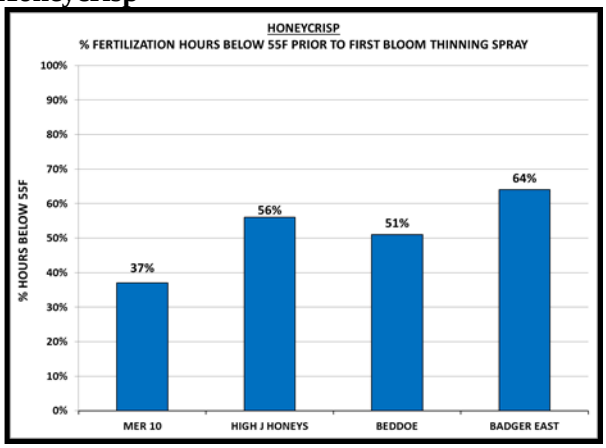
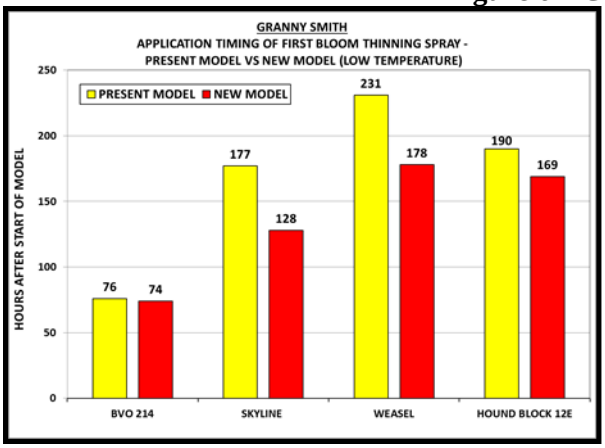


Figure 6 – Granny Smith



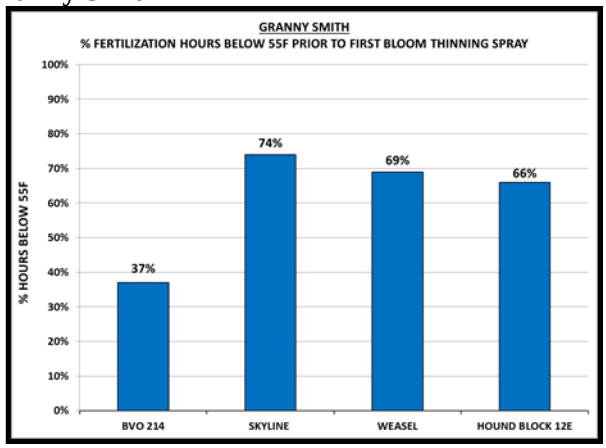
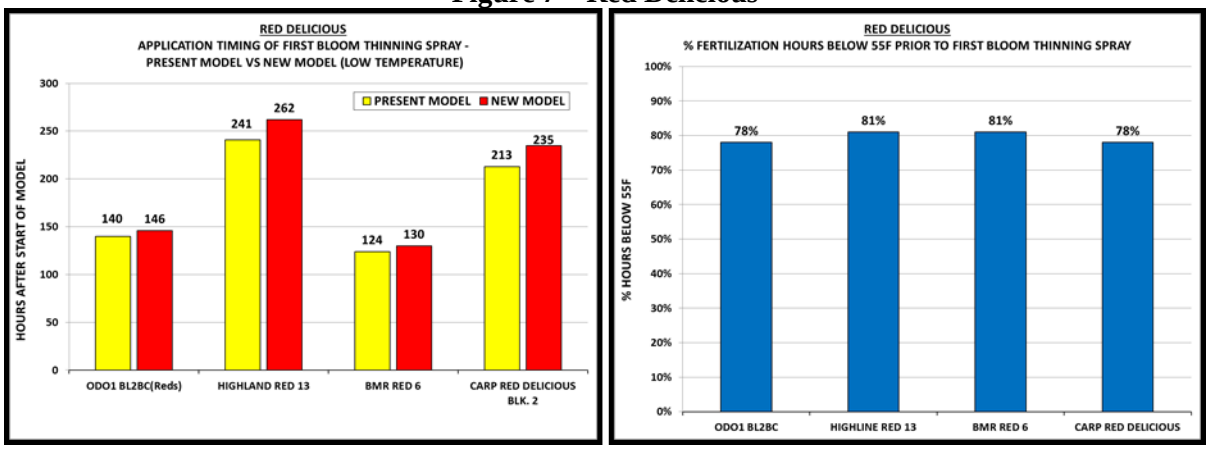


Figure 7 – Red Delicious



As shown in the figures above the actual application timing can be altered by the model user to adhere to more specific conditions in the field. The final decision for application timing always rests with the user in the field.

Literature Cited:

Yoder, K., R. Yuan, L. Combs, R. Byers, J. McFerson and T. Schmidt. 2009. Effects of temperature and the combination of liquid lime sulfur and fish oil on pollen germination, pollen tube growth, and fruit set in apples. HortScience 44(5):1277-1283.

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

Project Title: Crop load and canopy management of apple

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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	
Benefits	10,000	9,000	
Wages	50,000	35,000	
Benefits	17,000	12,000	
Equipment			
Supplies	1,000	500	
Travel	3,000	2,500	
Stemilt lab fees	2,000	1,500	
Statistical consulting	1,000	0	
Total gross costs	119,000	90,500	
Reimbursements	(119,000)	(87,000)	
Total net costs	0	3,500	

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

2015 HIGHLIGHTS:

K-Pax, an experimental formulation of lime sulfur demonstrated good thinning efficacy in an initial study (Table 2)

Metamitron can be an effective postbloom thinner in WA whether used alone at high rates or at lower rates with various spray adjuvants or thinning partners (Tables 5 & 6)

Metamitron can induce leaf burn in several apple cultivars, especially when tank mixed with dormant oil or a silicone-based surfactant (Figure 1); damage appears temporary and no long term harmful effects to the tree or fruit have been observed in WA trials

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

The efficacy of multiple spring applications of 100-200 ppm GA_3 to reduce return bloom is now well established in WA trials over several years including 2015; experimental formulations of GA also offer potential to help growers mitigate biennial bearing (data not shown)

2014 spring/summer applications of NAA or ethephon did not improve 2015 return bloom of Fuji (data not shown)

All formulations of prohexadione calcium evaluated effectively inhibited shoot growth in Fuji; acidifying the spray tank improved the performance of each material (data not shown)

BACKGROUND:

After years of robust efforts to evaluate various aspects of bloom and postbloom chemical thinning programs, our current focus is to screen new chemistries and provide collaborative support for external research programs working on crop load and canopy management. Most of our current trials are funded in part or whole by third party companies that contract our services to independently evaluate their products alongside industry standards. We continue to evaluate the relative success of chemical and mechanical thinning programs through three measurable targets which are directly tied to a grower's economic bottom line:

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

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

2015 chemical thinning programs are listed in Table 1; for those which show a range of possible rates, higher concentrations are typically reserved for cultivars known to be difficult to thin, such as Fuji and Golden Delicious. In most cases, additional chemical thinning treatments were left to the discretion of individual grower-cooperators, provided that each experimental plot received the same programs.

Table 1. Chemical thinning programs evaluated. WTFRC 2015.

BLOOM THINNERS (applied 100 gal water/A @ 20-40% &/or 80-90% bloom)
5-10% Rex lime Sulfur (LS)
6% K-Pax
4 gal ammonium thiosulfate (ATS)/A
2% Wilbur Ellis Supreme Oil (WES) + 10% lime sulfur
2% Crocker's Fish Oil (CFO) + 3% Lime Sulfur (LS)
400-800 ppm metamitron
POSTBLOOM THINNERS (applied 100 gal water/A at PF, 10mm, &/or 15mm)
48 oz Carbaryl 4L/A
48 oz Carbaryl 4L + 4-6 oz Fruitone L (NAA)/A
122-128 oz BA + 4-6 oz Fruitone L/A
FAL 551 24 oz (BA) + 4 oz Fruitone L
400-800 ppm metamitron
400 ppm metamitron + 4-6 oz Fruitone L (NAA)/A
400-800 ppm metamitron + 16 oz Regulaid/A
400-800 ppm metamitron + 6 oz Sylgard/A
400-800 ppm metamitron + 64 oz IAP dormant oil
400-800 ppm metamitron + 128 oz WES summer oil
400 ppm metamitron + 122 oz BA
400 ppm metamitron + 48 oz Carbaryl 4L/A
100 gal water + 16 oz Regulaid/A
100-400 gal water/A

BLOOM THINNING:

After a preliminary screen in 2014 revealed no phytotoxicity, we conducted a formal chemical 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. In its initial test, K-Pax thinned Gala fruit at least as well as a comparable rate of standard Rex Lime Sulfur (Table 2) and again showed no deleterious effects on fruit finish. Further testing of K-Pax is planned for 2016.

Table 3 represents two trials which featured both bloom and postbloom chemical thinning treatments. No bloom thinning treatment in either trial was effective at reducing fruit set or increasing fruit size with the exception of WES dormant oil + lime sulfur, which achieved both goals on Scilate (Envy). No postbloom thinning treatment was effective on Scilate, but we were encouraged to see that once again, no bloom or postbloom chemical thinning treatment seemed to induce russet on this sensitive variety. A separate trial featuring several metamitron treatments applied to Granny Smith during bloom and/or postbloom was not as successful as other 2015 trials of dedicated postbloom applications of metamitron (Table 5).

Table 2. Crop load and fruit quality effects of bloom chemical thinning programs. WTFRC 2015.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	%	g		%
Gala / M.7 - Bridgeport						
K-Pax	191 b	15 ns	27 ns	196 ns	93	76 ns
Rex lime sulfur	206 ab	15	24	202	90	79

Control	238 a	12	20	199	91	81

Table 3. Crop load and fruit quality effects of bloom + postbloom chemical thinning programs. WTFRC 2015.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	%	g		%
Scilate / M.9 Nic.29 - Prosser						
ATS	71 a	58 bc	21 ab	263 c	69	45 ns
Carbaryl 4L	50 ab	69 abc	18 ab	335 a	54	54
Carbaryl 4L + Fruitone L	61 ab	62 bc	22 ab	286 bc	63	45
Rex lime sulfur 5 gal	74 a	54 bc	26 a	265 c	69	40
Rex lime sulfur 10 gal	73 a	55 bc	27 a	258 c	70	65
Water + Regulaid	62 a	62 bc	22 ab	294 abc	62	53
Water only 100 gal/a	47 ab	70 ab	19 ab	299 abc	61	51
Water only 400 gal/a	78 a	53 c	26 a	275 bc	66	56
WES oil + Rex lime sulfur	29 b	79 a	15 b	318 ab	57	50
Control	76 a	56 bc	23 ab	263 c	69	61
Granny Smith 9 B / M.9 – Rock Island						
CFO + Rex lime sulfur	35 ns	67 ns	31 ns	200 ns	91	
Exilis Plus + Fruitone L	43	60	37	165	110	
Metamitron 400 ppm bloom	41	62	35	197	92	
Metamitron 400 ppm bloom + postbloom	39	64	34	190	96	
Metamitron 400 ppm postbloom	40	63	34	173	105	
Metamitron 800 ppm bloom	37	66	31	184	99	
Metamitron 800 ppm postbloom	40	65	31	194	94	
Control	43	60	37	168	108	

Table 4 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 4. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials. WTFRC 1999-2015.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	15 / 60 (25%)	10 / 63 (16%)	4 / 54 (7%)
NC99	15 / 32 (47%)	7 / 34 (21%)	2 / 28 (7%)
Lime sulfur	26 / 58 (45%)	12 / 52 (23%)	9 / 49 (18%)
CFO + LS	62 / 114 (54%)	27 / 105 (26%)	22 / 103 (21%)

JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	15 / 30 (50%)	5 / 29 (17%)	4 / 28 (14%)
ThinRite	7 / 22 (32%)	0 / 23 (0%)	0 / 12

[1]Does not include data from 2015 trials.

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

POSTBLOOM THINNING:

The primary focus of our 2015 chemical thinning work was on 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 early results indicated that two applications of 600-800 ppm are necessary to produce similar effects in Washington conditions. Employing this more aggressive approach, we produced our strongest set of results to date with metamitron.

Across several sites and cultivars, 2015 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 (Table 5). Additionally, we consistently observed increased thinning of Granny Smith when metamitron was partnered in the spray tank with a variety of adjuvants including silicone surfactants, non-ionic surfactants, dormant spray oil, and a summer spray oil (Table 5). In a separate trial on Jonagold, metamitron also performed well when tank-mixed with other thinning agents like BA, NAA, and carbaryl (Table 5).

Over the past few years, our research colleagues in the East and Europe have been incredulous that we had not yet observed significant phytotoxicity from metamitron, especially given our use of multiple applications of high rates. For the first time, we saw clear leaf burn at several sites in 2015, particularly where high rates of metamitron were combined with adjuvants with reputations for phytotoxicity risk such as silicone surfactants and heavyweight dormant oil. Figure 1 depicts examples of mild, moderate, and severe leaf damage from metamitron. As expected, even the most severely damaged trees quickly recovered and grew out of their "shocked" conditions to the point that it was difficult to identify those trees which had experienced phytotoxicity a few weeks after application. Further, the only effect on fruit finish in any thinning trial we were able to document was a tendency to reduce russet incidence by several thinning programs on Jonagold (Table 5).

Table 5. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2015.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	%	g		%
Fuji / M.9 337 – Royal Slope						
Carbaryl 4L + Fruitone L	99 ab	33 ns	43 ns	270 ns	67	66 b
Exilis Plus + Fruitone L	115 a	29	42	264	69	88 a
MaxCel + Fruitone L	98 ab	35	41	272	67	75 ab
Metamitron 400 ppm	104 ab	33	39	273	67	78 ab
Metamitron 400 ppm + Fruitone L	89 ab	37	43	278	65	89 a
Metamitron 800 ppm	81 b	42	40	276	66	89 a

Control	96 ab	38	36	259	70	89 a
Honeycrisp / M.9 337 Bench grafts - George						
Carbaryl 4L + Fruitone L	118 abc	38 ab	24 ns	249 abc	73	0 ns
Exilis Plus + Fruitone L	101 bc	47 a	22	237 abc	77	1
MaxCel + Fruitone L	120 abc	35 ab	27	235 bcd	77	0
Metamitron 400 ppm	111 abc	41 ab	24	255 ab	71	4
Metamitron 400 ppm + Fruitone L	101 bc	41 ab	29	247 abc	74	0
Metamitron 800 ppm	88 c	47 a	26	261 a	70	0
Control	143 a	34 ab	20	228 cd	80	1
Granny Smith 9 A / M.9 337 – Rock Island						
MaxCel + Fruitone L	30 cde	72 bcd	26 bcde	274 a	75	94 ns
Metamitron 400 ppm	45 ab	56 e	40 a	180 de	101	94
Metamitron 400 ppm + IAP oil	17 e	83 ab	17 e	216 abcd	84	86
Metamitron 400 ppm + Regulaid	36 bc	67 de	31 abc	204 abcde	89	93
Metamitron 400 ppm + Sylgard	19 e	81 ab	19 de	238 ab	76	89
Metamitron 400 ppm + WES oil	21 de	79 ab	20 cde	229 ab	79	94
Metamitron 800 ppm	36 bc	68 cde	30 abcd	187 cde	97	93
Metamitron 800 ppm + IAP oil	18 e	82 ab	18 e	234 ab	78	90
Metamitron 800 ppm + Regulaid	35 bcd	66 de	33 ab	198 bcde	92	89
Metamitron 800 ppm + Sylgard	16 e	84 a	15 e	234 ab	78	84
Metamitron 800 ppm + WES oil	24 cde	77 abc	22 bcde	223 abc	81	95
Control	50 a	55 e	41 a	175 e	104	89
Jonagold / M.26 – Rock Island						
Carbaryl 4L + Fruitone L	50 bc	65 abcd	24 b	296 a	61	53 ab
Exilis Plus + Fruitone L	77 ab	40 e	46 a	272 ab	67	61 a
Metamitron 400 ppm	66 abc	46 de	45 a	243 b	75	36 ab
Metamitron 400 ppm + Carbaryl 4L	29 c	76 a	20 b	271 ab	67	49 ab
Metamitron 400 ppm + Exilis Plus	56 abc	56 bcde	34 ab	259 b	70	44 ab
Metamitron 400 ppm + Fruitone L	41 bc	67 ab	26 b	253 b	72	44 ab
Metamitron 800 ppm	48 bc	68 abc	21 b	246 b	74	45 ab

Control	88 a	50 cde	27 b	247 b	74	28 b

Figure 1. Mild (L), moderate (C), and severe (R) leaf damage caused by metamitron applications. WTFRC 2015.

Our most effective postbloom thinning programs (Table 6) continue to feature BA, especially in combination with NAA, which is good news in light of the potential loss of registration for carbaryl as a postbloom chemical thinner. Although we are still learning how metamitron might be used most effectively, its trial performance thus far easily competes with established industry standard thinning programs.

Table 6. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2015.

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	17 / 63 (27%)	11 / 63 (17%)	6 / 57 (11%)
BA + NAA	15 / 36 (42%)	7 / 35 (20%)	4 / 27 (15%)
Metamitron	5 / 13	2 / 12	1 / 8

[1]Does not include data from 2015 trials.

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

2015 PLANT GROWTH REGULATOR PROGRAM HIGHLIGHTS:

Gibberellic acid (GA) for biennial bearing: 2015 results were consistent with prior findings in that we demonstrated significant bloom reduction from 2-4 applications weekly of 100-200 ppm GA_3 applied starting at 10mm fruitlet size (data not shown). Over the course of several years of study, these programs have been effective in roughly half of all trials, a rate of success which surpasses that of many other standard industry PGR programs that have been evaluated by our program. Other experimental formulations of GA were also effective in 2014-2015 trials and we continue to lobby PGR manufacturers to pursue necessary registrations or label amendments to accommodate this use pattern for apple growers seeking to promote annual cropping.

Naphthaleneacetic acid (NAA) or ethephon for bloom promotion: Despite years of trials evaluating a variety of formulations, application rates, and timings, we have never observed increased return bloom on any cultivar from spring/summer NAA programs. These results were corroborated by a 2014 trial evaluating new formulations of NAA in a biennial Fuji block: no NAA or ethephon treatments increased 2015 return bloom relative to the untreated control (data not shown).

Prohexadione calcium to inhibit shoot extension: A 2015 trial on vigorous Fuji trees demonstrated clear reductions in shoot extension which persisted throughout the season with spring applications of either Apogee (BASF), Kudos (Fine Americas), or a new experimental formulation of prohexadione calcium. The performance of each of the 3 products was improved with the addition of ammonium sulfate to reduce spray tank pH (data not shown).

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-14-101A

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

Co-PI:	Karen Killinger, Ph.D.	**Co-PI (2):**	Ines Hanrahan, Ph.D.
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Cooperators: This study involves partnerships with the WSU Wenatchee Tree Fruit Research and Extension Center and WSU Prosser Research and Extension Center for field studies, as well as industry partners and input from regulatory personnel. As of August 2015, Ines Hanrahan assumed PI status, since Dr. Killinger left WSU to take a position with FDA. Dr. Killinger remains as a co-PI on the project. The assistance of Tonia Green, Lauren Walter, Kyu Ho Jeong, Andy Liao and Manoella Mendoza are acknowledged and greatly appreciated. Special thanks to Dr. Meijun Zhu (WSU) for help with oversight of WSU based budgets upon Dr. Killingers departure.

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

Other funding sources

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

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

WTFRC Collaborative expenses:

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

Footnotes: Wages and benefits for assistance from WTFRC staff.

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

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

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

OBJECTIVES

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

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

SIGNIFICANT FINDINGS

- Preliminary, experimental data 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 two to three days after inoculation when applied within one week of commercial harvest.
- Treatment with overhead, evaporative cooling did not appear to enhance survival of generic *E. coli* on apples compared to the response on control apples that did not receive overhead cooling application.
- Field inoculation methods and staff training programs generated a consistent method for application of generic *E. coli* surrogates in an orchard setting.
- Evaluating weather conditions will be an important. Preliminary review of the data related to weather conditions indicated that maximum air temperature, solar radiation, relative humidity and leaf wetness warrant further analysis.
- Year 2 data further confirmed that canopy location is an important consideration in study design and analysis.
- At 2 hours post-inoculation, microbial levels varied dramatically. In one experiment, Golden Delicious apples had a slight microbial increase within 2 hours of inoculation.
- Generally, there is a rapid microbial decline within the first 48-72 hours after inoculation, followed by a much slower rate of microbial reduction.
- In an experiment that examined microbial reduction over 13 days, approximately 18-45% of sampled apples had detectable levels of generic *E. coli* present.

METHODS

Year 2.Objective 1. Method development.

Based on results from year one and input of experts, some methods were adjusted in 2015. Modifications included the following:

- Prior to inoculation, trees were carefully selected for moderate crop load, all damaged fruit was removed prior to inoculation due to crop load in Year 2. In 2014, variability among two canopy locations (high and low) was observed. In order to understand this variability better, we assessed apples from three canopy positions in 2015 for all experiments. These modifications were possible due to greater fruit availability in 2015.

- Further emphasis was given to crew training and quality control, such as careful selection of individuals tasked with inoculation (for even inoculation), repeated trouble shooting in the field, constant fed back loop between lab and field crew, detailed experimental flow plan to optimize sampling success at each time point.
- Increase of individual apple samples to 48-80 apples per time point.
- Addition of a two hour time point to study initial die-off rates in the field (upon complete drying).
- Notes on weather related variables during and following inoculation by field staff. WSU Ag WeatherNet data will be included in final data analysis.

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

- Orchards in two regions were used in year two of this study. Near Wenatchee, untreated and EC treated Golden Delicious, Gala and Fuji were examined with inoculation near harvest (short-term) and earlier in the season Golden Delicious and Gala (long-term). Near Prosser, untreated, EC and misted Fuji's were examined, short-term inoculation only.
- Tree selection was based on the amount of fruit distributed within each canopy location (high, inside, low).
- Each treatment (control, EC and mist) was divided in two replications for each individual field inoculation session.
- Surrogate, non-pathogenic *E. coli* strains were combined, transported and prepared for inoculation after sunset (highest risk for bacterial attachment).
- 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.
- Time points reflected industry harvesting practices (start of picking at dawn).

Study Design

- Antibiotic resistant *E.coli* was prepared in backpack sprayers.
- Inoculum was sprayed onto selected apple trees with apples at variable branch locations.
- Apple harvest time points were selected to reflect industry harvesting practices.
- After inoculation, apples were aseptically harvested at varying time points.
- After transport to the lab on ice, bacteria were removed by rubbing and shaking the apple vigorously in a peptone buffer for 2 ½ minutes.
- Apple rinsate was diluted and plated on appropriate media.
- Plates were grown overnight at 35°c for 24 hours.
- Colony counts were recorded and converted to $\log_{10}$ numbers for data analysis.

Year3. Objective 2.

Experiments for both locations (Prosser and Wenatchee) will be continued; multi-year examination is important to capture seasonal differences and potential influence on microbial response. Special emphasis will be given to: examination of microbial die-off near harvest, fruit location within canopy, examination of microbial die-off near harvest die-off under extreme weather conditions (i.e. rain or cloudy days). Due to industry concerns, Granny Smith will be added to the variety cocktail (Gala, Fuji, Golden Delicious continued). Additionally, we will explore the possibility of storing some samples to develop additional data on further die-off in storage.

RESULTS AND DISCUSSION

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

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

Only 5 apples examined prior to inoculation had detectable levels of generic *E. coli* and 11 had detectable levels of total coliforms.

In 2015, the study design from 2014 was repeated in the Wenatchee orchard, where short-term inoculation near harvest was performed on Gala and Golden Delicious varieties. The examination of canopy location was expanded from 2014, and another variety, Fuji, was added (data not shown). Furthermore, a second orchard location near Prosser, was added for the short-term inoculation study near harvest, and in this orchard it was possible to include untreated, EC and misted treatments. Only Fuji variety apples were available in the orchard near Prosser.

Gala, Golden Delicious, and Fuji (1 week pre-harvest die-off): For Golden Delicious at 10 hours, untreated with overhead, evaporative cooling, average reduction in generic *E. coli* was 2.0 log and for treated Golden Delicious over 3.0 log. (Figure 1). For Gala at 10 hours, untreated with overhead, evaporative cooling, average reduction in generic *E. coli* was over 2.5 log and for treated Gala over 3.5 log (Figure 1). For Fuji at 10 hours, average reduction in generic *E. coli* for untreated was almost 2 log and for misted and EC almost 3 log (Figure 2). For treated Gala and Fuji, generic *E. coli* were reduced at rates greater than or equivalent to the 0.5 log per day reduction proposed by FDA for two days, and for treated Golden Delicious three days. For untreated Golden Delicious and Fuji, die-off rates appeared to be slower than for treated fruit for up to 3 days.

In 2015, an examination of inoculation of immature fruit was also performed at the Wenatchee orchard for Gala and Golden Delicious varieties. Gala untreated apples averaged overall had over a 3 log reduction of generic *E. coli* occur during the first 8 hours post-inoculation (Figure 3). The reduction rate for generic *E. coli* varied dramatically between individual apples within the same variety, as seen in Figure 3. Although initial reduction rates were greatest in the first 8 hours post-inoculation, apple varieties had different reduction rates that can vary over time (Figure 4); differences in weather conditions and fruit developmental stage may have influenced these results. Canopy location of apples appeared to influence reduction of *E. coli*, especially after 36 hours, suggesting that UV exposure effects microbial survival (Figure 5).

Figure 1. Overall average of generic *E. coli* levels after inoculation near harvest (short-term) on Gala and Golden Delicious apples with (EC: treated) and without (UC: untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Values reported in $\log_{10}$

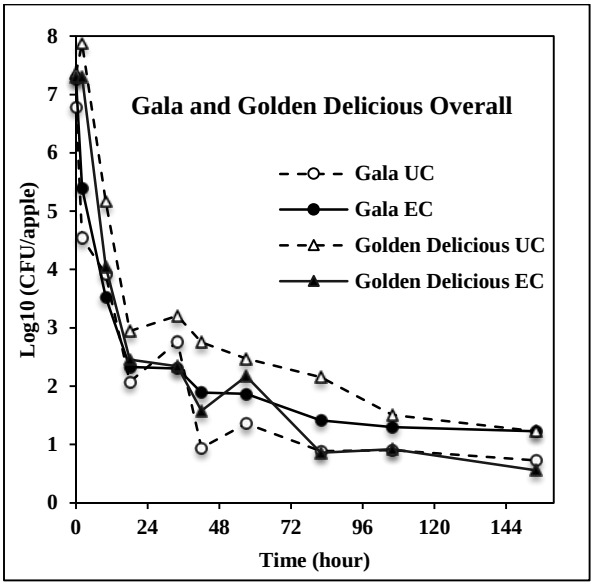


Figure 2. Overall average of generic *E. coli* levels after inoculation near harvest (short-term) on Fuji apples with (EC: treated), without (UC: untreated) and Mist overhead evaporative cooling water from an open surface water source near Prosser, WA. Values reported in $\log_{10}$ colony forming units.

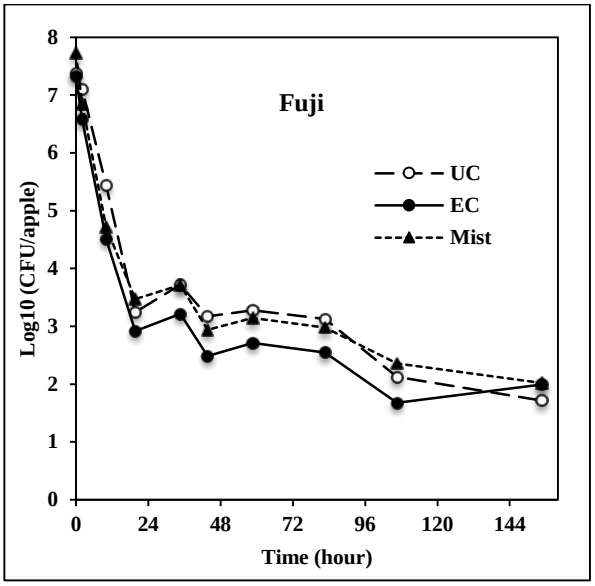


Figure 3. Average and individual generic *E. coli* levels after inoculation on immature fruit (long-term) on Gala apples without (UC: untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Values reported in $\log_{10}$ colony forming units (cfu)/apple (5=100,000 cfu/apple, 3=1,000cfu/apple).

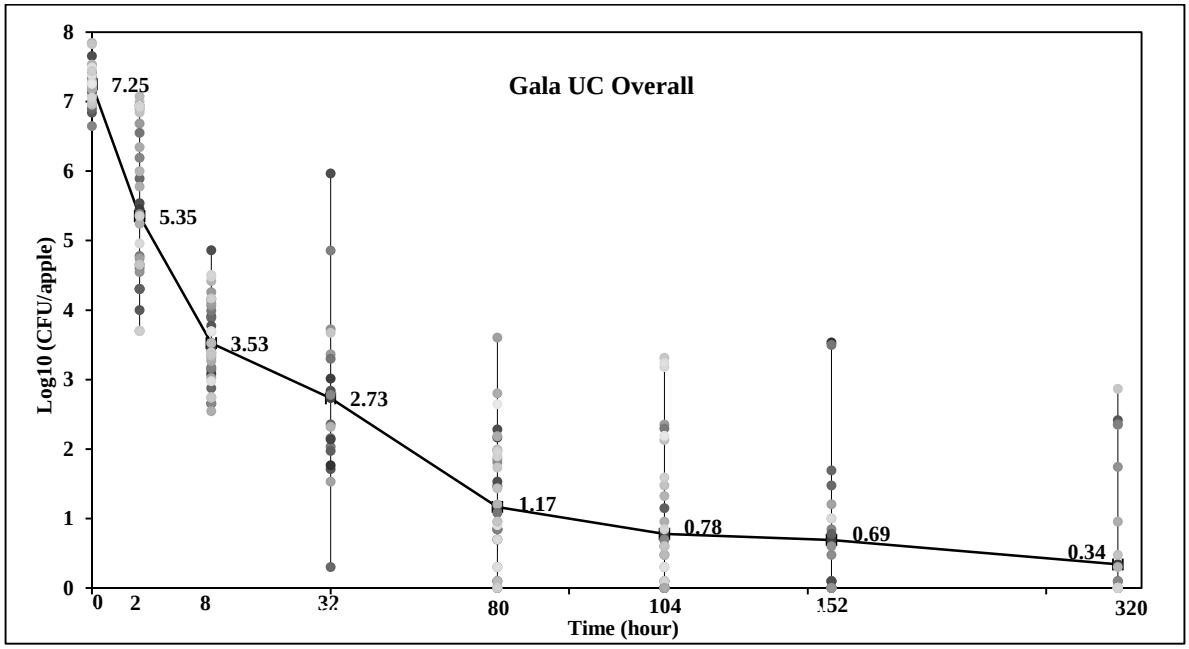


Figure 4. Overall average of generic *E. coli* levels after inoculation on immature fruit (long-term) on Gala and Golden Delicious apples with (EC: treated) and without (UC: untreated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Values reported in $\log_{10}$ colony forming units.

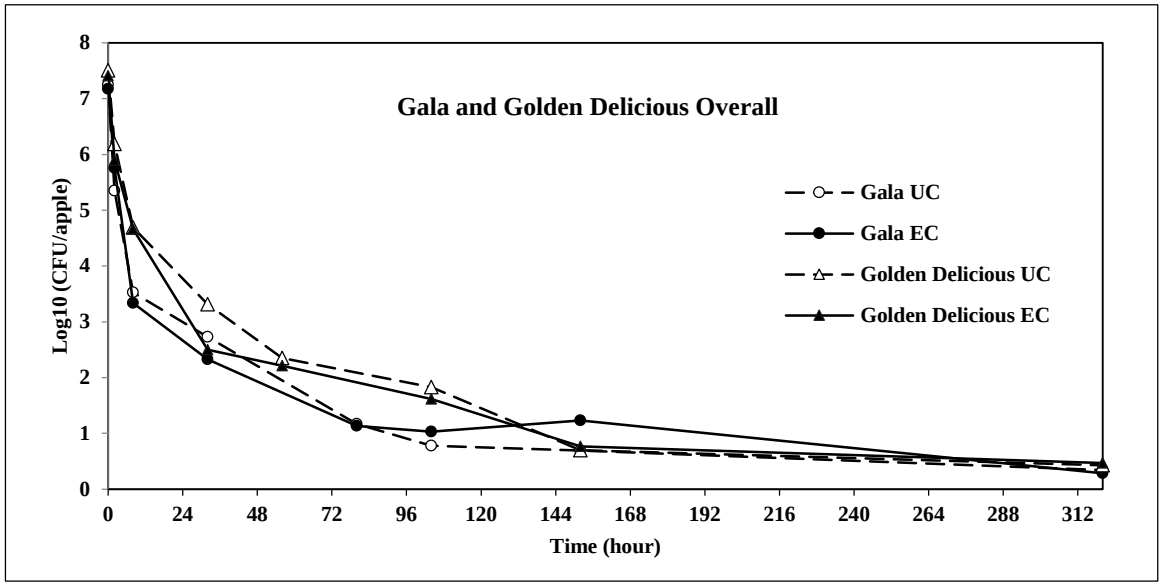
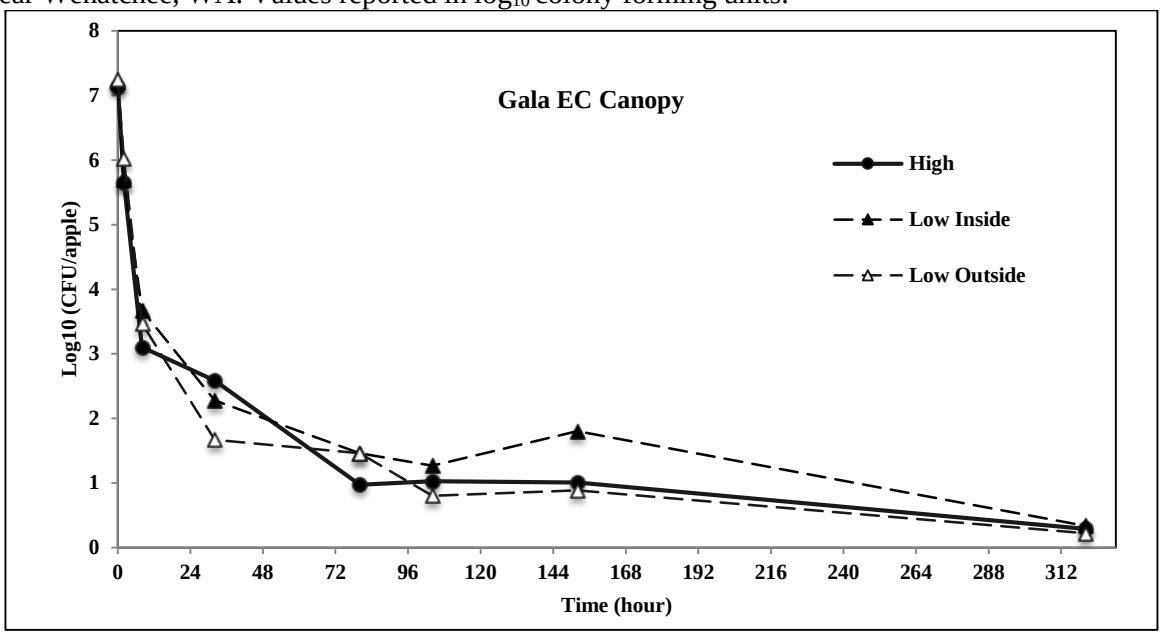


Figure 5. Overall average of generic *E. coli* levels after inoculation on immature fruit (long-term) on Gala apples with (EC: treated) overhead evaporative cooling water from an open surface water source near Wenatchee, WA. Values reported in $\log_{10}$ colony forming units.



CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-15-101

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

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Cooperators: Jim Mattheis, USDA-ARS; Stemilt Growers LLC, Oneonta StarrRanch Growers; Borton Fruits; CPC Fruit Company, MacDougall and Sons, Washington Fruit, Allan Godwin, Ray Fuller, CRO Orchards

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

Other funding sources: None

WTFRC Collaborative Expenses: 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 Corina Serban (Kalcsits) and 8.25% Research Associates (Sankaran and Khot).
[2]Wages for time slip summer wages for Corina Serban 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.

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

Objective 1

Fruit calcium is not necessarily linked to environment although it may increase the risk. Other factors seem to contribute more. These will be explored in detail in year 2 with detailed soil sampling, root measurements, and management assessments.

Nutrition and quality predictably varies within the tree, even in small, high-density plantings.

Early season estimates of fruit calcium content are correlated with fruit calcium at harvest on a tree level and field level.

Objective 2

Increasing calcium applications did not consistently increase fruit calcium in an orchard where fruit calcium levels were already high.

ABA can reduce transpiration in apples for extended periods but did not result in increased calcium in the fruit in this low vigor orchard.

These experiments need to be repeated in an orchard with more vigor.

Objective 3

Calcium isotope was applied at 7 points every two weeks during the season from June-August. This should identify times during the season when Ca absorption potential is greater. The samples are in the final stages for analysis.

METHODS

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

To study the environmental effect on tree physiology and resulting bitter pit development, we identified nine grower sites of equal age and similar management at a range in elevations (405 to 1857 feet above sea level across nine different sites). We measured soil and air temperature in each orchard. Sample trees were selected for uniform size and crop load thinned to three different crop loads (3, 5, 7 fruit cm^{-2}). Nine trees (three trees for each crop load) were harvested at each site and representative subsamples were taken from each tree for quality and nutritional analysis. To study bitter pit development in fruit from the above treatment blocks, samples (at harvest and postharvest) were analyzed using computed tomography (CT)-imaging, portable x-ray fluorescence, and Fourier-transform infrared (FTIR) spectroscopy techniques.

CT-imaging and FTIR spectroscopy will be used to analyze fruit for susceptibility to bitter pit. The pits formed at any locations within the fruit can be identified with the CT-based imaging technique (Figure 1). In addition, both traditional (ICP-MS) and non-destructive elemental analysis (X-ray

Fluorometer) was used to measure calcium, magnesium, potassium, and nitrogen concentrations in fruit and leaves.

Outcome/deliverables: Determine how Honeycrisp grown in different environments varies in nutritional balance and quality.

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

Secondly, we conducted a foliar calcium application trial near Quincy, WA with four different frequencies of application (monthly, biweekly, weekly and twice per week). There were 15 sampling trees selected from each test site (x 4 calcium treatments = 60 trees) that were selected for similar caliper. These sample trees were then hand thinned to a uniform crop load in June. At harvest, fruit subsamples (20 fruit) were taken from each tree. In the lab, quality analysis was done at harvest and then after 4 months of storage in addition to elemental analysis.

To evaluate the relationships between transpiration, fruit calcium and bitter pit, an experiment will be conducted to look at how fruit calcium is affected by transpiration. In 2015 and 2016, 10 trees will be selected within the calcium frequency trial (40 trees total). Half of the selected trees were sprayed monthly (June, July, August) with ABA to reduce transpiration. The other half will not be treated with ABA. Vegetative growth, fruit development and physiological status will be monitored throughout the growing season. Additionally, proximal and remote sensing based thermal images (Figure 3) were acquired using a high-resolution thermal infrared cameras to monitor differences in the transpiration rates between treatments. Ground-based spectral data was collected from the four calcium treatments (once a month, twice a month, once a week, twice a week). Each treatment was applied to about three rows of apples (aligned north-south). The visible-near infrared spectra (350-2,500 nm) was collected from the middle row of the treatments (Fig. 2b). The row from which data was collected comprised about 15 marked trees (5 trees treated with ABA to control transpiration, 5 control trees, and 5 trees marked to collect yield data). Four replicate spectra from multiple leaves were collected representing both east and west direction. Similarly, high-resolution thermal images were captured from the marked trees (two replicates, one in each direction). At harvest, leaves were sampled from each tree for carbon isotope analysis and elemental analysis. In addition, fruit was subsampled from each tree to measure elemental concentration and the frequency of bitter pit apples (visual symptoms).

Outcomes/Deliverables: Evaluate whether decreasing transpiration can increase fruit calcium and decrease the incidence of bitter pit.

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

Using a stable, calcium isotope tracer, the optimum timing of foliar calcium application was tested. At given intervals during the growing season, an isotopically labelled foliar calcium chloride treatment was applied to determine the optimum timing of calcium chloride applications that allow for the greatest fruit absorption of calcium spray. Three trees with similar crop loads and caliper were sprayed with labelled solution at two week intervals starting after June drop and continuing to harvest (7 total application treatments). Fruit volume and surface area measurements was estimated using caliper measurements made prior to each application. To measure absorption of applied calcium into fruit, the amount of calcium isotope tracer absorbed by the skin and cortex was measured. Comparisons will be made among timing of applications to determine the point in time when fruit calcium absorption from foliar sprays is the greatest.

Outcome/Deliverables: More informed guidelines on the timing of foliar calcium sprays in commercial apple orchards.

RESULTS & DISCUSSION

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

There were large differences in environment among the nine difference sites with a difference in more than 800 growing degree day (GDD) accumulated in the warmest site compared to the coolest site from May15, 2015 to September 1, 2015. Although there were large differences in environment and fruit elemental concentration between sites, there were no consistent correlations between bitterpit development, nutrition and environment. With the exception of one outlier site, there was a significant correlation between fruit calcium content in June and at harvest (Figure 1). The outlier site showed strong increases in calcium after the initial measurement. By identifying sample fruit early and measuring their location in the tree, calcium content is greater in the top of the tree compared to lower in the canopy. As expected, calcium and potassium content was significantly affected by crop load (Figure 3) indicating an optimum crop load for nutritional balance of around 7 fruit cm^{-2}. We will have lab analysis to support all of the non-destructive measurements made using the handheld x-ray fluorometer.

40 samples from 9 locations were scanned using X-ray Computer Tomography (CT) imaging system. Currently, we have developed two Matlab algorithms to process the CT images as a part of our ongoing WSDA project: 'Rapid detection technologies for in-field and post-harvest apple bitter pit management'. We will be utilizing these algorithms for analyzing the CT images from the 9 locations. In addition, these 40 samples are being processed to collect the Fourier Transform Infrared (FTIR) (7,800-350 cm^{-1}) spectra from flesh and skin (3 replicates each) to capture differences in calcium and magnesium peaks. We are currently developing a multivariate algorithm for pattern recognition from the FTIR spectra, which will also be used for FTIR spectral analysis. We anticipate to complete the FTIR sample analysis by end of December, 2015.

Table 1. Site characteristics for each of nine Honeycrisp orchards. * = 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	M26	69.11	68.2	2081

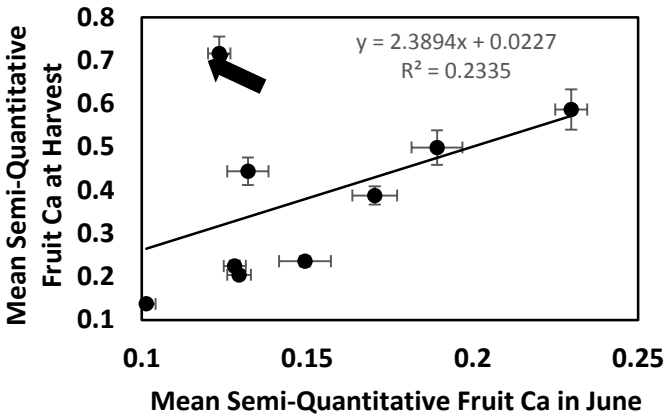


Figure 1. Mean semi-quantitative PXRF calcium counts for nine sites (72 apples for each site). Error bars represent standard error and the arrow represents an outlier site with strong positive increases in fruit calcium from June measurements to harvest.

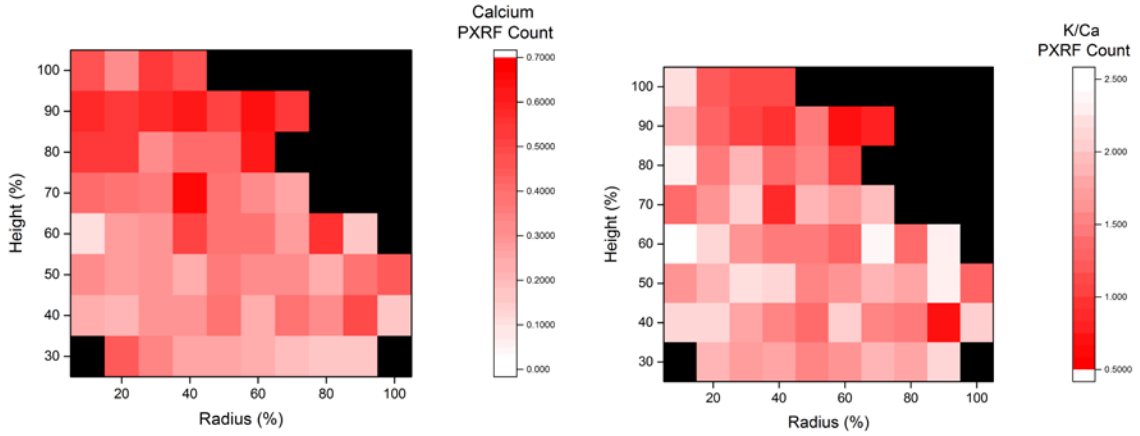


Figure 2. Calcium and Potassium: Calcium Ratio distribution in 'Honeycrisp' grown in 9 different locations across Washington State. Height (%) represents the relative height in the tree (100 % is the top of the tree) and radius (%) represents the distance from the trunk (0% is the trunk and 100% is the tip of the branch)

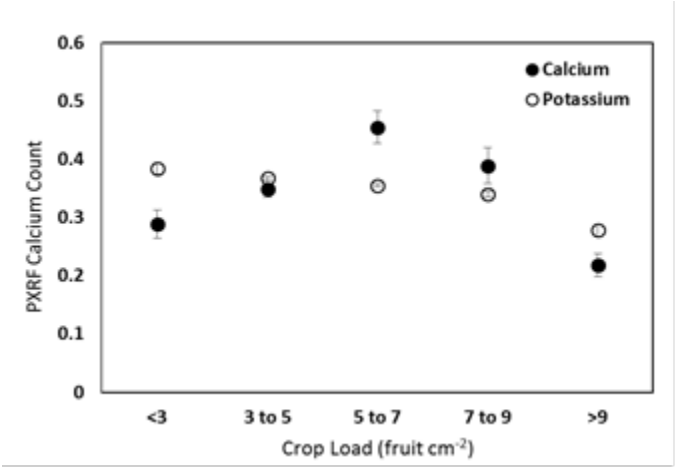


Figure 3. Semi-quantitative PXRF count for potassium and calcium with increasing crop loads measured at 9 different sites

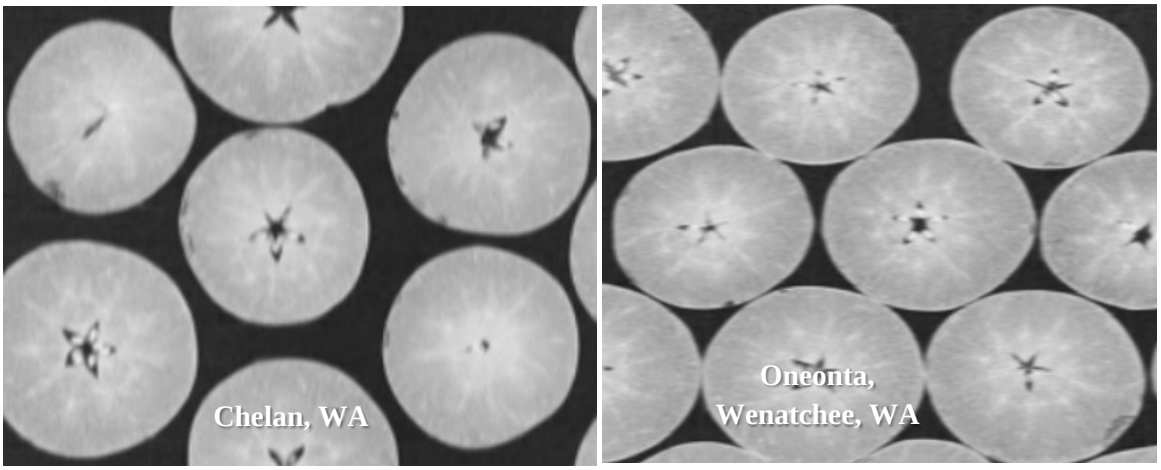


Figure 4. Sample single-layer CT images from two of nine locations showing bitter pit on the sub-surface/surface of the apples.

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

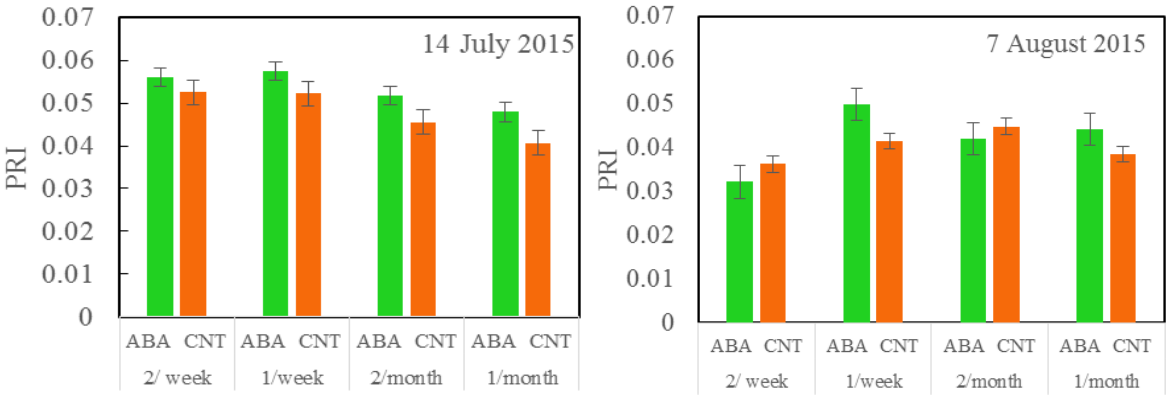
There were significant differences between treatments in the calcium frequency trial (Table 2). The differences were not consistent with differences in frequency of calcium application. However, this orchard, in general, had exceptionally high fruit calcium compared to orchards samples in Objective 1. This orchard had low vegetative vigor and was thinned to optimum crop loads (7 fruit cm^{-2}) in June and may explain the high fruit calcium content that was observed. At two months of storage, only 1.2% of total fruit has developed symptoms of bitterpit which could be predicted by the high fruit calcium and low K:Ca ratios observed in all of the fruit.

Ground and aerial based multi-spectral images were collected from a Honeycrisp Apple orchard in Quincy (Figure 5a) on 11 June 2015 (pre-treatment), 14 July 2015 (mid-season), and 7 August 2015 (late-season). The trees were harvested on 24 August, 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 some treatment effects. 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, and PRI values also increased with increase in calcium applications. However, this treatment effect on PRI values diminished later in the season as the leaves aged.

Table 2. Semi-quantitative fruit calcium and potassium measurements for different frequencies of calcium spray applications (±Standard Error N=15 trees)

Calcium Spray Frequency (/month)	Calcium	Potassium	Potassium:Calcium Ratio
1	0.58 ±0.021	0.33 ±0.004	0.8 ±0.028
2	0.93 ±0.021	0.36 ±0.004	0.43 ±0.012
4	0.60 ±0.021	0.33 ±0.004	0.74 ±0.022
8	0.76 ±0.019	0.34 ±0.003	0.67 ±0.026

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



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

Figure 5. Ground and aerial-based sensing for changes in leaf physiological responses to ABA treatments.

A new site will be chosen with a known history of bitterpit and low fruit calcium levels. In 2016, remote sensing data with ground-reference data (transpiration and fluorescence), we will be further able to evaluate the applicability of the technology in assessing the treatment effects. By using a site with more vegetative vigor and lower historical fruit calcium, the effect of differences in the frequency of calcium applications should be more apparent.

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

Figure 6. ^{44}Ca Application to apple fruit at bi-weekly intervals

For this objective, we are in the final processes of sending the samples for analysis. We expect to have the results in January or February and can share that data at that time. This objective will be repeated in 2016.

Research Outputs:

Jarolmasjed, S., Zúñiga Espinoza C., Sankaran, Kalcsits, L.A., Khot, L.R. 2015. Assessment of high throughput sensing techniques for pre- and postharvest apple bitter pit detection. 111th Annual Meeting of the WSTFA, December 7-9, 2015, Yakima, WA.

Jarolmasjed, S., Zúñiga Espinoza, C., Sankaran, S., Kalcsits, L.A., Khot, L.R. 2015. Sensing technologies in apple bitter pit management. CPAAS Open House, September 17, 2015, Prosser, WA (Poster).

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-15-104A

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

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Cooperators: McDougall & Sons, Inc.

Collaborators: Additional Co-PI: Sara Serra (WSU TFREC)

Total Project Request: Year 1: $84,102 **Year 2**: **$87,713**

Other funding sources: None

WTFRC Collaborative expenses:

Item	2015	2016
Wages and benefits[1]	8000	8000
Salaries and benefits[2]	5000	5000
Supplies	500	2500
Travel	500	500
Total	14,000	**16,000**

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

Budget: Kalcsits, Musacchi, Layne
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	4,000
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
[3]Frequent travel to orchard site (Quincy) where trials are being conducted.
[4]Goods and services include in-orchard temperature/humidity dataloggers, WSU TFREC fees for soil, leaf and fruit mineral nutrient analyses.

OBJECTIVES

1. In 2015, 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 microenvironment, photosynthesis, vegetative growth and tree stress.

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

SIGNIFICANT FINDINGS

- Photo-selective netting alters the light environment under the netting including the spectra of the incoming and scattered light.
- Netting strongly reduces wind, light energy and shows small reductions in temperature in the tree canopy.
- Absorptive surfaces (fruit, leaves, soil) are more impacted by netting than the air under the canopy.
- Netting increases fruit size and affects fruit quality. Netting strongly reduces sunburn compared to an uncovered control. However, more research is needed to discern the differences in fruit quality between the color treatments.

METHODS

Experimental Sites

Site 1: McDougall & Sons, Inc., Quincy, WA.

3rd leaf "Cameron Select Honeycrisp" on Bud-9 rootstock; trees trained on 4-wire V-trellis and spaced 2' x 12' (1815 trees/acre). The trees were planted in winter 2013. Trees were de-fruited during second leaf (2014) and there were non-fruited and fruited blocks of trees in each treatment in 2015. In 2014, the infrastructure (poles and cable) for 3 acres each of 22% shade pearl, blue and red netting was installed (GreenTek). The netting was first deployed in the spring of 2015. Trees were initially thinned heavily May 14th then thinned again at the end of June to a crop load of 4 fruit cm^{-2} so as not to limit growth of the tree. Fruit was harvested on August 26th, 2015 at full maturity in a single pick and all fruit was collected from the sample trees.

Site 2: WSU TFREC, Wenatchee, WA.

A shadehouse structure was built that has an uncovered control and pearl, blue and red netting (GreenTek). 2nd leaf 'Honeycrisp' on M-9 337 rootstock were planted and grown outside in 11 gal pots in 2014. Trees are spaced at 2' x 5' and each treatment has 4 rows of 11 trees (44 trees). The trees were established in 2015 and will bear fruit in 2016.

Objective 1 (Serra - Borghi - Musacchi): Determine characteristics of three net colors on light spectrum and their effects on the light quality and quantity of incoming radiation throughout the day (*2015*)

Canopy light interception measurements in the field

At the commercial orchard site (McDougall & Sons), canopy light interception was measured in July at midday (starting from 2 hours before solar noon) using a 1 m long light bar (Q2850 Licor) and a Li-1500 data logger to record data. Four "fruiting" blocks and four "non-fruiting" (all fruits have been

removed from the tree) have been selected for each colored net and the control (uncovered). Three adjacent trees (central tree facing west) trained at V system were measured for light in each block (fruiting/non-fruiting). For a reference light level, a PAR quantum measurement with the Q53292 quantum PAR sensor (Licor) was done to calculate the percentage of light intercepted by the canopy.

Light spectra under the nets
The 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). The open field readings were used as reference for the transmittance measurement. The entire data collection was performed orienting the surface of the detector perpendicular to the sun beams as reported in literature (Shahak and Gussakovsky, 2004, Kong et al., 2013). Along the north-south axis of each net dome, in two distinct positions (named north and south), the instrument was placed and pointed to the sun as previously described. Three transmittance measures were recorded in each spot. Transmittance of total light (%) for each colored net was expressed as total light under each net divided by the total light outside the net.

<u>Objective 2</u> (Kalcsits-Layne): Quantify the impact of nets on orchard microenvironment, photosynthesis, vegetative growth and tree stress.

Orchard environment
In May 2015 environmental sensors were installed under each netting treatment that monitor light intensity, wind speed, temperature and humidity above the trees and then replicated sensors were installed to measure in-canopy temperature and humidity and soil moisture and soil temperature throughout the growing season. To limit the risk of data loss or equipment failure, these sensors were monitored remotely for problems during the season.

Photosynthesis, tree stress and shoot growth
Photosynthetic rates were measured for trees in the shadehouses at WSU TFREC using a Li-Cor 6400XT gas exchange system in July and August. Leaves that were measured were fully sun exposed, mature and at the top of the tree to reduce variability. Sampling was performed on sunny days with no cloud cover and treatment sampling was randomized to limit bias in the timing of sampling. Leaf samples from both Quincy and WSU TFREC were collected and analyzed for carbon isotope ratios (an indication of tree stress and stomatal closure).

<u>Objective 3</u> (Kalcsits-Musacchi-Schmidt): Evaluate fruit and leaf nutritional balance and fruit quality under different light conditions.

Fruit quality
At harvest and after four months of storage fruit quality assessments were completed. Fruit was assessed for color and background color using a 1-4 scale established by the WTFRC. Then individual fruit were measured for dimensions and weight. Starch, brix and firmness were all measured on individual fruit. Fruit was also measured for sunburn using the WTFRC Honeycrisp Sunburn Scale. Then fruit was juiced and pooled per tree and analyzed for pH and titratable acidity.

Fruit (post storage) and leaf nutritional balance
Fruit will be measured with a portable x-ray fluorometer after 4 months storage and then pooled fruit samples (block) will be analyzed for potassium, calcium, magnesium and nitrogen to look for differences in netting on nutrient uptake and distribution. Leaves were collected in August from both Quincy and WSU TFREC and have been analyzed for nitrogen and will be analyzed for calcium, potassium and magnesium content to relate leaf content with fruit content in the different treatments.

RESULTS AND DISCUSSION

Canopy light interception measurements

These measurements have provided information about variation of light interception in an orchard environment under different netting treatments compared to a no-net control.
Trees grown under the three different colored nets intercepted similar percentage of light and was significantly different than the uncovered control (Fig 1A). This measure provides indirect information about the canopy development under different conditions; canopies under colored net developed better (up to +12%) than in standard uncovered condition. Producing fruit during the third year of growth severely effect canopy development in this orchard. Trees with a crop load of around 16 fruit/tree reported a 13% reduction in light interception due to a less developed canopy. There was a significant interaction of color x treatment revealed that control, blue and red nets showed a difference between fruiting and no fruiting treatment, while no difference was found under the white net between the two treatments (Fig. 1-B).

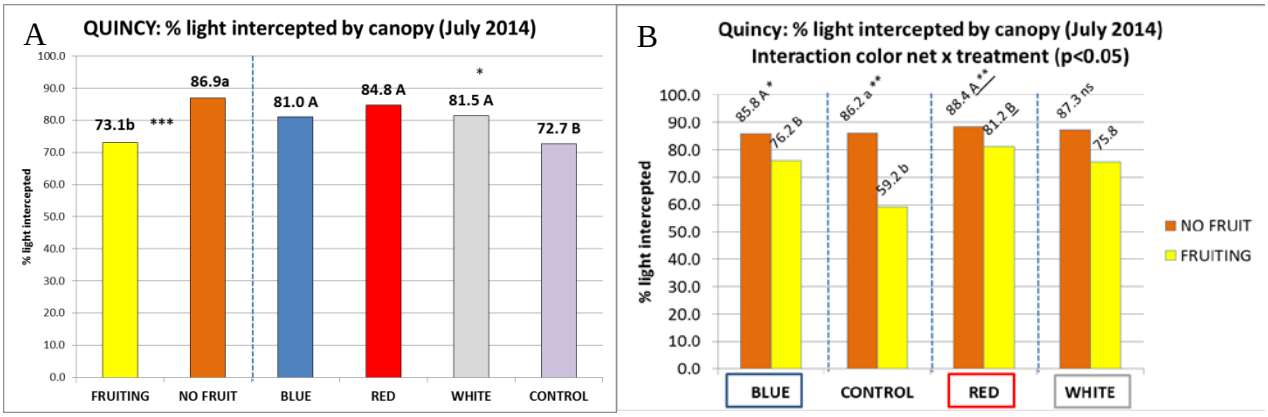
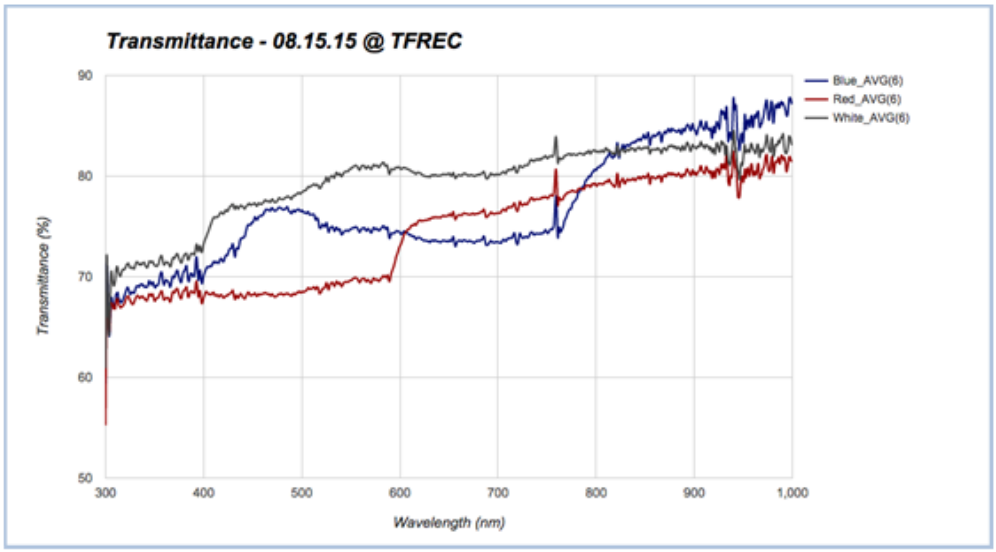


Figure 2: Light interception measures carried out in July 2015 in a commercial orchard in Quincy (site 1). Comparing 3 color photo selective nets against the non-covered control and fruiting block versus no fruiting ones (A). B) shows the interaction of color per treatment within each net and control. Significance: * p<0.05, ** p<0.01, ***p<0.001.

Light spectra under the nets

Measurement of the transmittance of light through the netting was consistent with previously published literature (Shahak and Gussakovsky, 2004). Blue net has a lower shading effect (more light is transmitted) in the range from 400 to 550 nm (PAR range) than the red net, which filters more than 30 % of the light up to 600 nm. Blue net resulted in a transmittance increase in the infrared range which was clearly different from the the red and pearl (white) nets. The white net seems to filter more the lowest wavelengths and shows an almost flat trend from 550 nm to higher wavelengths (Fig. 2 A). Scattered light measured under the netting shows a similar trend to the total light response, confirming the characteristics of the material (Fig. 2 B). In this case, however, the growing trend of the charts significantly increases at higher wavelengths of radiation, bringing the curve above 100%. This increment in the IR and NIR region in particular, is the result, of two phenomena affecting solar light: (i) part of the radiation inside the net is “trapped”, on its way out, reflected back by the net itself (green-house effect) ~~or~~ and (ii), in addition, part of the incoming light is scattered by the net, passing through the threads of the net. These two components especially increase the total amount of scattered light inside the net.



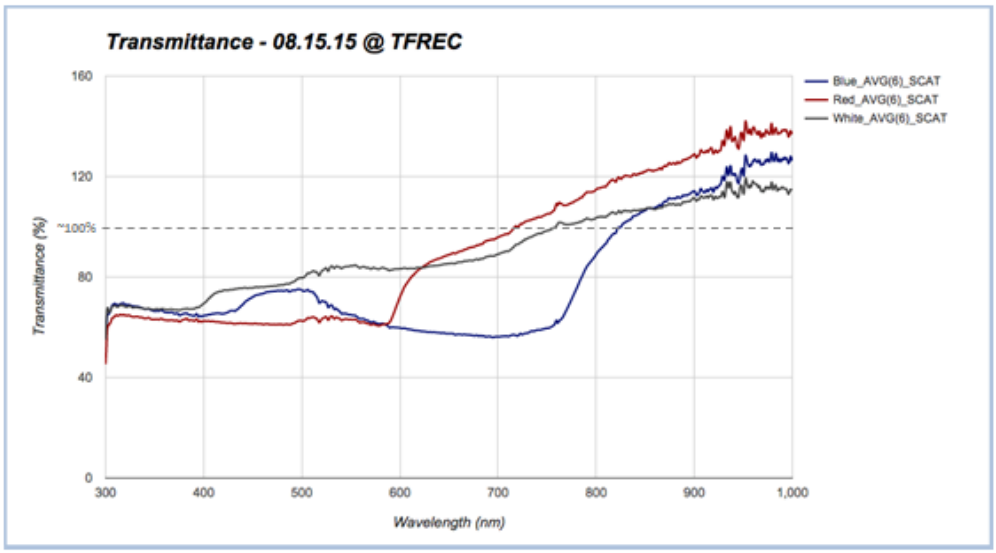


Figure 3: Spectra measurements made with spectroradiometer in TFREC (site 2). (Top) - Transmittance of total light (%) for each colored net is expressed as total light under each net / total light outside the net (open field) x 100. (Bottom) - Transmittance of scattered light (%) is expressed as diffuse light under the net / diffuse light in open field x 100.

Netting alters the orchard microenvironment

Netting reduced total light intensity by approximately 22% irrespective of net color (Table 1). Mean air temperature was higher in the uncovered treatment and there was no significant difference between net colors. Wind speed was 40% lower under the netting than in the uncovered control. Humidity was not different above the trees but was greater within the tree canopy under netting than the uncovered control (Table 1). The warmest maximum temperature and lowest mean minimum temperature was under the red netting where the largest day-night difference occurred (Table 2). Soil temperature was between 1 and 4 °F lower under netting when compared with the uncovered control (data not shown). The difference in soil temperature between red netting and the uncovered control was less than for the other two colors. Soil moisture was approximately 15% higher under netting

compared to the uncovered control indicating increased water use and stress in uncovered trees than trees covered with netting (data not shown).

Table 3. Mean above-canopy temperature, relative humidity, light intensity and wind speed for Blue, Pearl and Red netting compared to an uncovered control (84 days). Letters indicate significant difference determined by Tukey's HSD test.

	Mean Temp. (°F)	Relative Humidity (%)	Light Intensity (μmol m^{-2} s^{-1})	Wind Speed (miles $hour^{-1}$)
Control	**76.8 a**	**38.7 a**	**1804 a**	**8.1 a**
Blue	**76.3 b**	**38.4 a**	**1404 a**	**3.5 b**
Pearl	**76.3 b**	**37.6 a**	**1459 a**	**3.6 b**
Red	**76.1 b**	**37.9 a**	**1355 a**	**3.3 b**

Table 4. In-canopy temperature (°F) and relative humidity (%) for Blue, Pearl and Red netting compared to an uncovered control (84 days). Letters indicate significant difference determined by Tukey's HSD test.

	Mean Daily Temp. (°F)	Maximum Daily Temp. (°F)	Minimum Daily Temp. (°F)	Max-Min Temp. Difference (°F)	Relative Humidity (%)
Control	76.8 a	90.6 a	63.1 a	27.5 a	41.4 a
Blue	76.3 b	90.7 a	62.9 a	27.9 ab	43.4 b
Pearl	75.9 c	89.3 a	62.3 b	27.0 a	43.9 b
Red	76.4 bc	91.1 b	62.5 b	28.7 b	43.9 b

Netting increases photosynthetic rates and shoot growth

Above, we reported decreases in soil temperature and increases in soil moisture under netting. We also observed significantly higher leaf nitrogen content in trees under blue and pearl nets compared to the red and uncovered control. Leaf nitrogen content was 2.35% in trees under blue and pearl netting compared to 2.25% in leaves from trees in the uncovered control. Early spring budbreak meant that spring growth had stopped and bud-set had occurred earlier than normal, occurring just after initial shoot measurements were made immediately after netting deployment in May. Shoot growth was greater under the netting than outside the netting, particularly for lateral shoots. Shoots were 12% longer under netting than the uncovered control. Similar patterns were observed for trees in the shadehouses but for the younger trees in the shadehouses, the difference in shoot growth between the netting treatments and the uncovered control was much greater. Trees under blue netting were the only trees with a second flush of shoot growth in July. Trees grown under pearl and blue netting had more growth than trees under red netting or the control. At all times of day that photosynthetic rates were measured, rates were higher overall in trees under netting compared to trees in the control (Figure 3).

Netting reduces sunburn and increases fruit size but red netting does not affect fruit color

Netting reduced sunburn. 70% of uncovered fruit was classified as free of sunburn or in the Y1 class of the WTFRC Honeycrisp sunburn classification system (Table 3). 90.5, 88.8 and 94% of fruit from trees under the red, pearl and blue netting, respectively, were free of sunburn or in the Y1 class. Fruit

size was larger from trees under the netting even though the crop load was the same. Blue netting appeared to delay maturity and color development compared to the red netting or no netting as shown by reduced red color development and also reduced breaking of the background color compared to the control (Table 4). After storage, a better assessment can be made of the impact of netting on postharvest fruit quality.

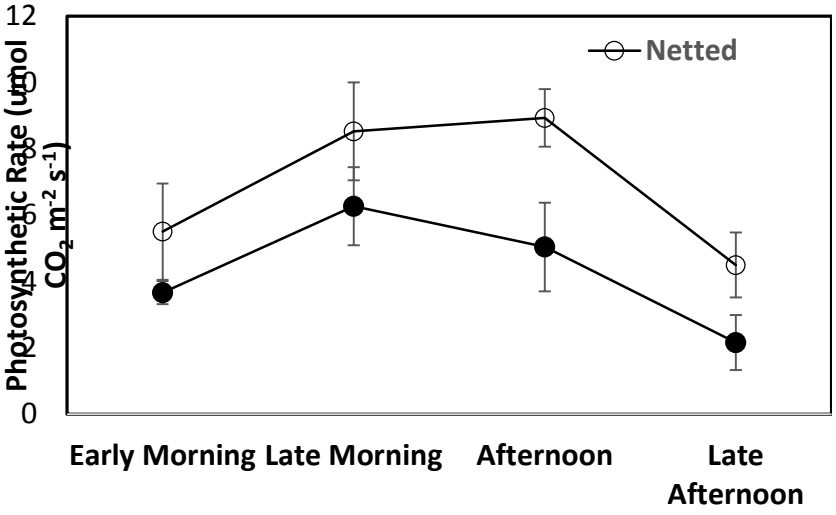


Figure 4 Photosynthetic Rates of 'Honeycrisp' apple measured using a Li-Cor 6400XT at 4 different times of day.

Table 5. Sunburn assessment of harvested 'Honeycrisp' fruit from Quincy trial.
Letters indicate significant difference determined by Tukey's HSD test.

	Y1+Clean	Y2	Y3	Tan	Black
Control	69.9% a	12.5% b	14.7% b	3.2% b	0%*
Red	90.5% b	5.8% a	2.9% a	0.2% a	0%
Pearl	88.8% b	5.5% a	4.6% a	0% a	0%
Blue	94.0% b	3.9% a	1.5% a	0% a	0%

***Much of the black class fruit had fallen before harvest. In 2016, this fruit will be collected and included in the analysis.**

Table 6. Average fruit size, color, background color and starch ratings for harvested fruit with no netting (control) compared to Red, Pearl or Blue netting.

	Size (g)	Color (1-4)	Background Color (1-4)	Starch (1-6)
Control	234 a	2.81 c	1.72 b	4.69 a
Red	261 bc	2.60 bc	1.74 b	5.07 bc
Pearl	279 c	2.53 ab	1.56 ab	4.96 b
Blue	246 ab	2.38 a	1.47 a	5.19 bc

2016 Plans

In 2016, we will continue as planned with the three stated objectives. We will pursue the effects of different light spectra on physiological processes such as photosynthesis and heat stress. We will look at how the changes in soil environment transfer to changes in fruit and leaf nutrition. We will quantify the differences in water-use between the different treatments and continue to provide more information on the physiological benefits of using netting in apple orchards in Washington State.

2015 WTFRC APPLE PESTICIDE RESIDUE STUDY

Visible residues of fruit treated with overhead cooling (L), Raynox (C), and Eclipse (R) at harvest

For the fifth consecutive year, the Washington Tree Fruit Research Commission (WTFRC) conducted a trial to evaluate pesticide residues on 'Gala' apples. Fourteen insecticide/acaricides and nine fungicides were applied using a Rears airblast sprayer according to either an "aggressive" (maximum label rates at minimum retreatment and pre-harvest intervals) or "standard" (typical industry rates and timings) protocol. Products evaluated for the first time in 2015 included Nealta, Exirel, Merivon, Vivando, and Entrust. Plots from both protocols were divided for one of three additional factorial treatments: 1. Overhead cooling 2. Raynox (Pace Intl.), a waxy sunburn protectant or 3. Eclipse (D & M Chem), a calcium carbonate and boron fertilizer with sunburn protective properties. Raynox and Eclipse were applied according to their respective label specifications. One plot also received the aggressive spray protocol with no additional treatment (control). Fruit samples were delivered the day after harvest to Pacific Agricultural Labs (Portland, OR) for chemical analysis.

Measured residues vs. maximum residue levels (MRLs) for uniformly applied STANDARD industry pesticide programs utilizing typical rates, timings, and retreatment intervals on apples with overhead cooling (OHC), Raynox (320 oz/a) or Eclipse (3 gal/a) applied at 35 and 14 days before harvest (dbh). 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2015.

Chemical name	Trade name	Application rate	Application timing(s)	OHC fruit	Raynox treated fruit	Eclipse treated fruit	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Penthiopyrad	Fontelis	20	35	<0.01	0.012	<0.01	0.5	0.4 (many)
Flubendiamide	Tourismo	16	35	<0.02	<0.02	<0.02	1.5	0.8 (many)
Buprofezin	Tourismo	16	35	<0.01	<0.01	<0.01	3	1 (Taiwan)
Spirotetramat	Ultor	14	35	<0.01	<0.01	<0.01	0.7	0.7 (many)
Fluopyram	Luna Sensation	5.5	35	<0.01	0.011	<0.01	0.3	0.3 (CAN,MEX)
Trifloxylstrobin	Luna Sensation	5.5	35	<0.01	<0.01	<0.01	0.5	0.5 (CAN,MEX)
Etoxazole	Zeal	2	35	0.011	0.011	<0.01	0.2	0.07 (many)
Spirodiclofen	Envidor 2SC	18	35	0.017	0.016	0.016	0.8	0.8 (many)
Myclobutanil	Rally 40WSP	10	35	0.013	0.014	0.015	0.5	0.5 (many)
Emamectin benzoate	Proclaim	4.8	35	<0.01	<0.01	<0.01	0.025	0.02 (many)
Metrafenone	Vivando	15.4	35	<0.01	<0.01	<0.01	1.5	0.01 (Taiwan)
Fluxapyroxad	Merivon	5.5	35	0.012	0.017	0.013	0.8	0.8 (CAN,MEX)
Pyraclostrobin	Merivon	5.5	35	<0.01	0.011	<0.01	1.5	0.5 (many)
Cyantraniliprole	Exirel	13.5	35 & 21	0.032	0.030	0.024	1.5	0.8 (many)
Spinosad	Entrust	3	35 & 21	0.019	0.026	0.014	0.2	0.1 (many)
Cyflumetofen	Nealta	13.7	35 & 21	0.015	0.010	0.010	0.3	0.3 (CAN,MEX)
Difenoconazole	Inspire Super	12	28	<0.01	<0.01	<0.01	5	0.01 (India)
Cyprodinil	Inspire Super	12	28	<0.01	<0.01	<0.01	1.7	0.05 (Vietnam)
Flutriafol	Topguard	10	28	0.012	0.011	<0.01	0.4	0.2 (Hong Kong)
Bifenazate	Acramite	16	28	0.015	0.028	0.015	0.7	0.2 (China)
Lambda-cyhalothrin	Warrior II	2.56	28	<0.05	<0.05	<0.05	0.3	0.2 (many)
Hexythiazox	Onager	20	28	0.014	0.016	0.013	0.4	0.4 (many)
Pyridaben	Nexter	6.6	28	0.022	0.023	0.015	0.5	0.5 (many)
Ziram*	Ziram 76DF	96	21	0.130	<0.1	<0.1	7	2.5 (Taiwan)
Fenpropathrin	Danitol	18	14	0.020	0.043	0.030	5	0.5 (Taiwan)
Thiophanate-methyl**	Topsin 4.5FL	16	14	<0.01	0.032	0.015	2	3 (many)

[1] Top markets for WA apples; 17 Sep 2015. http://www.nwhort.org/AppleMRLs.html, https://www.globalmrl.com/

* Dithiocarbamate residues cannot be directly measured; total Ziram values are estimates based on analysis of the degradation product CS_2

** Thiophanate-methyl values reported are sum totals of thiophanate-methyl and carbenzadim residues

Results of this lone unreplicated trial are shared for informational purposes only and should not be construed as endorsements of any product, reflections of their efficacy against sunburn, any insect, acarid, or fungal pest, or a guarantee of similar results regarding residues for any user. Apple growers should consult their university extension staff, crop advisors, and warehouses to develop responsible pest control programs.

TRIAL DETAILS

- 8th leaf 'Pacific' Gala / M.9 Nic.29 trained to central leader/spindle on 3' x 10' spacing
- 2 x 25 gal Rears Pak-Blast sprayer calibrated to 100 gal / acre
- All pesticides applied with 8 oz Regulaid / 100 gal water / acre
- No precipitation recorded on site during trial
- Overhead cooling settings: 15 min. on/15 min. off from noon to 6PM from start of trial (July 16) to harvest (Aug 19) at a rate of 0.11"/hour for an approx. total of 11" of water applied throughout the study

Measured residues vs. maximum residue levels (MRLs) for uniformly applied AGGRESSIVE pesticide programs utilizing maximum rates, and minimum preharvest and retreatment intervals on apples with no additional treatment (Control), overhead cooling (OHC), Raynox (320 oz/a) or Eclipse (3 gal/a) applied at 35 and 14 dbh. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2015.

Chemical name	Trade name	Application rate	Application timing(s)	Control fruit	OHC fruit	Raynox treated fruit	Eclipse treated fruit	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Penthiopyrad	Fontelis	20	35 & 28	0.015	<0.01	0.011	<0.01	0.5	0.4 (many)
Hexythiazox	Onager	24	28	0.020	0.014	0.016	0.013	0.4	0.4 (many)
Pyridaben	Nexter	10.67	28	0.041	0.034	0.029	0.029	0.5	0.5 (many)
Lambda-cyhalothrin	Warrior II	2.56	28 & 21	<0.05	<0.05	<0.05	<0.05	0.3	0.2 (many)
Flutriafol	Topguard	12	28 & 14	0.023	0.023	0.019	0.015	0.4	0.2 (Hong Kong)
Fenpropathrin	Danitol	21.3	28 & 14	0.120	0.120	0.120	0.074	5	0.5 (Taiwan)
Difenoconazole	Inspire Super	12	21 & 14	<0.01	<0.01	<0.01	<0.01	5	0.01 (India)
Cyprodinil	Inspire Super	12	21 & 14	<0.01	<0.01	<0.01	<0.01	1.7	0.05 (Vietnam)
Flubendiamide	Tourismo	17	21 & 14	0.057	0.059	0.042	0.040	1.5	0.8 (many)
Buprofezin	Tourismo	17	21 & 14	0.024	0.027	0.025	0.014	3	1 (Taiwan)
Fluopyram	Luna Sensation	5.8	21 & 14	0.010	0.012	<0.01	<0.01	0.3	0.3 (CAN,MEX)
Trifloxylstrobin	Luna Sensation	5.8	21 & 14	<0.01	<0.01	<0.01	<0.01	0.5	0.5 (CAN, MEX)
Emamectin benzoate	Proclaim	4.8	21 & 14	<0.01	<0.01	<0.01	<0.01	0.025	0.02 (many)
Myclobutanil	Rally 40WSP	10	21 & 14	0.032	0.029	0.024	0.018	0.5	0.5 (many)
Spirotetramat	Ultor	14	21 & 7	0.038	0.022	0.024	0.022	0.7	0.7 (many)
Cyflumetofen	Nealta	13.7	21 & 7	0.037	0.026	0.027	0.016	0.3	0.3 (CAN,MEX)
Spinosad	Entrust	3	21 & 7	0.035	0.031	0.048	0.022	0.2	0.1 (many)
Etoxazole	Zeal	3	14	0.016	0.020	0.020	0.012	0.2	0.07 (many)
Ziram*	Ziram 76DF	128	14	<0.1	0.120	<0.1	<0.1	7	2.5 (Taiwan)
Metrafenone	Vivando	15.4	14 & 7	<0.01	<0.01	<0.01	<0.01	1.5	0.01 (Taiwan)
Cyantraniliprole	Exirel	20.5	14 & 5	0.032	0.040	0.035	0.027	1.5	0.8 (many)
Spirodiclofen	Envidor 2SC	18	7	0.065	0.047	0.058	0.033	0.8	0.8 (many)
Bifenazate	Acramite	16	7	0.029	0.018	0.033	0.030	0.7	0.2 (China)
Thiophanate-methyl**	Topsin 4.5FL	20	7 & 1	0.099	0.082	0.111	0.100	2	3 (many)
Pyraclostrobin	Merivon	5.5	7 & 1	0.053	0.036	0.050	0.036	1.5	0.5 (many)
Fluxapyroxad	Merivon	5.5	7 & 1	0.056	0.043	0.057	0.040	0.8	0.8 (CAN,MEX)

[1] Top markets for WA apples; 17 Sep 2015. http://www.nwhort.org/AppleMRLs.html, https://www.globalmrl.com/

* Dithiocarbamate residues cannot be directly measured; total Ziram values are estimates based on analysis of the degradation product CS_2

** Thiophanate-methyl values reported are sum totals of thiophanate-methyl and carbenzadim residues

CONCLUSIONS

As expected, residues measured for all pesticides were well beneath EPA tolerances for domestic fruit, but for the first time in 5 years, **no residues were found in excess of any MRL for a major export market** for WA apples. Credit for this result lies in both the continued relaxation and standardization of MRLs in several foreign markets which formerly held more stringent standards as well as the incidence of lighter residues (relative to previous findings) for many products, particularly fungicides. While our study experienced normal wind conditions and no rainfall this year, intense heat and sunshine throughout the trial may have accelerated the UV degradation of sensitive chemistries this year, producing lesser residue levels. As in previous studies, the application of Raynox, Eclipse, or overhead cooling did not significantly affect pesticide residues. Reports from previous pesticide residue studies on apple and cherry which provide a broader context for these results are available on the WTFRC website at www.treefruitresearch.com. For more resources on MRLs, visit the Northwest Horticultural Council website, www.nwhort.org.

For more information, contact Tory Schmidt (509) 669-3903 or email tory@treefruitresearch.com

WASHINGTON TREE FRUIT RESEARCH COMMISSION

CONTINUING PROJECT REPORT **YEAR:** 2015

Project Title: Programs to increase packouts of apples

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

Cooperators:
WTFRC internal program: Manoella Mendoza, Tory Schmidt, Sandy Stone, Kyle Tynan
Scientists: Jaqueline Gordon, Lauren Brandt (both WSU)
Product suppliers: Extenday Inc.
Others: Grower collaborators, WTFRC seasonal crew and interns, Glade Brosi (Stemilt)

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

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

Item	2014	2015	2016
Salaries	7,120	13,817	10,000
Tax		4,990	
Wages	12,351		10,000
Equipment + supplies		749	
RCA rental	1,800	1,800	1,800
Total gross costs			
Reimbursements			
Total net costs	**21,271**	**21,356**	**21,800**

Footnotes:
Entire budget is based on fiscal year August 1st– July 31st the following year, i.e. 2015 reflects costs from Aug.1, 2014 until July 31st, 2015.
Salaries: incl. benefits,proportional time spent on outlined projects for Hanrahan, Mendoza, Schmidt, Tynan
Wages: incl. benefits, covers timeslip expenses, benefit rate at 42%
Supplies: experimental fruit, storage boxes and trays donated, Extenday supplied shade nets
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. Determine harvest maturity and storage behavior of red strains of Honeycrisp.* (no data in 2015)
2. Investigate practical use parameters for the DA meter in Honeycrisp orchards. Determine if the I_{AD} index correlates to common maturity parameters.* (no data in 2015)
3. Document Honeycrisp fruit quality in local store displays.
4. Determine easy and cost effective ways to measure titratable acidity.
5. Determine dry matter content of Honeycrisp apples. (NEW in 2015)
6. Determine spatial distribution of bitter pit within the tree in Honeycrisp orchards. (NEW in 2015)
7. Conduct consumer taste panels for Honeycrisp apples. (NEW in 2015)
8. 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 3: Honeycrisp were available until July in local stores. Eating quality remains of concern and fruit from the southern hemisphere did not show improved olfactory acceptability.

Objective 4: Titratable acidity can be determined reliably and cost effectively with several “off the shelf” tests.

Objective 5: A range of dry matter content in Honeycrisp apples was observed in 2015, with weak correlation to final eating quality and to physical measurements at harvest or after storage.

Objective 6: No bitter pit appeared preharvest in the orchards observed, but in storage fruit fruit from earlier picks and low canopy positions developed significantly more bitter pit symptoms.

Objective 7: Fruit assessed within the experiment performed well in taste panels, regardless of storage treatment or orchard location.

Objective 8: Ongoing collaborative efforts across disciplines, institutions, and regions leverage funding and increase relevance and impact of research in the areas of fruit quality and food safety.

METHODS

Supermarket survey: Eight Yakima supermarkets were visited monthly from February until July 2015. Visual and sensory quality of fruit was determined.
Titratable acidity test kits: Two tube tests (Accuvin Titratable Acidity and BSG Wine Acid Test)were compared to an automated titrator (Methrom).
Dry matter content: Slices were cut from the middle of 20 apples/ treatment of each of four replications, then dried them for 48 hours at 70°F. The remaining fresh fruit sample was used to determine TA, SSC and flavor.
Bitter pit: Fruit in two orchards was marked in three positions (top, middle, bottom) and evaluated frequently from July until harvest for bitterpit onset and incidence rate. Secondly, fruit from all locations within tree was harvested sequentially in three picks and stored for up to four months (HC protocol). Bi-weekly observations revealed the sequence of bitterpit symptom appearance and final bitter pit severity.
Taste panel HC: We harvested fruit from three orchards at optimum CA quality and stored it for one week @50F, then 36F for 6 months.

The six treatments included:

a) CA;
b) CA + 1-MCP;
c) 9,8,6 weeks of RA followed by CA (different for each orchard);
d) same as c. with 1-MCP treated fruit;
e) 2 weeks RA followed by CA; f) same as e. with 1-MCP treated fruit.

At harvest and after storage common maturity parameters and disorders were assessed. In the lab two people tasted each apple to determine off flavor. A consumer taste test (remaining fruit, 50+ pieces) was conducted of fruit from treatment a-d with 120 participants. Panelists rated fruit based on overall appearance, taste/flavor, texture, overall eating experience.

RESULTS & DISCUSSION

The fruit quality program has continued to focus on Honeycrisp fruit quality in 2015, but the general scope was scaled back based on industry direction. In addition, Ines Hanrahan also took on responsibility as the principal investigator and laboratory manager for Karen Killinger's lab upon her departure in July.

Supermarket survey: Eight Yakima area supermarkets were visited eight times from February 15 until July 22, 2015. All locations carried fruit until May 15th, and three stores continued to sell Honeycrisp until the end of our observations on July 22, 2015 (not shown). Most notably, several stores started to carry fruit from Chile in July. Fruit eating quality from Chile was acceptable, but not outstanding compared to much older local fruit still available in stores at this time. Overall, eating quality of fruit has improved compared to 2014, with 70-100% of stores carrying fruit of acceptable (bland) or excellent (apple flavor) eating quality at any time, except in late July (Figure 1).

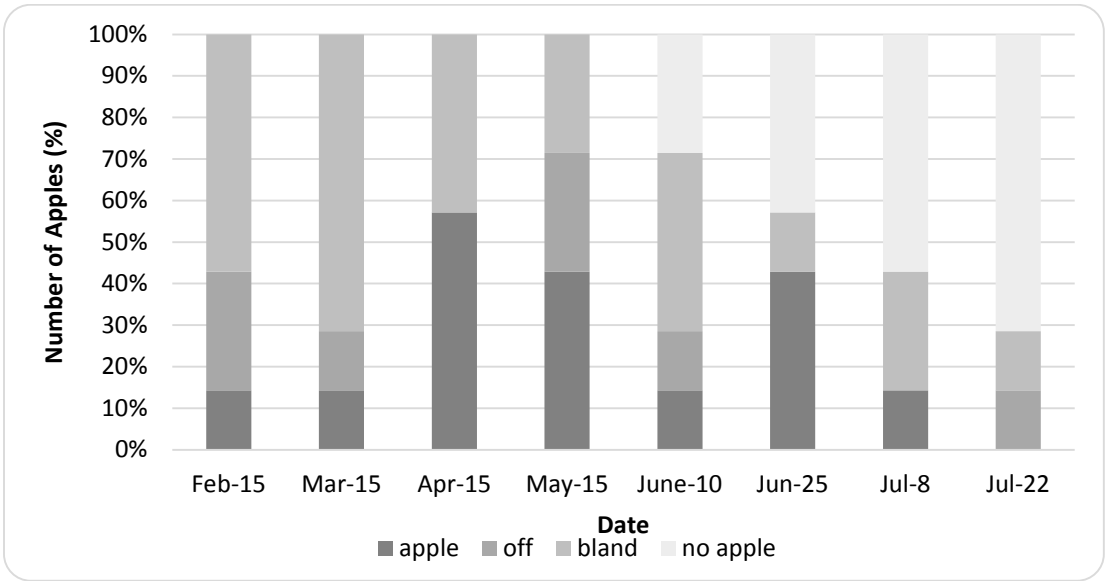


Figure 1: Taste of fruit (8 stores total) for Honeycrisp purchased in Yakima area supermarkets between February 15 and July 22, 2015. Scale: apple=typical fresh HC taste, off=off flavor, bland=no off flavor but lacking taste, no apple= no apples available in some stores.

Titratable acidity test kits: To ensure good storability and eating quality of Honeycrisp apples after CA storage, it is advisable to select lots with higher titratable acidity (TA) levels at harvest. Malic acid is the prevalent acid in apples. It is used as metabolic substrate during storage and is typically highest in fully mature fruit. Traditionally, TA could only be determined with specialized titration equipment. This method is time consuming and expensive, thus not practical for commercial packing and quality control. The Accuvin TA test kits (TT) (www.resultsnowtests.biz) (results reported last year) and a BSG Wine Acid Test (WAT) were compared to an automated titrator (Methrom) used at the WTFRC quality lab in Wenatchee (Figure 2). Both tests (TT and WAT) are less accurate than the Methrom (Table 1), but the relative ease of use would make them suitable for use in QC departments.

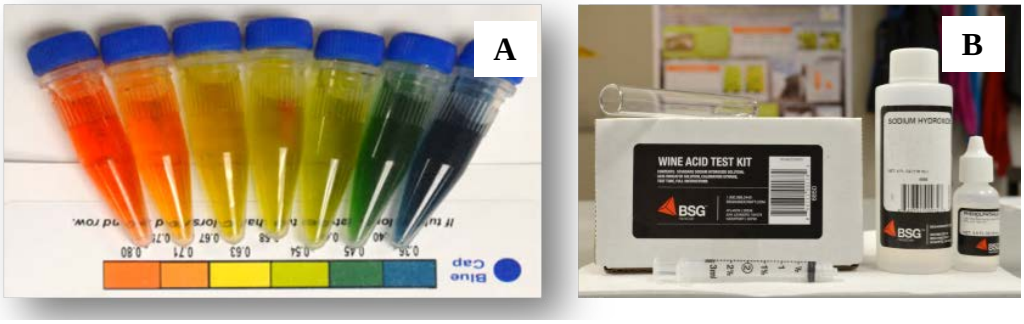


Figure 2: Titratable acidity test kits: A= Accuvin; B=BSG Wine kit

Table 1: TA test kit results 2015.

	AccuvinTitrable Acidity	BSG Wine Acid Test
Avg. time per sample	1 min.	3 min.
Accuracy	73 %	87 %
Cost per 100 samples	$ 166.80 - 250.00 (depending on box size)	$ 27.73
Training	easy	intermediate

Dry matter content: Dry matter content (DMC) of fruit includes all constituents excluding water, i.e. the accumulated sugars, cell walls, proteins and starch that are present in the apples. DMC has been suggested as additional fruit quality parameter to be assessed after storage. Further, DMC has been correlated with quality of various fruit species at harvest. Consumer preference in previous studies has shown a correlation to higher DMC levels.

We determined DMC for several lots of Honeycrisp after storage (3 and 6 months, with and without 1-MCP) and obtained a range of values from 12-23% (not shown). In 2014 the difference in overall DMC content of fruit was higher between orchards, than within the same orchard (covered with net vs. control). However, we did see a slightly increased DMC in netted sections of the same orchard (not shown). When correlating final fruit eating quality to DMC we found fruit with higher DMC to maintain apple flavor better during storage and fruit with lower DMC had comparatively more apples with off flavor (Figure 3).

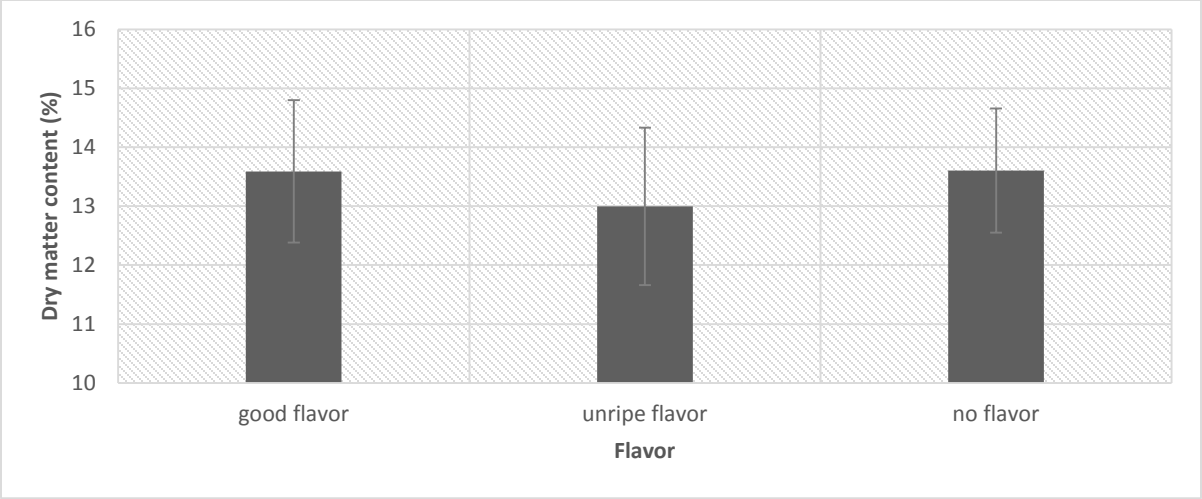


Figure 3: DMC after 3 month in storage in relation to final eating experience. Data includes one orchard location, several storage treatments and combines fruit from netted and un-netted sections in 2015.

Bitter pit: No bitter pit was observed in 2015 during the growing season in the orchards selected for the study. During storage, bitter pit appeared after 1-2 weeks of storage on fruit from top and bottom locations (5-10% of fruit affected). After 12 weeks of storage, apples from the 1st and 2nd pick showed the highest amount of bitter pit (Figure 4). Bitter pit was found more frequently in fruit from the top and bottom locations within trees.

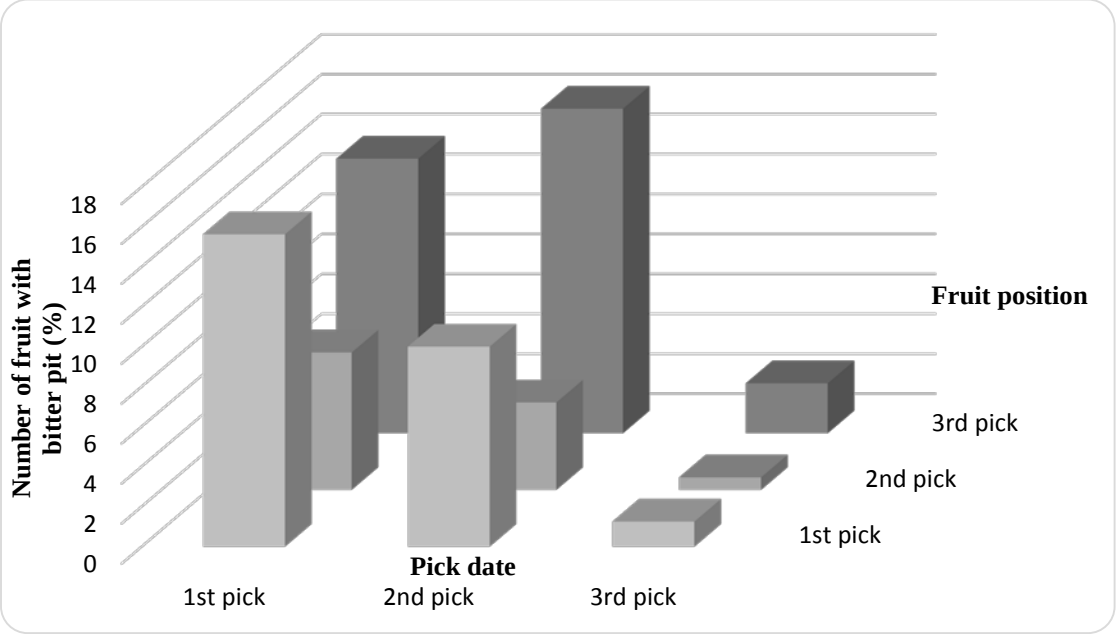


Figure 4: Bitter pit after 3 months in RA storage in 2015

Taste panel HC: We conducted three taste panels in March of 2015. Participants were recruited during a master gardeners meeting and from WSU Extension office staff. Overall, none of the fruit received consistently high or low scores. Although fruit within one tasting was selected from the same orchard, participants rated appearance as different amongst the samples. (example in Fig. 5). In general, fruit from all orchards ranked highest for overall eating experience and appearance if treated with the industry standard of CA+1-MCP. We have noted 'fresher' appearance of fruit treated with 1-MCP after long term CA storage in previous experiments.

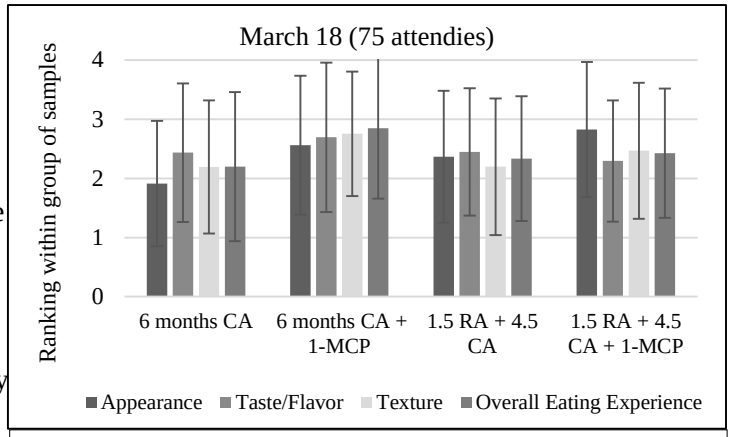


Fig. 5: Consumer ratings for Honeycrisp fruit subjected to CA storage with differing amounts of time spent in RA initially.

Collaborative research

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

Table 2. 2015Hanrahan/WTFRC collaborations on pre-and post- harvest fruit quality and food safety projects.

COLLABORATOR(S)	PROJECT	COMMENTS/HANRAHAN ROLE
NEW: Killinger*	Listeria environ. monitoring	PI
Killinger	Overhead cooling	PI (see AP-12-108)
Evans/Auvil	WSU Breeding: P3	Collaborator storage evaluation
Mattheis/Rudell	Extend HC storage life	CO-PI
Killinger	Microbial safety of bins	Collaborator on CPS project
NHC/WSU*	China apple export	Collaborator harvest + storage
NEW: Atwill*	Yakima Valley Irrig.	Collaborator
NEW: Suslow*	Microbial indicator	Collaborator

*project costs completely covered by companies/external projects